Working papers from the international symposium:

Beyond Antimicrobial Growth Promoters in Food Animal Production

Research Centre Foulum
6-7 November 2002

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Organised by:

Danish Institute for Food and Veterinary Research and

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Preface

An international invitational symposium *Beyond antimicrobial growth promoters* was held on 6-7 November 2002 at the Danish Institute of Agricultural Sciences, Research Centre Foulum. The symposium was organised by Danish Institute for Food and Veterinary Research and Danish Institute of Agricultural Science with the support of the World Health Organization. A total of 140 participants from 12 countries participated in the symposium, which included 32 scientific presentations in 6 sessions with the following headings:

- Effects of the termination of antimicrobial growth promoter use on bacterial resistance to antimicrobials
- Effects of the termination of antimicrobial growth promoter use on animal welfare and productivity
- Consequences of termination of antimicrobial growth promoters use for animal health and the use of antimicrobials in food animals for therapy and prophylaxis
- Effects of the termination of antimicrobial growth promoter use on food prices and the competitiveness of agricultural industries
- Consequences of termination of antimicrobial growth promoters use for the environment
- Alternatives to the use of antibiotic growth promoters
- International speakers

This report contains all working papers prepared by the presenters in the symposium. The papers are also available at www.who.int

In conjunction to the symposium the World Health Organization organised an independent expert review of the Danish experiences. The expert reviewers report is also available from www.who.int

This report has been prepared for publication by Susanne Carlsson DFVF and Lotte Tind Pedersen DIAS.

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1. Effects of termination of AGP use on antimicrobial resistance in food animals

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Abstract

Antimicrobial agents are in the production of food animals used for therapy and prophylactics of bacterial infections and in feed to promote growth. The use of antimicrobial agents for food animals may cause problems through the selection for resistance among bacteria pathogenic for animals or humans (zoonotic bacteria). The emergence of resistant bacteria and resistance genes because of the use of antimicrobial agents are well documented and there is in general a close association between usage and resistance. Especially the selection and spread of glycopeptide resistant Enterococcus faecium (GREF) has come under increased scrutiny in recent years. Today there is compelling evidence that the use of the glycopeptide avoparcin for growth promotion for food animals has selected for GREF among the food animal reservoirs and that this have played some role in the epidemiology of GREF causing infections in man. In Denmark it has been possible to reduce the usage of antimicrobial agents for food animals significantly, through banning of antimicrobial agents for growth promotion and restrictions on the usage for therapy. The use of avoparcin was banned in 1995 and a decrease in the occurrence of GREF among food animals has been observed. Also in other countries a decrease in resistance among both food animals and humans has been observed following the ban of avoparcin. In general a decrease in resistance have followed the reduction in usage of other antimicrobial agents used for growth promotion.

Introduction

The introduction of antimicrobial agents in human clinical medicine and animal husbandry has been one of the most significant achievements of the 20th century. The first antimicrobial agents were introduced in the 1930's, and a large number of new compounds were discovered in the following decades. However, shortly after the introduction, resistance began to emerge and in all known cases emergence of antimicrobial resistance have followed the introduction of new antimicrobial compounds (Levy, 1982). It has now become clear that antimicrobial resistance poses a threat to human and animal health and should be taken seriously.

Modern food animal production depends on the use of large amounts of antibiotics for disease control. This provides favourable conditions for selection, spread and persistence of antimicrobial-resistant bacteria capable of causing infections in animals and humans. During the last decade there has been an increased awareness of the potential problems selection of antimicrobial resistance among food producing animals could have on human health.

Already in the late 1960'ties the Swann Committee pointed to the potential problems with selection of antimicrobial resistance through the usage of antimicrobial agents for growth promotion (Swann, 1969). Following this, a number of antimicrobial agents that were used for therapy were banned for use for growth promotion in most European countries. However, a large number of antimicrobial agents were still used or became introduced for use as growth promoters in the following decades.

During the 1980'ties and early 1990'ties pathogenic bacteria resistant to an increased number of antimicrobial agents emerged world-wide. As a consequence antimicrobial agents belonging to classes that not previously have had clinical interest gained increasing importance. One of these agents was the glycopeptide antimicrobial, vancomycin that was discovered in the early 1960'ties. Vancomycin became in the 1980'ties

one of the most important antimicrobial agents for the treatment of infections in humans with multiple resistant Gram-positive bacteria.

In 1993 glycopeptide resistant enterococci (GRE) were isolated from food animals in England (Bates et al., 1993) and soon after in Germany (Klare et al., 1995). This was surprising because no glycopeptide antibiotics had been used for treatment of infections in food animals. However, another glycopeptide antibiotic, avoparcin, had been used for several years for growth promotion in several countries around the world and it was suggested that the occurrence of GRE might be related to this usage.

In 1994 no studies had been conducted to monitor the occurrence of antimicrobial resistance to agents used for growth promotion in Denmark. Inspired by the findings in England and Germany, the Danish Veterinary Institute conducted a small survey on the occurrence of GRE among conventional and organic poultry (Aarstrup, 1995) in Denmark. The first finding of GRE was confirmed on January 25th 1995. The findings resulted in much scientific and public attention and in April 1995 the Danish Pig and Poultry Producers Organizations decided voluntarily to stop the use of avoparcin for growth promotion. In May 1995 this was followed by a general ban by the Danish Ministry of Agriculture and Fisheries. The ban was imposed to preserve the efficacy of vancomycin as a drug of last resort in human medicine.

Within the European Union, the European Commission approves antimicrobial agents for growth promotion centrally and until recently 11 different substances were approved (Table 1). In 1986 Sweden banned the use of all antimicrobial agents for growth promotion. When Sweden in 1994 joined the European Union they were granted five years before they had to adapt to the common guidelines. For Denmark no such exception was in place. A member state can only temporarily ban the use of a product if new information has shown danger to human health. The Danish ban in 1995 was controversial and was not supported by the EU Scientific Committee on Animal Nutrition. The ban was, however, upheld by the European Council of Ministers. In January 1996 Germany banned the

use of avoparcin and in 1997 a general ban on the use of avoparcin followed in all of EU. The finding of VRE among food animals in Denmark prompted a number of initiatives by the Danish authorities to limit the increase of antimicrobial resistance. Since 1995 the Danish Veterinary Institute has conducted or been involved in a number of monitoring and research programmes with the overall aim to determine the occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion.

In the following an overview of the use of antimicrobial agents for growth promotion food animals, and the effects of the reduced usage over time is given. Special emphasis is given to data from Denmark.

Usage of antimicrobial agens for food animals

The first observations on the inhibitory effect of penicillium mould on bacteria seems to have been made by Sir John Burden-Sanderson in 1871 and Joseph Lister in 1872 (Fraser-Moodie, 1971; MacFarlane, 1984). In 1928 Alexander Fleming made similar observations (Fleming, 1929) and when it later became possible to purifying penicillin (Fleming) the way for the use of penicillin for therapy was opened. Since then a large number of other antimicrobial agents have been discovered and introduced for human and veterinary therapy and in the course of the last 50 years antimicrobial agents have become the keystone in the therapy of bacterial infections in humans and animals.

In Denmark greater amount of antimicrobial agents were until recently used in animal feed for growth promotion than for treatment of diseases (Aarestrup et al., 1998a; Aarestrup et al., 1998b). Avoparcin was banned in Denmark because of the selection of vancomycin resistant enterococci and the potential risk of spread of this resistance through the food chain to humans. In 1997, avoparcin was banned in all EU-countries.

In January 1998, virginiamycin was banned in Denmark because of cross-resistance to Synercid, a streptogramin used for human treatment. In December 1998, the European Commission

decided to ban the use of bacitracin, spiramycin, tylosin and virginiamycin for growth promotion from the 1st of July 1999. In Denmark the food animal industries decided to voluntarily stop all use of antimicrobial growth promoters from the end of 1999. This has had major influence on the amounts of antimicrobial agents used in Denmark (Table 1 and 2).

Table 1. Usage of growth promoters in Denmark during 1990 through 2001

Growth promoter	1992	1994	1995	1996	1997	1998	1999	2000-01
Avilamycin	853	433	1,665	2,740	670	7	91	3
Avoparcin*#	17,210	24,117	5,690	-	-	3,945	-	-
Bacitracin#	5,657	13,689	7,910	8,399	8,544	1,803	63	-
Carbadox#	7,221	10,012	1,181	1,985	4,153	6	293	-
Flavomycin	1,299	77	48	18	93	935	665	11
Monensin	3,700	4,755	5,007	4,741	3,08	28,445	0	-
Olaquindox#	21,193	22,483	16,213	13,486	17,595	113	9,344	-
Salinomycin	0	213	850	759	460	0	0	-
Spiramycin#	0	95	507	15	3	13,148	0	-
Tylosin#	26,980	37,111	52,275	68,350	62,009	892	1,827	-
Virginiamycin**#	15,537	2,801	2,590	5,055	10,644	49,294	-	-
Total	99,650	115,786	93,936	105,548	107,179	1998	12,283	14

*: Banned in May 1995 in Denmark
**: Banned in January 1998 in Denmark
#: Approval suspended in EU in 1999

Table 2. Estimated usage of antimicrobial agents for treatment of infections in food animal production in Denmark

Antimicrobial agent	1992	1994	1996	1997	1998	1999	2000	2001
Aminoglycosides	8,500	8,600	7,100	7,000	7,800	7,500	10,400	11,900
Macrolides	12,900	11,400	7,600	6,700	7,100	5,300	15,400	14,300
Penicillins	6,700	9,400	7,200	13,100	14,300	14,700	14,800	17,100
Syntetic penicillins	2,500	4,400	5,800	6,200	6,700	6,600	7,600	9,300
Sulfonamides	5,900	5,600	2,100	1,400	1,000	1,000	1,000	800
Sulfa/TMP	7,900	9,500	4,800	6,900	7,700	6,800	7,000	7,400
Tetracyclines	22,000	36,500	12,900	13,700	12,100	16,200	24,000	27,900
Others	6,800	4,400	600	650	3,800	3,800	300	5,500
Total	73,200	89,900	48,000	55,700	57,300	61,900	80,600	94,200

Selection for resistance

Infections with enterococci in animals are rare and are also rarely treated with antimicrobial agents (Aarestrup et al., 2002). However, as normal inhabitants of the intestinal tract they are exposed to antimicrobial selection every time the animals are subjected to antimicrobial treatment or given antimicrobial agents for growth promotion or prophylaxis. A large number of studies have shown that the use of antimicrobial agents whether in high or low concentrations will select for resistance. Already in 1951 Starr & Reynolds reported the selection of streptomycin resistant coliforms in the intestinal tract of turkey experimentally fed streptomycin. In the 1950'ties studies confirmed that the use of tetracycline in concentrations for treatment or growth promotion selected for tetracycline resistance among group D streptococci in chickens

(Barnes, 1958; Elliott & Barnes, 1959). More recently, epidemiological studies and feeding experiments have clearly shown that the use of different antimicrobial growth promoters, the glycopeptides (avoparcin), macrolides (tylosin) and oligosaccharides (avilamycin), will select for resistance among enterococci in poultry and pigs (reviewed in Aarestrup, 2000b).

Effects of interventions

There is only limited information available about the effects of terminating the usage of an antimicrobial agent on the occurrence of antimicrobial resistance. Following the ban of tetracycline for growth promotion in The Netherlands in 1974 a decrease in the occurrence of tetracycline resistance in salmonellae isolated from food animals and humans were observed (van Leeuwen et al., 1979).

In Denmark the DANMAP program for surveillance of antimicrobial resistance was established in 1995 (Aarestrup et al., 1998a; Aarestrup et al., 1998b). The program determines the susceptibility of antimicrobial resistance among selected pathogenic bacteria and bacteria isolated from healthy food animals, and are co-ordinated with similar programs determining resistance among bacteria from food and humans. The establishment of this programme has made it possible to follow the changes in occurrence of antimicrobial resistance as a consequence of changes in usage.

Glycopeptide resistant enterococci

The occurrence of resistance among E. faecium from pigs and broilers has largely followed the consumption of the different agents. Thus, since the ban of avoparcin in 1995 the occurrence of vancomycin resistance has decreased significantly among enterococcal isolates from broilers, whereas no significant change occurred in pigs (Aarestrup et al., 2001) (Fig. 1). One explanation for the persistence of glycopeptide resistant enterococci (GRE) among the pig population in Denmark could be that the production of broilers in Denmark is performed in all in all out facilities with thorough cleaning of housing facilities after each flock, whereas the production of pigs takes place in facilities in continu-

ous use. However, all GRE isolated from pigs were also simultaneously resistant to macrolides and tetracycline. These two antimicrobials have been commonly used for growth promotion and therapy, respectively, in the pig production in Denmark. It was since shown that all GRE isolated from pigs in Denmark belonged to the same clone and that the genes encoding resistance to macrolides (ermB) and glycopeptides (vanA) were located on the same mobile DNA-element (Aarestrup, 2000a).

Fig. 1. Glycopeptide resistance among *E. faecium*
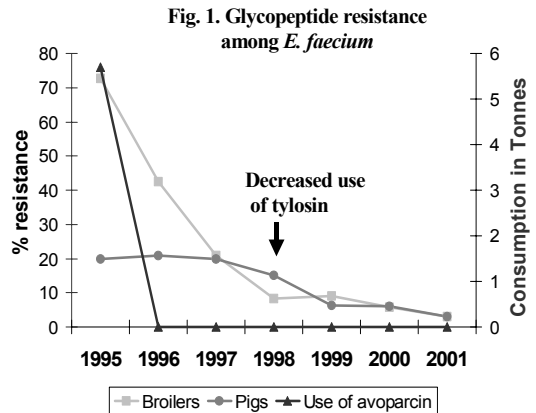
The consumption of tylosin for growth promotion decreased substantially during 1998. During 1999 and 2000 a significant decrease in the occurrence of GRE among E. faecium isolates from pigs have been observed (Aarestrup et al., 2001). These findings strongly suggest that the persistence of GRE among the pig population was caused by the continued use of macrolides, mainly tylosin, for growth promotion and therapy.

In Germany a decrease in the GRE carrier rate among healthy humans in the community in the period 1994 to 1997 has been observed (Klare et al., 1999). In 1994 the carrier level was found to be 12%. This level has decreased to approx. 4% in 1997.

Similarly a decrease in the occurrence of VRE among poultry products in Italy has been observed during 18 months after the ban of avoparcin (Pantosti et al., 1999).

Also in The Netherlands a decrease has been observed among broilers, pigs and humans from 1997 to 1999 (van den Bogaard et al., 2000). A plausible explanation for this is a reduced exposure to GRE from food as a result of the withdrawal of avoparcin from animal feed.

These observations show that the occurrence of GREF among food animals has decreased after the selective pressure, the use of avoparcin, has been removed. However, in the case of resistance to vancomycin among isolates from pigs in Denmark it has also been shown that if the resistance genes are located on the same genetic element, resistance can persist as a consequence of co-selection.

Prevalence of GRE in The Netherlands
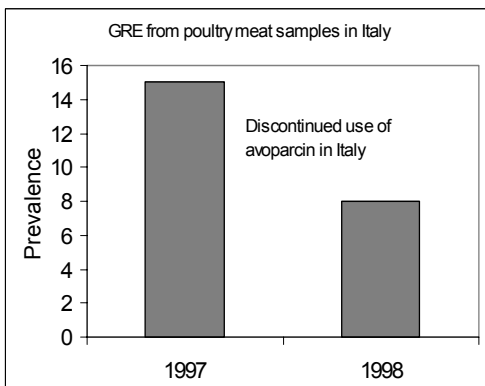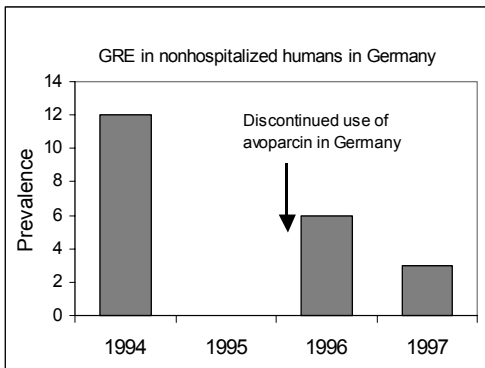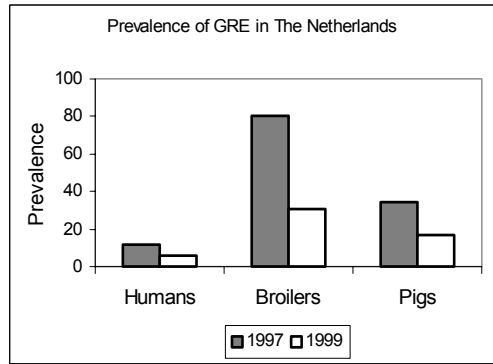
Macrolides

For several years the macrolide tylosin was the most widely used AGP for pigs in Denmark. In 1995 the occurrence of resistance among E. faecium isolates from pigs was 80%, in 1996 it was 93%, and in 1997 it was 87%. Among E. faecalis isolates resistance was 94% in 1995, 91% in 1996, and 91% in 1997. The use of tylosin decreased considerably during 1998 and 1999. This was almost immediately followed by a decrease in the occurrence of resistance among both E. faecium and E. faecalis isolates from pigs (Fig.4). Thus, in 1998 resistance among E. faecium isolates had decreased to 71%, and that among E. faecalis isolates had decreased to 79%. Resistance have decreased further to 26 and 32%, respectively, in 2001. Macrolides are still widely used for treatment of infections in pigs, and thus future trends in the occurrence of resistance cannot yet be predicted.

The occurrence of macrolide resistance among E. faecium isolates from broilers has also been very high, reaching a maximum of 76% in 1997. It decreased to 15% during 2001, concomitantly with the more limited use of virginiamycin. Relatively minor amounts of the macrolide spiramycin have been used for growth promotion in broilers. However, virginiamycin is a natural combination of two structurally unrelated molecules (groups A and B), and group B has the same mechanism of action as antimicrobials belonging to the macrolides.

GRE in nonhospitalized humans in Germany

GRE from poultry meat samples in Italy

Fig. 5 Resistance to tylosin among enterococci from pigs

*: 4de quarter only

E. faecium — E. faecalis — Use of Tylosin
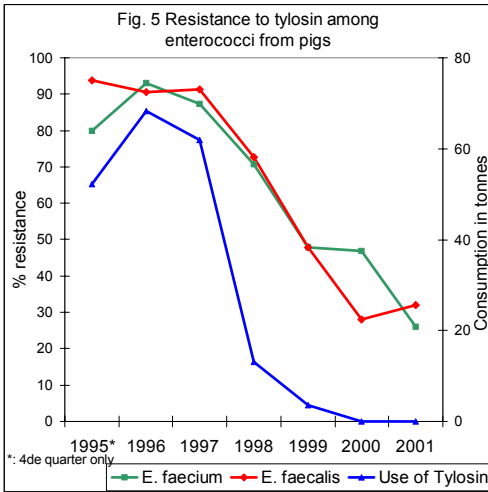
Enzymes methylating the target site of macrolides, lincosamides, and streptogramin B have been observed to be the most common cause of resistance in enterococci. In addition, it has recently been shown that some of the genes encoding resistance to group A and group B, respectively, are genetically linked. Thus, it seems likely that the use of virginiamycin as a growth promoter for broilers may have selected for macrolide resistance in E. faecium.

Oligosaccharides
Avilamycin has primarily been used for growth promotion in the broiler production in Denmark. The consumption of avilamycin for growth promotion increased from 10 kg in 1990 to 2,740 kg 1996 and decreased in the following years to only 7 kg in 1998. The occurrence of resistance among isolates from broilers increased from 64% at the end of 1995 to a maximum of 81% during the last half of 1996. Since then, the occurrence of resistance decreased to 5% in 2001 (Fig. 6). Thus, the occurrence of resistance has closely followed the consumption.

Streptogramins
The use of virginiamycin increased between 1995 to 1997. The increase was seen mainly in broilers and was associated with an increase in the occurrence of resistance in E. faecium.

Thus, virginiamycin resistance among E. faecium isolates from broilers increased from 27% in 1995 to 66% in 1997. In January 1998, the use of virginiamycin was banned in Denmark, and the occurrence of resistance subsequently decreased to 27% in 2001 (Fig.7). More variation in the occurrence of virginiamycin resistance can be observed among E. faecium isolates from pigs. Thus, resistance decreased from 60% in 1995 to 36% in 1997, increased in 1998 to 56%, but has since decreased to 6% in 2001.

Fig. 6 Avilamycin resistance among *E. faecium*

*: 4th quarter

Broilers — Use of Avilamycin

Fig. 7 Virginiamycin resistance among *E.faecium*

*: 4th quarter

Broilers — Pigs — Use of Virginiamycin

Taken together all these different observations show that the occurrence of resistance will decrease after the selective pressure has been removed. However, in the case of resistance to vancomycin among isolates from pigs it has also been shown that if the resistance genes are lo-

cated on the same genetic element, resistance can persist as a consequence of co-selection.

Discussion and conclusion

In the 1970'ties enterococci were not considered important human pathogens and prior to 1986 they were in general considered susceptible to glycopeptides. However, shortly after the first observations of GRE this resistance spread rapidly world-wide. The most effective way to limit the spread of antimicrobial resistance and thereby extend the usefulness of antimicrobials is through a restricted use of antimicrobial agents. As a consequence it has been recommended that antibiotics that select for resistance against antibiotics used for human therapy no longer should be used in animal husbandry. Especially the use of growth promoters should be limited to agents that are of no interest for therapeutic use (Aarestrup, 2000b).

When avoparcin was approved for use as growth promoter in animals, this group of antibiotic was not seen as potentially important in human medicine. Thus, it was at that time difficult to foresee that due to the development of antimicrobial resistance among bacteria causing infections in humans these classes of antimicrobial agents became very valuable. The resistance that has already been created by the use of avoparcin as growth promoter will most likely reduce and shorten the lifespan of vancomycin as therapeutic in humans. The safest way to limit the development of resistance to antimicrobial agents seems to be to limit the use of antimicrobial agents as much as possible. Thus, all unnecessary use of antimicrobial agents should be strongly discouraged. However, it is and will also in the future be necessary to use antimicrobial agents for treatment of infectious diseases in food animals and we will also in the future observe the selection of antimicrobial resistance among bacteria from food animals.

It is not known which resistance problems we may face in the future. However, to implement timely actions, and to limit the emergence of antimicrobial resistance and the consequences for human and animal health, it is necessary to obtain and maintain scientific knowledge regarding factors affecting the occurrence, emergence and spread of resistance. At present knowledge of antimicrobial resistance in food animals is in most countries incomplete or even absent. An organised monitoring of antimicrobial resistance carried out by an international network of laboratories should be implemented in order to be able to identify and report emerging resistance problems at the earliest possible stage. In this way we might be able to implement interventions before emerging resistance causes major problems.

The importance of the non-human reservoir of enterococci for infections in humans has not been quantified. There is today compelling evidence that enterococci are transferred between the reservoirs and that the populations colonising and infections humans and animals share the same gene pool. Different studies have shown that it is possible at least to some degree to limit the occurrence of resistance by removing the selective pressure. It is, however, not known whether the occurrence of resistance will ever reach the same low level as before the antimicrobials were introduced. Most probably a low frequency of resistant bacteria will persist and make up a reservoir from where resistance problems easily can be selected if antibiotics are introduced again.

References

Aarestrup FM. 1995. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb. Drug Res. 1, 255-257.

Aarestrup FM. 2000a. Characterization of glycopeptide resistant *Enterococcus faecium* from broilers and pigs in Denmark. Genetic evidences that the persistence of GRE among the pig population is associated with co-selection by resistance to macrolides. J. Clin. Microbiol. 38, 2774-2777.

Aarestrup FM. 2000b. Occurrence, selection and spread of resistance to antimicrobial agents used for growth promotion for food animals in Denmark. APMIS Suppl. 101, 1-48.

Aarestrup FM, Bager F, Madsen M, Jensen NE, Meyling A, Wegener HC. 1998a. Surveillance

of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. APMIS 106, 606-622.

Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A, Wegener HC. 1998b. Resistance to antimicrobial agents used for animal therapy in pathogenic , zoonotic and indicator bacteria isolated from different food animals in Denmark: A baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). APMIS 106, 745-770.

Aarestrup FM, Seyfarth AM, Emborg HD, Pedersen K, Hendriksen RS, Bager F. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob. Agents Chemother. 45, 2054-2059.

Aarestrup FM, Butaye P, Witte W. 2002. Non-human reservoirs of enterococci. In: Gilmore M (ed.). The Enterococci: Pathogenisis, Molecular Biology and Antibiotic Resistance. 1st Edition. ASM Press, Washington DC, 55-99.

Barnes, EM. 1958. The effect of antibiotic suppleents on the faecal streptococci (Lancefield group D) of poultry. Brit. Vet. J. 114, 333.

Bates J, Jordens JZ, Selkon JB. 1993. Evidence for an animal origin of vancomycin-resistant enterococci. Lancet 342, 490-491.

Elliott SD, Barnes EM. 1959. Changes in serological type and antibiotic resistance on Lancefield group D streptococci in chickens receiving dietary chlortetracycline. J. Gen. Microbiol. 20, 426-433.

Fleming A. 1929. On the antibacterial action of cultures of a penicillum, with special reference to their use in the isolation of B. influenzæ. Br. J. Exp. Path. 10, 226-236.

Fraser-Moodie W. 1971. Struggle against infection. Proc. R. Soc. Med. 64, 87-94.

Klare I, Heier H, Claus H, Bohme G, Marin S, Seltmann G, Hakenbeck R, Antanassova V, Witte W. 1995. *Enterococcus faecium* strains with vanA-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. Microb. Drug. Resist. 1, 265-72.

Klare I, Badstubner D, Konstabel C, Bohme G, Claus H, Witte W. 1999. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb. Drug Resist. 5, 45-52.

Levy SB. Microbial resistance to antibiotics. An evolving and persistent problem. Lancet 1982; July 10, 83-8.

MacFarlane G. 1984. Alexander Fleming: The man and the myth. Harvard University Press, Cambridge.

Pantosti A, Del Grosso M, Tagliabue S, Macri A, Caprioli A. Decrease of vancomycin-resistant enterococci in poultry meat after avoparcin ban. Lancet 1999, 354, 741-2.

Starr MP, Reynolds DM. Streptomycin resistance of coliform bacteria from turkeys fed streptomycin. 51st General Meeting, The Society of American bacteriologist, Chicago, Illinois, May 27-31, 1951; 15.

Swann MM. 1969. Joint committee on the use of antibiotics in animal husbandry and veterinary medicine. H.M.S.O., London.

van den Bogaard AE, Bruinsma N, Stobberingh EE. 2000. The effect of banning avoparcin on VRE carriage in The Netherlands. J. Antimicrob. Chemother. 46, 146-147.

van Leeuwen WJ, van Embden J, Guinee PA, Kampelmacher EH, Manten A, van Schothorst M, Voogd CE. 1979. Reduction of the number of tetracycline resistant strains of *Salmonella* in the Netherlands. Tijdschr. Diergeneeskd. 104, 923 927.

2. Effects of the termination of antimicrobial growth promoter use on antimicrobial resistance in bacteria from foods

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Abstract

Antimicrobial growth promoters (AGP) were previously used extensively in the production of broilers and pigs in Denmark. This study is a presentation of data on resistance towards AGP in *Enterococcus faecium* isolates from Danish broiler meat, Danish pork, and vegetables collected at the retail level in the period from 1996 to 2001. The data originate form the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme - DANMAP. Data from broiler meat show that the discontinued use of AGP has had a positive effect on the occurrence of AGP resistance. Following the termination of AGP a decrease was observed in resistance towards avilamycin, virginiamycin and avoparcin in *E. faecium* from broiler meat. Data from pork indicate that the level of resistance in *E. faecium* from pork to virginiamycin and macrolides has been constant during the period 1996-2001, regardless of the termination of AGP use. This is in contrast to corresponding data from pigs, where a rather good association between the phasing out of AGP and resistance trends in faecal isolates of *E. faecium* from pigs at slaughter has been observed. Possible explanations to this could be that it is different clones that prevail in faecal and food samples. Enterococci from pork may originate from other sources in the food supply chain and distinct clones of enterococci may possess different potential for survival in the food production environment. From vegetables, *E. faecium* exhibiting resistance towards AGP and other antimicrobial agents at low frequencies have been isolated. These data indicate that a there is a certain level of antimicrobial resistance in many types of food. The observation is furthermore important since many vegetables are consumed uncooked. The present data indicate that along with the use of antimicrobial agents in food production other factors like cross contamination and survival and growth during food processing may affect the levels of antimicrobial resistant bacteria in foods. The data further show that the sensitivity of the applied isolation procedures should be considered when evaluating resistance data. However, data about antimicrobial resistance in bacteria from food at retail level represents, together with knowledge about consumption patterns, a measure for human exposure to resistant bacteria deriving from foods.

Introduction

The surveillance of antimicrobial resistance in bacteria from food was initiated in 1996 as part of the DANMAP programme. One of the aims of the programme is to monitor the level of antimicrobial resistance in bacteria from farm animals through the food supply chain to humans. Antimicrobial agents, like tylosin, virginiamycin and avoparcin, were previously used extensively as growth promoters in food animals in Denmark. One of the problems with AGP is the exhibition of cross-resistance to important antimicrobial agents that are used for treatment of humans e.g. tylosin/erythomycin (macrolides), virginiamycin/pristinamycin (streptogramins) and avoparcin/vancomycin (glycopeptides). In the DANMAP programme resistance in faecal enterococci are used as an indicator of the level of antimicrobial resistance in Gram-positive bacteria and in this paper data on resistance in *E. faecium* isolated from foods towards AGP are presented.

Materials and Methods

Food samples were collected at retail outlets by Regional Veterinary and Food Control Authorities (RFCA) between 1996 and 2001. The collection of samples was planed annually by the Danish Veterinary and Food Administration

(DVFA), and encompassed different groups of domestically produced raw foods including pork, broiler meat, and vegetables. The food samples were investigated qualitatively for the presence of enterococci by the RFCA. Five grams of food were added 45 ml of azide dextrose broth followed by incubation at 44°C for 18-24 hours. The broth culture was seeded on to Slanetz-Bartley agar and incubated for 48 hours at 44°C. The agar plates were examined for growth and one typical red colony was purified on blood agar. This isolate was identified to species level and susceptibility tested at DVFA. Only one strain of *E. faecium* from each positive sample was susceptibility-tested. The strains were tested for resistance to the following AGP: avoparcin, avilamycin, virginiamycin, macrolides, and bacitracin. The susceptibility testing of strains isolated in 1999-2001 was performed by determination of minimal inhibitory concentration (MIC) in microtitre wells (Sensititre, Trek Diagnostic Systems Ltd.). The strains isolated in 1996-1998 were tested for susceptibility to AGP by the standard agar (Müller-Hinton II agar) dilution method in accordance with NCCLS or by tablet diffusion tests (Sensitabs, Rosco Diagnostica). The diffusion tests were applied for virginiamycin and bacitracin. The following breakpoints were used: avoparcin 32 μg/ml, avilamycin 16 μg/ml, virginiamycin 8 μg/ml (inhibition zone 25 mm), macrolides 8 μg/ml, and bacitracin 128 μg/ml (inhibition zone 17 mm). Whole broilers from the retail level were specifically investigated for presence of *E. faecium* with high-level vancomycin resistance. Five grams of skin was incubated at 37°C for 18-24 hours in 45 ml of azide dextrose broth and subsequently seeded on Slanetz-Bartley agar added 50 μg/ml vancomycin. Typical red colonies were identified as *E. faecium* and the isolated strains were analysed by PCR for presence of *van*A, a gene that confers high-level resistance to vancomycin. Data about consumption of antimicrobials in the Danish livestock have been obtained from DANMAP reports.

Results and Discussion

E. faecium in raw foods

It is usually possible to isolate enterococci from raw foods and *E. faecium* is also a part of the normal microflora of many raw foods. With the applied method *E. faecium* was isolated from approximately 25% of raw pork samples and approximately 33% of the raw broiler meat samples. It is also possible to isolate *E. faecium* from vegetables, but the prevalence varies between different products. The most frequently contaminated vegetables were sprout products, in which *E. faecium* could be isolated from 60% of the analysed samples.

Effects of the termination of AGP use on antimicrobial resistance in E. faecium in pork

The percentages of virginiamycin and macrolide resistance among 186 *E. faecium* strains from Danish pork are presented in Figure 1 together with the consumption of the AGP in kg of active compound.

A
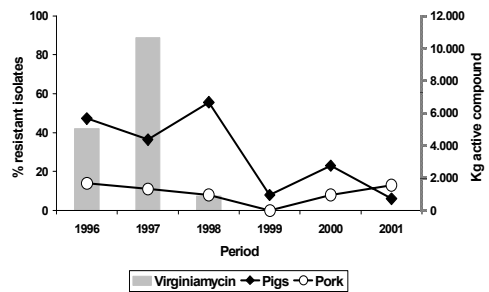
B
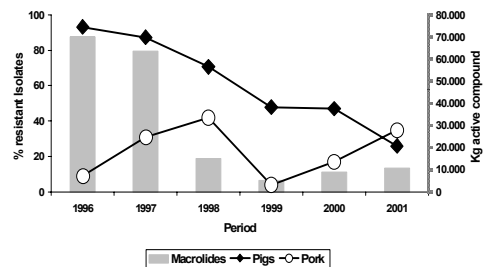
Figure 1. Trends in virginiamycin resistance (A) and macrolide resistance (B) among *Enterococcus faecium* from pigs and Danish pork and the usage of the growth promoters virginiamycin (A) and macrolides (B). Pig data were obtained from Danish Veterinary Institute

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The data show, that the level of resistance for both virginiamycin and macrolides is quite constant during the period, regardless of the termination of AGP use. Avoparcin resistance has not been detected in isolates from pork in the period 1996-2001. The constant level of resistance in *E. faecium* from pork differs from the level of resistance monitored in animals at slaughter, where a decrease has been observed (DANMAP, 2001). The reason for this difference is not clear. A possible explanation could be that it is different clones that prevail in faecal and food samples. Enterococci from pork may originate from other sources in the food supply chain than faeces from pigs and it is also possible that different clones of enterococci have different potential for survival and growth in the food production environment.

Effects of the termination of AGP use on antimicrobial resistance in E. faecium from broiler meat
The resistance in 181 strains of *E. faecium* isolated from Danish broiler meat towards avilamycin, avoparcin, and virginiamycin together with the consumption of the AGP in kg of active compound is presented in Figure 2. The data show that the level of resistance has decreased after the use of AGP in broiler production has been suspended. The decline in occurrence of AGP resistance among *E. faecium* from poultry meat is in accordance with corresponding data on *E. faecium* isolated from faecal samples of broilers at slaughter in Denmark (DANMAP, 2001; Emborg et al., 2003).

A

B
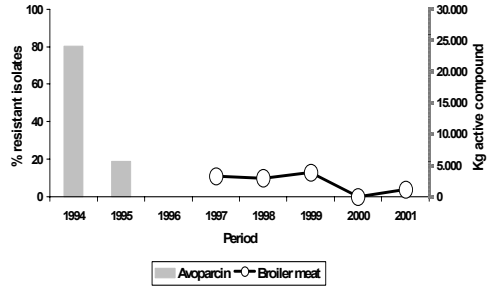
C
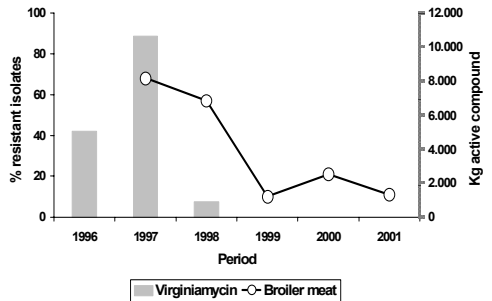
Figure 2. Trends in avilamycin resistance (A), avoparcin resistance (B), and virginiamycin resistance (C) among *Enterococcus faecium* from Danish broiler meat and the usage of the corresponding AGP
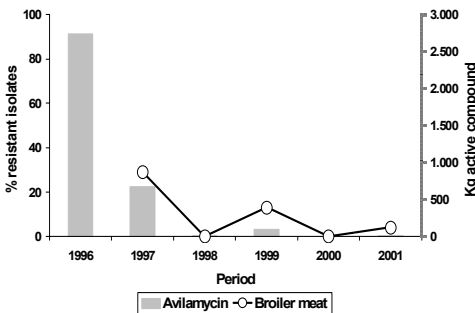
Antimicrobial resistance in E. faecium from vegetables
E. faecium exhibiting resistance towards AGP were isolated at varying frequencies. The percentages of resistance detected in 119 *E. faecium* isolated from raw vegetables were 54% for macrolides, 10% for virginiamycin, 3% for avilamycin, and 3% for bacitracin. The majority of the resistant strains from vegetables were isolated from different sprout products. The observation is important because many vegetables are consumed uncooked. The data furthermore indicate that a there is a certain level of antimicrobial resistance in many types of food.

Sensitivity of resistance monitoring
The level of resistant bacteria from food samples can be monitored in different ways. In the DANMAP surveillance bacteria are isolated

from foods by methods, which do not include antibiotic selection. This approach makes it possible to monitor the relative prevalence of antimicrobial resistance in bacteria. However, the method is not suitable for monitoring resistance in bacterial populations where only a small proportion is resistant. In these cases antibiotic selection improves the sensitivity of the detection methods. In 1996 and 1997 the prevalence of vancomycin resistant enterococci VRE) was studied by comparing the method applied in the DANMAP surveillance with a isolation procedure that applied selective enrichment and inoculation on Slanetz & Bartley agar supplemented with a breakpoint concentration of vancomycin. The studies included 90 and 60 broilers respectively and the results of the investigations are presented in Figure 3.
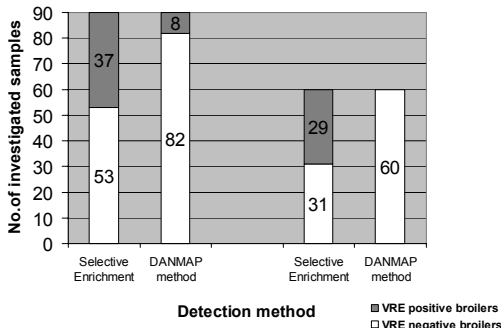
Figure 3. Detection of vancomycin resistant *E. faecium* in raw broiler meat collected at retail outlets determined by two different methods. The data are generated in two studies, which included 90 and 60 broilers respectively. In the selective method vancomycin was applied as a selective agent in the isolation of *E. faecium* from foods whereas the *E. faecium* strains isolated by the DANMAP method were isolated without the use of vancomycin

VRE were recovered from 41% and 48% of the samples with the selective enrichment procedure, whereas the DANMAP method only resulted in the recovery of VRE from 9% and 0% of the investigated samples. A similar selective method was applied in a recent study of VRE in Danish broiler flocks. In this study VRE were demonstrated in up to 74% of broiler flocks five years after the use of avoparcin were prohibited (Heuer et al. 2002).

Human exposure

Data about antimicrobial resistant bacteria from food at retail level, represents, together with knowledge about consumption patterns, a measure for the potential human exposure to resistant bacteria deriving from foods. Antimicrobial resistance in *E. faecium* may affect human health in different ways. It is well known, that *E. faecium* sometimes cause opportunistic infections and in these cases antimicrobial resistance may impede efficient therapy. Another aspect is the exposure of humans to bacteria that contain mobile antibiotic resistance genes. The potential of subsequent transfer of genes to the resident microbiota in the gastrointestinal tract may add to the development of bacteria carrying new resistances. Recently, it was shown that antimicrobial resistant *E. faecium* strains isolated from broilers and pork ingested by human volunteers resulted in sustained intestinal carriage, where the ingested strains were shedded for at least 14 days post ingestion (Sørensen et al. 2001). Other studies have shown that resistance genes are readily horizontally transferred both *in vitro* and *in vivo*. A study performed with gnotobiotic rats has demonstrated transfer of *vat*D gene (virginiamycin resistance) between isogenic strains of *E. faecium* in the gastrointestinal tracts (Jacobsen et al. 1999).

Conclusions

This study is presenting baseline data on AGP resistance in *E. faecium* strains isolated from foods in Denmark. Together with knowledge about consumption patterns these data are a measure for the potential human exposure to resistant bacteria from foods. In Denmark the termination of AGP use has been followed by a reduction of the resistance in *E. faecium* from broiler meat towards virginiamycin, avoparcin and avilamycin. In *E. faecium* from pork the resistance levels towards virginiamycin and macrolides have been quite constant during the period, despite of the termination of AGP use. The

data furthermore indicate that a there is a certain level of antimicrobial resistance in many types of food.

References

DANMAP, 2001. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark. ISSN 1600-2032.

Emborg, H.-D., Andersen, J.S., Seyfarth, A.M., Andersen, S.R., Boel, J. and Wegener, H.C., 2003. Relation between the occurrence of resistance to antimicrobial growth promoters among Enterococcus faecium isolated from broilers and broiler meat. Int. J. Food Microbiol. 84, 273-284.

Heuer, O.E., Pedersen, K., Andersen, J.S., Madsen, M., 2002. Vancomycin-Resistant Enterococci (VRE) in Broiler Flocks 5 years after the Avoparcin Ban. Microbial. Drug. Resistance. 8, 133-138.

Sørensen T.L, Blom, M., Monnet, D.L., Frimodt-Møller, N., Poulsen, R.L. and Espersen, F., 2001. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. New Engl. J. Med. 345, 1161-1166.

Jacobsen, B.L., Skou, M., Hammerum, A.M. and Jensen, L.B., 1999. Horizontal transfer of the satA gene encoding Streptogramin A resistance between isogenic *Enterococcus faecium* strains in the gastrointestinal tract of gnotobiotic rats. Microbial Ecology in Health and Disease 11, 241-247.

3. Effects of the termination of AGP use on antimicrobial resistance in humans

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Abstract

The EU-ban on some of the antimicrobial growth promoters (AGP) and the subsequent voluntary stop in the use of all AGP in Denmark reduced the consumption of antimicrobials with around 2/3 of the total amount of antibiotics used for veterinary purposes, or about 80-90 tons of antibacterial compounds. This had an almost immediate impact with a marked reduction in the frequency of resistance in enterococci isolated from various production animals towards several of the drugs used for growth promotion. The impact of these changes in enterococcal resistance has been less marked in Danish human medicine, mostly because enterococci are relatively rare etiologies in serious infections in humans in Denmark. In Danish enterococci isolated from blood cultures in 2000 and 2001 no vancomycin resistant enterococci (VRE) were present, but this was also very seldom seen before the AGP ban. In other European countries with higher pre-ban VRE rates a reduced carrier rate of VRE in human volunteers has been reported.

Reduced resistance frequency against bacitracin was seen in these strains compared to pre-AGP ban strains.

The marked reduction in macrolide AGP use can be monitored in a reduced resistance rate against macrolides in *Campylobacter* spp in both Denmark and other European countries. This lower resistance rate has beneficial consequences in that it can be shown, that macrolide resistance in *Campylobacter* is related to a more serious outcome, even death, from *Campylobacter* infections.

After the AGP ban an increase in veterinary therapeutic use of antibiotics has been recorded in Denmark, especially in tetracycline. The impact of this increased use might be the reason for increases in tetracycline resistance recorded in some *Enterobacteriaceae* such as *Salmonella* spp. Whether this has had any clinical impact remains to be proven.

Further studies are needed to monitor the effect in humans of the ban on AGP use.

Introduction

In July 2002 the first vancomycin-resistant *Staphylococcus aureus*, i.e. based upon the *vanA* gene, was reported from the USA (Anonymous, 2002). The strain had probably received the *vanA* gene by conjugation from a *vanA* bearing *Enterococcus* sp. residing in the same catheter-related infection in a dialysis patient (Anonymous, 2002). This was exactly what everybody had feared would happen if vancomycin-resistant enterococci (VRE) were allowed to roam freely particularly in settings, where selective and promotive factors were at hand such as an immunodepressed patient with foreign body infections frequently treated with broad-spectrum antibiotics. And this was the ultimate risk determinant brought forward by opponents to the wide use of antibiotic growth promoters in Europe in the mid 1990´ies, which included avoparcin, a glycopeptide with complete cross resistance towards vancomycin.

Surprisingly – or luckily – the political system was sensitive to the expert recommendations. Perhaps it is ironical that the *vanA* positive *S. aureus* should develop in the USA, which had not registered avoparcin for use as AGP, but which has on the other hand not yet taken action against AGP´s in their food production industry.

The ban on some of the antimicrobial growth promoters (AGP) in the EU countries combined with the subsequent voluntary withdrawal of all AGP from the food production industry in

Denmark resulted in one of the most marked reductions in antibiotic use ever seen – at least in our country. In Denmark this amounted to a decrease in antibiotic use of more than 115 tons from 1994 to 2000 or 20 tons more than the total therapeutic use of antibiotics in the veterinary field in 2001 (DANMAP, 2001). The three most important drugs used for AGP were in ranking order the macrolide, Tylosin, the glycopeptide, Avoparcin, and Bacitracin. The impact of this change on antibiotic resistance especially for human medicine is the scope in this presentation. This may be monitored in susceptibilities of Gram-positive bacteria such as enterococci and in zoonotic bacteria as *Campylobacter* spp. Another effect of reduced antibiotic pressure might be a change in prevalences of certain pathogens innately resistant towards the misused drugs.

The reduction in AGP use has lead to a hopefully temporary increase in the use of therapeutic antibiotics, especially tetracycline. This increase may on the other hand have a detrimental effect such as increases in resistance towards tetracycline and perhaps other antibiotics in clinically important bacteria, e.g. *Salmonella*.

Some of the problems with studying the above mentioned pathogens are that bacteria such as enterococci have not previously been part of intense screening programs prior to the AGP, which means that there is a paucity in pre-ban data to compare with recent post-ban data.

Enterococcus spp.

The increasing prevalence of and clinical importance of vancomycin resistant enterococci (VRE) were the main reasons for focusing on AGP in the beginning of the 1990´ies. The prevalence of VRE increased particularly in the USA, and to some extent in some European countries. The suspicion on AGP´s was substantiated by studies investigating the VRE carrier-rate in volunteers not receiving – or working with antibiotics, such as in hospitals and the like; carrier rates of up to 38% of such individuals were reported (DANMAP, 2001; Ieven et al., 1999), indicating that these bacteria were

present in the community outside hospitals, and cultures from poultry and pork provided the link (DANMAP, 2001).

What is the present post-ban prevalence of VRE in Europe and other continents ? One study specifically investigated the carrier-rates at the time around the AGP ban and again in 2001 (Ieven et al., 2001) and found a reduction in the rate of *vanA* positive *E. faecium* from 5.7% in 1996 to 0.6% in a hospitalised patient population in Belgium. This reduction was linked to the AGP ban (Ieven et al., 2001).

Prevalence rates of faecal carriage in human volunteers, i.e. not connected with hospitals, of VRE from various parts of the World after 1998 are shown in Table 1. Generally, the rates are lower in Europe and Australia than in the USA. In Europe VRE rates are lower in Scandinavian countries than in Southern Europe, but generally the rates are quite low in Europe. This could indicate that the withdrawal of a huge selective force such as the glycopeptide Avoparcin has reduced or maintained a low prevalence of VRE in Europe. The reason for the extremely high rates of VRE in hospital isolates in the USA must be found in the antibiotic use and infection control measures in that country.

Table 2 shows resistance rates of various AGP´s in *E. faecium* and *E. faecalis* from Danish studies of human volunteers in 1996 (DANMAP, 2001) and an ongoing prospective study of human volunteers, started in 2002. Furthermore, Table 2 compares these rates with results from a strain material from blood culture isolates from all over Denmark in 2000-01. No VRE´s were found, but erythromycin resistance is prevalent in these strains, which probably is connected with the human use of macrolides, i.e. around 2 DDD/1000ginhabitants/day (DANMAP, 2001).

Campylobacter spp.

Campylobacter are well known zoonotic bacteria causing infections in humans and it has in Denmark clearly overtaken the role from *Salmonella* as the most common etiology in human diarrhoeal disease. In some cases the diarrhoea

develops into serious disease, which may cause the death of the patient. Tchamgoue et. al. (2001) reported 121 *Campylobacter* patients admitted to a hospital in France, of whom 106 (88%) suffered from bacteraemia. Serious complications including endocarditis and meningitis amongst others developed in some of the patients, and 18 (15%) of the patients died.

In an ongoing study of the Danish human *Campylobacter* cases reported to the Statens Serum Institut, it can be demonstrated, that erythromycin resistant isolates are connected with a significantly higher mortality than other cases (K Mølbak, personal communication).

Resistance towards erythromycin and fluoroquinolones has been amply demonstrated in these bacteria and this has been connected with the use of these antibiotics in animal husbandry. Therefore it is interesting to follow especially the macrolide resistance rate in these pathogens, since this might indicate whether the drop in macrolide use as growth promoters has had any consequence for antibiotic resistance rates. Actually, erythromycin resistance rates in *Campylobacter coli* from pigs have decreased from 60% in 1996 to 30% in 2001, while showing a more subtle decrease in the same type of strains from broilers (DANMAP, 2001). Similarly, erythromycin resistance in *C. jejuni* from broilers have decreased from 5% in 1996 to 0 in 2001. Eryhtromycin resistance in human *C. jejuni* isolates still remains around 5%, which is connected with import from foreign travel. Similar trends have been reported from Norway (Kruse et al., 2001).

Salmonella spp.

Although the total antibiotic consumption has decreased significantly in Denmark as a consequence of the AGP ban, some increase in therapeutic use of antibiotics for animal husbandry has been recorded (DANMAP, 2001). Use of tetracycline constitutes a major part of this increase (24 tons in 2000, 27,9 tons in 2001). Whether this increasing use has any impact for human disease has not been studied in detail. In human domestic isolates of Salmonella Typhimurium, however, resistance towards tetracycline increased from 20% in 1996 to 40% in 2001, and this increase was parallel for ampicillin and chloramfenicol, which might indicate that a major part of these resistance traits are prone to co-selection. In this respect, increase in tetracycline use may select for other and more important types of antibiotic resistance in these pathogens.

Conclusion

The major reduction in antimicrobial consumption after the ban of AGP´s has resulted in a decrease in antibiotic resistance rates towards the AGP´s especially the glycopeptides. The rate of VRE in Denmark was low to non-existent prior to the ban, probably connected with the low antibiotic use on the human side both inside- and outside hospitals. This extremely low prevalence of VRE has been maintained in Denmark and the rest of Scandinavia. Somewhat higher VRE prevalence rates are reported from the rest of Europe, but these rates are low – in some cases clearly lower than five years ago. This low prevalence of VRE in the community is probably the reason for the general low prevalence of these bacteria in hospitals, in contrast to the very high frequencies of VRE (~ 50%) reported from in the USA.

The decrease in AGP macrolide use has had a beneficial impact on the macrolide resistance rates in *Campylobacter*, which may be proven to result in lower mortality from infections caused by this pathogen. The resulting increase in therapeutic use of antibiotics -noticeably tetracycline - for animal husbandry may have resulted in increased resistance e.g. in *Salmonella* spp. There is focus on this increased consumption in Denmark, and we expect further reductions in therapeutic antibiotic use in the coming years, and further efforts will be concentrated on maintaining the low antibiotic consumption for humans.

Table 1. Prevalence rates or frequencies of vancomycin resistant enterococci (VRE) in rectal samples, or otherwise noted, reported from various parts of the World from after 1998, i.e. after the ban on antimicrobial growth promoters in the EU. Only reports on vancomycin resistant E. faecalis or E. faecium are included

Reference	Country	Year	Type of samples	VRE rate (%)	Comments
This paper	Denmark	2002	Faeces HV*	0/27 (0)	
Torell et al., 1999	Sweden	1999	Faeces HV*	1/670 (0,1)	One VRE imported from Africa
Wendt et al., 1999	Germany	1999	Faeces HV*	(0,9)	
Van den Braak et al., 2000	Holland	1998	Faeces, Ptts	15/1.112 (1.4)	No increase from 1995
Gambaretto et al., 2000	France	2000	Faeces, HV*	20/169 (12)	Majority E. gallinarum.
Ieven et al., 2001	Belgium	2001	Faeces, Ptts	3/353 (0,6)	
Nourse et al., 2000	Ireland	2000	Faeces HV*	0/116 (0)	VanA E.
Lombardi et al., 2001	Italy	2001	Isolates from Faeces, Ptts	6/90 isolates (7)	E. faecium isolates studied
Novais et al., 2001	Portugal	2001	Isolates from Faeces HV*	21/354 isolates (6)	Enterococcal isolates studied
Del Campo et al., 2001	Spain	2001	Faeces HV*	0/42 (0)	
Padiglione et al., 2000	Australia	2000	Faeces HV*	2/1.085 (0,2)	Both vanB E. faecium
Moet et al., 2001	USA	1997-01	Blood culture isolates	E. faecium 50%, E. faecalis 2.3%	> 3.500 enterococcal isolates
Garey et al., 2001	USA	2001	20.194 enterococcal isolates, origin not specified	VR E. faecalis 7% VR E. faecium 50%	

*HV, healthy volunteers

Table 2. Resistance to various antibiotics used as growth promoters in Danish enterococcus spp. from healthy volunteers (1996 & 2002) and from blood cultures (2000-01)

	Resistant/total (%)					
	E. faecium			*E. faecalis*		
	Human volunteers		Blood culture	Human volunteers		Blood culture
Year	1996	2002	2000-01	1996	2002	2000-01
Antibiotic:						
Flavomycin[1]	-	-	-	1/49 (2)	2/27 (7)	0/24 (0)
Avilamycin (Evernimicin)	0/15 (0)	3/19 (16)	0/29 (0)	0/49 (0)	0/27 (0)	0/24 (0)
Virginiamycin (quinopris-tin/dalfopristin)[2]	7/24 (29)	0/19 (0)	0/29 (0)	-	-	-
Tylosin (Erythromycin)	2/25 (8)	1/19 (5)	16/29 (55)	8/97 (8)	10/27 (37)	7/24 (29)
Avoparcin (Vancomycin)	0/24 (0)	0/19 (0)	0/29 (0)	0/49 (0)	0/27 (0)	0/27 (0)

1) *E. faecium* is intrinsic resistant to flavomycin

2) *E. faecalis* is intrinsic resistant to virginiamycin

References

Anonymous. Staphylococcus aureus resistant to Vancomycin – United States, 2002. MMWR 2002, 51, 565-7.

DANMAP 2001- Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. Available on: http://www.vetinst.dk

Ieven M, Vercauteren E, Descheemaeker P, van Laer F, Goosens H. Comparison of direct plating and broth enrichment culture for the detection of intestinal colonization by glycopeptide-resistant enterococci among hospitalized patients. J. Clin. Microbiol. 1999, 37, 1436-40.

Ieven M, Vercauteren E, Lammens C, Ursi D, Goosens H. Significant decrease of GRE colonization rate in hospitalized patients after avoparcin ban in animals ? 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, 2001, abstract LB-8.

Torell E, Cars O, Olsson-Liljequist B, Hoffman BM, Lindback J, Burman LG. Near absence of vancomycin-resistant enterococci but high carriage rates of quinolone-resistant ampicillin-resistant enterococci among hospitalised patients and nonhospitalized individuals in Sweden. J. Clin. Microbiol. 1999, 37, 3509-13.

Wendt C, Krause C, Xander LU, Loffler D, Floss H. Prevalence of colonization with vancomycin resistant enterococci in various population groups in Berlin, Germany. J. Hosp. Infect. 1999, 42, 193- 200.

Van den Braak N, Ott A, van Belkum A, Kluytmans JA, Keoleman JG, PSpanjaard L, Voss A, Weersink AJ, Vandenbroucke-Grauls CM, Buiting AG, Verbrugh HA, Endtz HP. Prevalence and determinants of fecal colonization with vancomycin resistant Enterococcus in hospitalised patients in the Netherlands. Infect. Control. Hosp. Epidemiol. 2000, 21, 520-4.

Gambaretto K, Ploy MC, Turlure P, Grelaud C, Martin C, Bordessoule D, Denis F. Prevalence of vancomycin-resistant enterococci in fecal samples from hospitalised patients and nonhospitalized controls in a cattle-rearing area of France. J. Clin. Microbiol. 2000, 38, 620-4.

Nourse C, Byrne C, Murphy H, Kaufmann ME, Clarke A, Butler K. Eradication of vancomycin

resistant Enterococcus faecium from a paediatric oncology unit and prevalence of colonization in hospitalised and community-based children. Epidemiol. Infect. 2000, 124, 53-9.

Lombardi G, Brigante G, Coli A, Bonsignori M, Stefani S, Toniolo A. Emerging nosocomial infections caused by vancomycin-resistant Enterococcus faecium in Northern Italy. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, 2001, abstract K-1952

Novais C, Coque TM, Sousa JC, Ferreira HN, Peixe LV. The community and the environment: Insights about recent epidemiology of enterococcus resistance in Portugal. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, 2001, abstract C2-1117.

Del Campo R, Ruiz-Garbajosa P, Sanchez-Moreno MP, Canton R, Baquero F, Coque TM. Antimicrobial resistance phenotypes and resistance genes in Enterococcus spp. Isolated from healthy volunteers and food handlers in Spain. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, 2001, abstract C2-1877

Padiglione AA, Grabsch EA, Olden D, Hellard M, Sinclair MI, Fairley CK, Grayson ML. Fecal colonization with vancomycin-resistant enterococci in Australia. Emerg. Infect. Dis. 2000, 6, 534-6.

Moet G, Biedenbach D, Pfaller M, Jones RN. Occurrence of blood stream infection pathogens and key resistances from Sentry Antimicrobial Surveillance program, a 5-year report. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, 2001, abstract C2-299.

Garey KW, Manduru M, Gupta V, Danziger LH. Nationwide survey of pathogen frequency and antimicrobial susceptibility patterns from community hospitals throughout the United States. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, 2001, abstract C2-79.

Tchamgoue S, Neau D, Ragnaud J, Megraud F. Extra-digestive manifestations of infection due to Campylobacter: a retrospective multicenter study of 121 cases. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, 2001, abstract L-770.

Kruse H, Nødtvedt A, Hofshagen M, Stavnes T, Lassen J. Prevalence of resistance in Campylobacter jejuni from humans in Norway linked to travel abroad. 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, 2001, abstract LB-14.

4. Effects of the termination of antibiotic growth promoters use on antimicrobial resistance in pig farms: Macrolide-resistance among enterococci in finishers

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Summary

In the Danish pig industry, tyrosin (a macrolide) has been commonly used as an antimicrobial growth promoter (AGP) for weaners and growers-finishers since the mid 80's. The use of tylosin as antimicrobial growth promoter was ceased in 1999, when the use of AGP in the pig production was withdrawn. The use of tylosin as AGP in pig production might have caused large reservoirs for resistant bacteria or genes encoding macrolide-resistance that can be transferred to human pathogens.

The purpose of the presented study was to identify and quantify a possible effect of withdrawing tylosin as AGP on the occurrence of macrolide-resistance in the faecal enterococci population in finishers at Danish pig farms. The study was based on results of bacteriological examination of faecal samples from 670 pigs at 69 different farms. The samples were collected from each farm at one to three occasions, and at each occasion samples were collected from 10 finishers. The dates of cessation use of tylosin as AGP for weaners and/or growers-finishers were collected by interviewing the pig-producer.

The effect of ceasing the use of tylosin as AGP was assumed to be multiplicative with the duration of time that had passed after cessation and was estimated in 1) a logistic model with the detection of macrolide-resistant enterococci within finishers as dependent variable and 2) in a linear model with the degree of macrolide-resistance in the enterococci population in finishers as dependent variable. The estimates were adjusted for a potential within-farm clustering of the occurrence of macrolide-resistance by adding farm as a random effect in the models.

This study showed that the occurrence of macrolide-resistance in enterococci from faecal samples from finishers is high, and the rate of decrease in the occurrence of macrolide-resistance after withdrawal tylosin as AGP to pigs is slow, and takes place over several years.

The study also showed that, concerning macrolide-resistance, correlation between finishers that have been exposed to the same farm-specific management factors is low, indicating that pig-specific factors have major influence on the occurrence of macrolide-resistance. Future epidemiological research concerning antimicrobial resistance in the pig production should be focused on identifying risk factors at both the pig-level and the farm-level.

Introduction

The main focus of this written contribution is to illustrate the effects of the termination of antibiotic growth promoters (AGP) use on antimicrobial resistance in Danish pig farms. Parallel to this, the contribution describes the application of epidemiology concepts for illustrating the effect. In Denmark different types of antibiotics has been used as AGP within the pig production since the 70's, whereas the antibiotic tylosin has been one of the most commonly used for both weaners and growers-finishers since the mid 80's. Tylosin is a macrolide antibiotic, just as erythromycin and azitromycin, which are important antibiotics in the treatment of infections with staphylococci and streptococci in humans. Bacterial enzymes that alter the target site of the macrolides are the most common cause of resistance in staphylococci, streptococci and enterococci, and the genes encoding these enzymes are commonly transferable. Therefore, the use

of tylosin as AGP in pig production may result in large reservoirs for resistant bacteria or genes encoding macrolide-resistance that can be transferred to human pathogens.

In the beginning of 1998, the use of AGP for growers-finishers was withdrawn, and the cessation of using AGP within the entire pig production became complete in 1999, when the use of AGP for weaners was withdrawn. Regarding the effect of ceasing the use of tylosin as AGP on the occurrence of macrolide-resistance in the Danish pig production, information at the national level is available in DANMAP (DANMAP, 2001). However, due to the design of the surveillance system, the DANAMP-data can neither be used for estimating the effects of ceasing the use of tylosin as AGP on the occurrence of macrolide-resistance at farm-level or pig-level, nor estimate the variation of occurrence of macrolide-resistance in the enterococci population between farms, and between pigs within farms.

To obtain these estimates, an observational study was performed in a number of pig farms, and the results concerning the occurrence of macrolide-resistant enterococci from this study will be used to illustrate the effects of the termination of AGP use on antimicrobial resistance in Danish pig farms. The results were statistically analysed with the specific aim to estimate the effect of cease the use of tylosin as AGP among 1) weaners and 2) growers-finishers. A secondary objective was to quantify the variation of occurrence of macrolide-resistant enterococci between farms, and between finishers within farms.

Material and methods

Materials
The actual study is a part of a study performed to evaluate the effect of terminating the use of AGP in the pig production on antimicrobial resistance, pig health and performance. One important intention in this study was to evaluate the effect of different factors present during the nursing and weaning period, e.g. the use AGP, on occurrence of macrolide-resistance among finishers. On this background, to be able to collect valid data about early exposure of the finishers, the study was restricted to integrated pig production units - farms.

The selection criteria used in the identification of the study population is given in the written contribution by Per Bundgaard Larsen at this symposium. Briefly, identification of pigs for collection of faecal samples to be used for examining the occurrence of macrolide-resistance in the enterococci population was performed by a stratified sampling procedure. Sixteen veterinary swine-practitioners participated in the study. Among the clients of each veterinarian, 2-17 farms were identified, in total 120 farms. The farms were either at one single locality, or situated at two localities (at a sow- and weaning unit, and at a growing-finishing unit). Faecal samples were collected from each farm at one to three occasions. At each occasion, samples were collected from the rectum of 10 finishers.

Information about the use and ceased use of AGP in the farms was collected by interviewing the pig-producer. The dates when the farms stopped using different AGP for weaners and growers-finishers, respectively, were also collected at the interview. The date for final stop of AGP use to weaners was validated with the firms delivering the feedstuff.

The date of the final termination of using AGP to weaners ranged from the 1 Jul. 1996 to the 25 Feb. 2000 (25% of the farms ceased using AGP before 31 Mar. 1999, 50% of the farms before the 1 Aug. 1999 and 75% of the farms before the 1 Sept. 1999). In the 120 farms, the final AGP used for weaners was olaquindox/carbadox (83 farms), tylosin (18 farms) and avilamycin (9 farms). The information was missing for 7 farms. Many farms had been using several AGP's in rations. For 16 of the farms, which have not been using tylosin immediately before cessation of AGP, it was possible to collect the date when tylosin was last used as AGP for weaners. The date of ceased use of AGP for growers-finishers ranged from the 1 Jul. 1994 to the 1 Sept. 1999 (25% of the farms ceased using AGP before the 1 Jan. 1998, 50% of the farms ceased using AGP before the 1 Jan. 1998 and 75% before the 1 Mar. 1998). The final AGP

used to the growers-finishers was tylosin (47 farms) and olaquindox/carbadox (4 farms). The information was missing for 69 farms.

The methods used for bacteriological examination of the faecal samples are described in detailed by Aarestrup and Carstensen (1998). Enterococci were enumerated in three replicates in the dilution 10^{-2}, 10^{-3} and 10^{-4} on two different selective and indicative substrates: Slanetz agar and Slanetz agar containing 20 mg/ml of erythromycin. The number of enterococci-like colonies was counted and recorded.

Statistical methods
Only farms with known date of ceased using tylosin as AGP for weaners or to growers-finishers were of concern in the statistical summarisation and analyses of data. The purpose of the statistical analyses was to identify and quantify a possible effect of withdraw tylosin as AGP on the occurrence of macrolide-resistance in the faecal enterococci population in finishers. In the statistical models it was assumed that the possible effect was multiplicative with the duration of time passed after ceased use of tylosin as AGP (interval $_{AGP-sampling}$).

The effect of ceasing the use of tylosin as AGP on the occurrence of macrolide-resistance concerned 1) probability for detection of macrolide-resistant enterococci within finishers and 2) the degree of macrolide-resistance in the enterococci population in finishers with detectable macrolide-resistant enterococci.

A logistic model (model 1) was used to estimate the effect of ceasing the use of tylosin as AGP on the probability (p) to detect macrolide-resistant enterococci. The effect of farm was included as random effect (u_{farm}). The estimation procedure was implemented in SAS, proc nlmixed.

$\log (p/(1-p)) = \beta_0 + \beta_{day\ AGP-sampling} * $ interval $_{AGP-sampling} + u_{farm}$ (model 1)

where: $\beta_{day\ AGP-sampling}$ = linear effect of each day in the interval between ceasing the use of tylosin as AGP and faecal sampling.

The degree of macrolide-resistance was defined as the proportion of macrolide-resistance enterococci within each faecal sample – the macrolide-resistance index. It was calculated as the maximum likelihood estimate in a Poisson model with uncountable plates considered as right-censored (model 2). The estimation procedure was implemented in SAS, proc nlmixed.

$\log(CFU) = \beta_0 + \beta_{dilution}(-(\log\ dilution)) + \beta_{resistance}$(presence of erythromycin in the agar) (model 2)

where: β_0 = log(number of CFU per gram faeces); $\beta_{dilution}$ = effect of dilution; $\beta_{resistance}$ = log(macrolide-resistance index).

To estimate the effect of ceasing the use of tylosin as AGP on the degree of macrolide-resistance the calculated logarithmic macrolide-resistance indices were used as the outcome in a linear model, where farm was included as a random effect (u_{farm}). The estimation procedure was implemented in SAS, proc mixed. To adjust the analysis for the fact that the indices were determined with different accuracy the analysis was weighted by the reciprocal of the standard error of the estimated $\beta_{resistance}$.

$\log$(macrolide-resistance index) $= \beta_0 + \beta_{day\ AGP-sampling}$ (interval $_{AGP-sampling}$) $+ u_{farm} + \epsilon_{pig}$ (model 3)

where: $\beta_{day\ AGP-sampling}$ = linear effect of each day in the interval between ceasing the use of tylosin as AGP and faecal sampling.

Given that the age of the animals at sampling was a result of the sampling schedule, and that age might influence the occurrence of resistance, the effect of age of the animals at sampling was forced into model 1 and 3.

The degree of similarity in occurrence of macrolide-resistance between pigs within the same farm was expressed as the intra-unit correlation coefficient (ICC). The ICC expressing the similarity between pigs concerning the probability of detecting macrolide-resistant enterococci in faeces was calculated as the ratio between the variance component at farm-level and the sum of the variance components at farm- and pig-

level using the latent approach and the estimates obtained in the logistic model. The ICC expressing the similarity between pigs concerning degree of macrolide-resistance was calculated as the ratio between the variance component at the farm-level and the sum of the variance components at the farm- and pig-level using the estimates of the variance components in the linear model.

Results

From the 69 farms with known date of cessation of using tylosin as AGP for weaners and/or for growers-finishers, results from the bacteriological examination were available from 670 pigs. The faecal samples were collected in the period 18 Feb. 1999 – 31 Oct. 2000. The crude proportion of pigs with erythromycin-resistant enterococci was 68% (458/670). In fig 1, the proportion of pigs with erythromycin-resistant enterococci at different times after ceased used of tylosin as AGP for weaners and growers-finishers, respectively, are illustrated. Among the pigs with erythromycin-resistant enterococci, the mean of the proportion of macrolide-resistant

enterococci in the pigs was 34%. In fig. 2 the proportion of erythromycin-resistant enterococci in individual pigs are scattered against time after ceased used of tylosin as AGP for weaners and growers-finishers, respectively.

The parameter-estimates obtained in model 1 and 3 are presented in table 1.

Using the estimated confidence limits in the logistic model of the effect of ceasing the use of tylosin as AGP for weaners, the average probability to detect macrolide-resistant enterococci in faecal samples could be predicted to be halved (from 94% to 47%) within a period between 2 to 9 years.

Due to that the response variable in the linear model was the logarithm of degree of macrolide-resistance, the average rate of decreasing degree of macrolide-resistance in the pig production could be expressed as half-life ($\log2/(-(\beta_{day\ AGP-sampling}))$). Using the estimated confidence limits of the effect of ceasing the use of tylosin as AGP for weaners ($\beta_{day\ AGP-sampling}$), the half-life was estimated to be between 0.5 and 1.5 year.

Table 1. The parameter-estimates in 1) the logistic model describing the probability of detecting erythromycin-resistant enterococci in a faecal sample from a finisher and in 2) the linear model describing the degree of erythromycin-resistant enterococci in a faecal sample from a finisher

Parameters	Logistic model		Linear model	
	interval $_{AGP\text{-}sampling}$ weaners [a]	Interval $_{AGP\text{-}sampling}$ growers-finishers [b]	Interval $_{AGP\text{-}sampling}$ weaners [a]	interval $_{AGP\text{-}sampling}$ growers-finishers [b]
Random effects				
u_{farm}	1.44	2.67	0.46 [c]	0.51 [c]
ϵ_{pig}	1	1	1.25 [c]	1.51 [c]
Fixed effects				
β_0	2.59	4.51	0.77	1.03
$\beta_{day\ AGP\text{-}sampling}$	-0.00321 *	-0.00468 *	-0.00221 *	-0.00141*

[a] using data where date of ceasing the use of tylosin as AGP for weaners are known
[b] using data where date of ceasing the use of tylosin as AGP for growers-finishers are known
[c] the estimates of the random effects are from the non-weighted analyses
* the estimate is significant different from zero at a 0.05-level of significance
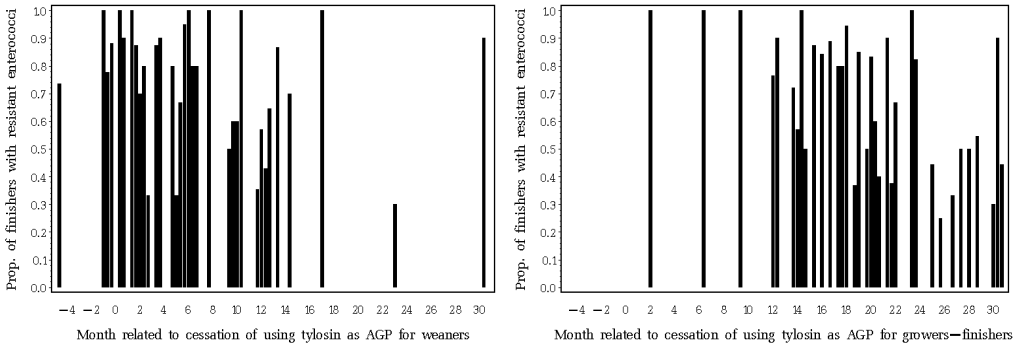
Fig. 1. Bar charts summarising the proportion of pigs with erythromycin-resistant enterococci (vertical axis) in relation to month that had passed after cessation of using tylosin as AGP (horizontal axis) for weaners (left column) and for growers-finishers (right column)
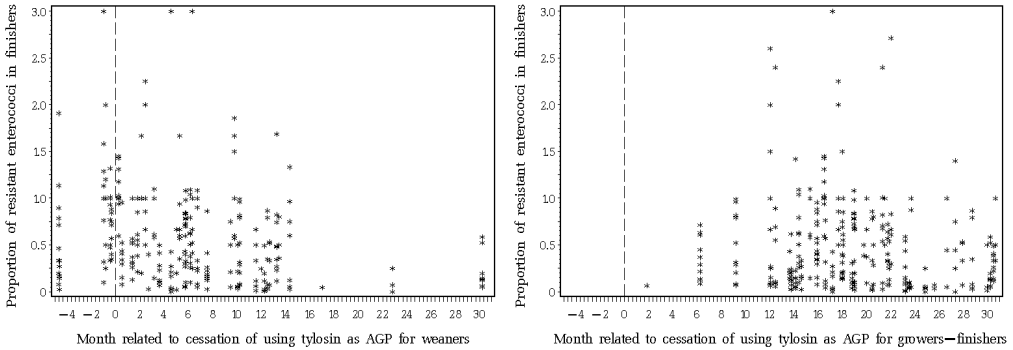
Fig. 2. Scatter plots of proportion of erythromycin-resistant enterococci in individual pigs in which erythromycin-resistant enterococci were detected (vertical axis) in relation to month that had passed after cessation of using tylosin as AGP (horizontal axis) for weaners (left column) and growers-finishers (right column)

Using the estimated variance components for farm-level and pig-level the correlation between pigs in the same farm concerning detection of macrolide-resistance was 30%, and the correlation between pigs in the same farm concerning the degree of macrolide-resistance was 27%.

Only in 12 farms both the date of ceasing the use of tylosin as AGP for weaners and for growers-finishers were available. In these farms the amount of time that had passed after ceasing the use of tylosin as AGP for weaners and for growers-finishers had a high in-farm correlation (72%). As a consequence of the strong within-

farm correlation between the time that has passed after cessation of using tylosin as AGP for weaners and growers-finishers, it was not possible in this study to distinguish the effect of ceasing the use of tylosin as AGP in weaners and the effect of ceasing the use of tylosin as AGP in growers-finishers on the occurrence of macrolide-resistance.

During analysing the data, efforts were made to detect a more complex effect then a simple linear of ceasing the use of tylosin as AGP. The effect was added as a cubic polynomial into the models, but without increasing the fit of the

models to the data. Also, the effect of events of mass-medication with tylosin or lincomycin in the farms were added to the models as a binary effect, but the estimated parameters did not indicate any effect of mass-medication on occurrence of macrolide-resistance.

Conclusion

Based on the results presented it can be concluded that the rate of decreasing occurrence of macrolide-resistance after cessation the use of tylosin as AGP to pigs is slow, and takes place over several years. The relative high occurrence of macrolide-resistance in the pig production, presumably provoked by the use of tylosin as AGP for decades, will last for several years after the cessation. Several factors have been proposed to have a maintaining effect on the occurrence of macrolide-resistance. Using tylosin as a therapeutic antibiotic will make the microbiological environment more suitable for tylosin-resistant enterococci compared to non-resistant enterococci, and this will provoke macrolide-resistance in the same way as tylosin used as AGP. Also factors not related to antibiotics might influence on the occurrence of macrolide-resistance, e.g. the effect of season. Furthermore, except from an indirect effect of disease preventing factors due to less use of tylosin as a therapeutic, production systems known to prevent transmission of pathogenic bacteria in pig production, e.g. all in/all out production, might also reduce the transfer of antibiotic-resistant bacteria between pigs. However, in this study a relatively low between-pig within-farm correlation in the occurrence of macrolide-resistance was found. The low correlation between finishers that have been exposed to the same farm-specific management factors indicate that pig-specific factors, or even sample-specific factors, have major influence on the occurrence of macrolide-resistance. Future epidemiological research should be focused on identifying risk factors at both the farm-level and the pig-level. Formulation of causal path diagrams, and evaluation of which type of effect potential risk factors included in the path diagrams have, are of special importance.

References

DANMAP 2001. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. [available from the Zoonosis Centre homepage: http://www.vetinst.dk]

Aarestrup, F.M. & Carstensen, B. 1998. Effect of tylosin used as a growth promoter on the occurrence of macrolide-resistant enterococci and staphylococci in pigs. Microb. Drug. Resist. vol. 4, 307-312.

5. Occurrence and persistence of vancomycin resistant enterococci (VRE) in Danish broiler production after the avoparcin ban

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Abstract

The glycopeptide growth promoter avoparcin was banned from animal production in Denmark in 1995 due to concern for the transfer of vancomycin resistant enterococci (VRE) from food animals to humans. This presentation contains results from two studies of the occurrence and persistence of VRE in Danish broiler production after the avoparcin ban.

In the first study we investigated the occurrence of VRE in broiler flocks in the absence of the selective pressure exerted by the use of avoparcin. One hundred and sixty-two broiler flocks from rearing systems with different history of avoparcin exposure were investigated for the presence of VRE. Using a direct selective plating procedure VRE were isolated from 104 of 140 (74.3 %) broiler flocks reared in broiler houses previously exposed to avoparcin on conventional and extensive indoor broiler farms. In contrast only 2 of 22 (9.1 %) organic broiler flocks reared on free-range farms with no history of previous exposure to avoparcin were VRE-positive. Furthermore the occurrence of VRE over time in flocks reared in broiler houses previously exposed to avoparcin was investigated. No significant decrease in the proportion of VRE positive flocks was observed during the study period (1998-2001). This study demonstrated extensive occurrence of VRE in broiler flocks more than five years after the avoparcin ban in Denmark, indicating that VRE may persist in the absence of the selective pressure exerted by avoparcin.

In the second study we investigated the persistence of VRE on broiler farms in the absence of the selective pressure exerted by avoparcin. Environmental samples were obtained from five broiler houses after depopulation, cleaning and disinfection of the houses between rotations, and two consecutive broiler flocks from each house were sampled by taking cloacal swabs from the broilers at the time of slaughter. Vancomycin resistant *E. faecium* isolates obtained from broiler flocks and broiler houses were subjected to molecular typing by pulsed-field gel electrophoresis (PFGE). VRE with indistinguishable or highly similar PFGE-profiles were isolated from consecutive broiler flocks and from environmental samples from the houses in which the flocks were reared, whereas VRE-isolates from different broiler houses, and from flocks reared in different houses appeared to be genetically unrelated. These findings indicated that VRE was transmitted between consecutive broiler flocks by clones of resistant bacteria surviving in the broiler houses despite cleaning and disinfection between rotations. Thus, the extensive occurrence of VRE in broiler flocks after the avoparcin ban may be explained by persistence of VRE in the broiler house environment.

Introduction

Vancomycin resistant enterococci (VRE) have emerged as an important cause of hospital acquired infections. This has been ascribed to an increase in the use of cephalosporins and vancomycin in hospital settings (Moellering, 1992), but also to the possible transfer of resistant bacteria or mobile resistance determinants to humans, from food animals fed avoparcin as a growth promoter. The glycopeptide avoparcin has been used for growth promotion in animal production in many countries and the association between the use of avoparcin as a feed additive in broiler production and the occurrence of VRE in broilers is well documented (Bager et al., 1997; Kruse et al., 1999). The use of avoparcin was banned in Denmark and Norway in

1995, in Germany in 1996 and in all of the EU in 1997. The enforcement of the ban was expected to result in a decline of VRE in food animals and thereby to reduce human exposure to VRE via the food chain.

However, studies from Belgium (Butaye et al., 1999), Norway (Kruse et al., 1999) and Denmark (Bager et al., 1997) conducted shortly after the avoparcin ban gave indications that VRE in poultry flocks would not readily disappear. In these studies sampling was done from few month until one and a half year after the avoparcin ban, yet VRE were frequently isolated from fecal samples from poultry. Norwegian studies have documented extensive occurrence of VRE on poultry farms three years after the avoparcin ban (Borge et al., 2000), and persistence of VRE in the poultry environment despite absence of the selective pressure exerted by the use of avoparcin has been demonstrated (Borgen et al., 2000).

A decline of VRE prevalence in broilers after the avoparcin ban has been reported from The Netherlands (van den Bogaard et al., 2000), and in Denmark data from the antimicrobial resistance monitoring and research programme (DANMAP) have shown a decrease in the occurrence of VRE in broilers between 1995 and 2000 (Anonymous, 2001). A direct comparison of VRE prevalence results from different studies will obviously be hampered by differences in sampling scheme, sample size, isolation procedures and sample material used. The DANMAP surveillance programme was not designed specifically to detect VRE in broilers, and the purpose of the programme is to monitor resistance to a range of different antimicrobial substances in bacterial isolates randomly collected from Danish broiler flocks. The use of media supplemented with vancomycin has been recommended for VRE screening procedures (HIC-PAC, 1995) and several studies have shown that successful recovery of VRE from fecal samples is highly dependent on the isolation method used (Butaye et al., 1999a; Butaye et al., 1999b; Ieven et al., 1999).

In the first study we aimed to investigate the occurrence of VRE in broiler flocks over time after the avoparcin ban, and to compare VRE prevalence in broiler flocks from rearing systems with different history of avoparcin use. In the second study we aimed to investigate the persistence of VRE in the broiler house environment, in the absence of the selective pressure exerted by avoparcin, and to investigate the possible transmission of VRE between consecutive broiler flocks by clones of resistant bacteria surviving in the broiler houses despite cleaning and disinfection.

Materials and methods

First study
Broiler farms
Thirty-one broiler houses on 24 farms with a recorded history of previous use of avoparcin were included in the study. Seventeen of these farms employed conventional rearing and seven farms employed extensive indoor rearing. In order to gain knowledge of VRE prevalence in flocks from farms with no recorded history of use of avoparcin, organic broiler flocks from 12 free-range farms were included in the study.

Sampling procedure
One hundred and forty broiler flocks from farms previously exposed to avoparcin (86 conventional broiler flocks and 54 extensive broiler flocks) and 22 organic broiler flocks from free-range farms with no history of avoparcin use were sampled between October 1998 and February 2001. In order to enable investigation of the occurrence of VRE over time on farms previously exposed to avoparcin, more broiler flocks from each broiler house were sampled during the study period. The number of flocks sampled from each house on conventional and extensive farms ranged from three to seven. From each free-range farm one to three organic broiler flocks were sampled.

From each broiler flock ten broilers were sampled individually. The sample size considerations were based on the assumption that the within flock prevalence of VRE-positive birds in positive flocks would be 50% or higher. Samples consisted of cloacal swabs taken from the broilers on arrival at the abattoir.

Isolation of enterococci

Swabs were immersed in 1 ml veal infusion broth supplemented with 4 % new-born calf serum. The swabs were left for 10 -15 min in the broth. Subsequently, 10 ml of the broth from each swab were streaked directly onto two Slanetz and Bartley agar plates (Oxoid), one supplemented with vancomycin (50 ml/ml) for selective isolation of VRE (SBv), the other not containing vancomycin (SB) to confirm growth of enterococci from the samples. The plates were incubated at 37°C for 48 h.

Second study
Broiler houses and broiler flocks

On the basis of results from the first study, four broiler houses (A, B, C and D) known to have produced VRE-positive broiler flocks in previous rotations, and one broiler house (E) known to have produced VRE-negative broiler flocks for previous rotations, were selected for sampling. Environmental samples were taken in the boiler houses by streaking sterile cotton swabs against the surface of the floor, floor cracks, feed bowls and water cups. Ten samples were obtained in each broiler house. At the time of sampling the broiler houses were depopulated, cleaned and disinfected.

Two broiler flocks from each of the five broiler houses were sampled by taking cloacal swabs from the broilers at the time of slaughter. Ten broilers from each flock were sampled individually. All samples from broiler flocks and environmental samples from broiler houses were collected from July 1999 to December 1999. VRE-isolates obtained from a broiler house and from flocks reared in the house were regarded as epidemiologically related.

Broiler feed and hatchery

In order to investigate possible routes of introduction of VRE to broiler flocks and broiler houses, 100 samples were obtained at four sampling occasions from the incubators at a major Danish hatchery supplying farm A, B and D. Each sample from the hatchery consisted of approx. one gram of dust, litter and eggshell. Furthermore, samples of broiler feed were ob-

tained from the feed stock on farm A and B at the time of environmental sampling of the broiler houses.

Isolation of enterococci

Samples from broiler flocks were processed as in the first study. In order to facilitate isolation of VRE from environmental samples from broiler houses and samples of broiler feed and hatchery samples, these were incubated at 37°C over night in Enterococcosel broth (Becton Dickinson) before plating. The broth used for feed and hatchery samples was supplemented with vancomycin (20 mg / ml). Subsequently, 10 ml of the broth from each swab or sample were streaked directly onto SBv agar plates for selective isolation of VRE, and SB agar plates to confirm growth of enterococci from the samples. The plates were incubated at 37°C for 48 h.

Details on identification of enterococci, susceptibility testing, VanA PCR, Pulsed-field gel electrophoresis (PFGE) and statistical analysis may be found in the following publications: (Heuer, 2002; Heuer et al., 2002).

Results and discussion
First study

By direct plating on SBv agar plates VRE were isolated from 104 of 140 (74.3%) broiler flocks from farms previously exposed to avoparcin (57 of 86 (66.3%) conventional broiler flocks and 47 of 54 (87.0%) extensive indoor broiler flocks). From organic broiler farms with no history of previous use of avoparcin VRE were isolated from 2 of 22 (9.1%) broiler flocks (Table 1). Selective isolation of VRE by the use of media containing vancomycin (SBv agar plates) substantially increased the sensitivity of VRE detection. By the selective isolation procedure VRE were detected in 106 of 162 (65.4%) flocks, whereas by the non-selective procedure 17 of 162 (10.5%) flocks were VRE-positive. Thus, only 17 of 106 (16%) flocks confirmed to be VRE-positive by selective isolation were detected using the non-selective procedure. During the study period more broiler flocks from each broiler house were sampled in order to enable

analysis of the occurrence of VRE over time. This was done using a poison regression model with the number of VRE positive samples from each flock as the dependent variable and time as the explanatory variable. Time was not significant in the regression analysis (P=0.165), thus no significant decrease in the proportion of VRE-positive samples was observed during the study period. The same conclusion was reached using a binary outcome (VRE positive/VRE negative) for each flock in a logistic regression model with time as explanatory variable (P=0.423).

In previous Danish studies based on data from the DANMAP programme, a decline of VRE prevalence in broiler flocks after the avoparcin ban from 72.7% in 1995 to 5.8% in 2000 has been reported (Aarestrup et al., 2001; Anonymous, 2001; Bager et al., 1999). However, the isolation procedure used with samples from broilers in the DANMAP does not select for VRE and only a single swab sample from each broiler flock is tested (Anonymous, 2001; Bager, 2000), whereas in the present study ten samples from each flock were subjected to selective isolation of VRE. Thus, the difference of VRE prevalence may be explained by the use of different isolation procedures. In the present study selective isolation of VRE by the use of media containing vancomycin substantially increased the sensitivity of VRE detection, and allowed a more accurate determination of flock prevalence of VRE compared to non-selective isolation (Table 1).

Assuming the differences in VRE prevalence observed in the DANMAP and in the present study may be explained by the use of different isolation procedures, the decline of VRE prevalence in broiler flocks recorded in the DANMAP programme after the avoparcin ban may reflect a quantitative reduction of VRE in the samples, while qualitatively the prevalence of VRE-positive broiler flocks may have remained at a high level. However, valid inferences concerning a quantitative reduction of VRE in broilers after the avoparcin ban should be based on quantitative investigations.

The extensive occurrence of VRE in broiler flocks reported in this study indicates that consumers may still be exposed to VRE from poultry products despite the avoparcin ban, and the spread of vancomycin resistance from broiler products to humans should remain a matter of concern. Further investigations of the epidemiology of VRE and its quantitative occurrence in broiler flocks is needed in order to evaluate the effect of the current intervention.

Second study
The results of isolation of VRE from broiler flocks and from the floor and equipment in the houses are given in Table 2. A total of 69 vancomycin resistant *E. faecium* isolates were recovered. All isolates originated from different samples.

Table 1. Prevalence of vancomycin resistant enterococci (VRE) in 162 broiler flocks from farms previously exposed and unexposed to avoparcin

	Direct plating on Slanetz and Bartley agar plates containing vancomycin (SBv)		Direct plating on Slanetz and Bartley agar plates not containing vancomycin (SB)	
	Avoparcin exposed (conventional/-extensive broiler flocks)	**Avoparcin unexposed** (organic broiler flocks)	**Avoparcin exposed** (conventional/-extensive broiler flocks)	**Avoparcin unexposed** (organic broiler flocks)
VRE positive	104	2	17	0
VRE negative	36	20	123	22
Total	140	22	140	22

Table 2. Isolation of VRE (*E. faecium*) from broiler flocks at slaughter and from environmental samples obtained in broiler houses after depopulation, cleaning and disinfection of the houses

Broiler house	Broiler flocks	Floor	Floor cracks	Water cups	Feed bowls
A	+	+	+	+	-
B	+	+	+	-	-
C	+	+	+	-	-
D	+	+	+	-	-
E	-	-	-	-	-

("+" denotes successful isolation of VRE)
("-" denotes no VRE isolated)

A total of 41 different PFGE-profiles were observed. Interpretation of profiles using the criteria proposed by Tenover et al. (1995), resolved 34 PFGE-types. Examples of PFGE-profiles from broiler flocks and broiler houses are shown in Figure 1. Indistinguishable or highly similar PFGE-profiles were observed among VRE-isolates from consecutive broiler flocks reared in the same house, and among VRE-isolates from broiler flocks and the houses in which they were reared. In contrast PFGE-profiles of epidemiologically unrelated VRE-isolates showed extensive heterogeneity.

All hatchery samples and samples of broiler feed were VRE-negative. Avoparcin was never used in the parent stock, and the broiler feed was heat-treated at 81° C prior to delivery (Anonymous, 2001). The findings are in agreement with the results of a Norwegian study (Borgen et al., 2000), in which 41 samples from a hatchery and feed mill samples were VRE-negative. Although the hatchery samples investigated in the present study were not obtained at hatching of the actual broiler flocks sampled for the study, the results did indicate that neither hatchery nor broiler feed was a likely source of VRE.

House A; Lane 1-2: VRE from two consecutive broiler flocks.
House B; Lane 3-4: VRE from two consecutive broiler flocks.
House C; Lane 5-9: VRE from two consecutive broiler flocks and from environmental samples.
House D; Lane 10-11: VRE from a broiler flock and an environmental sample.
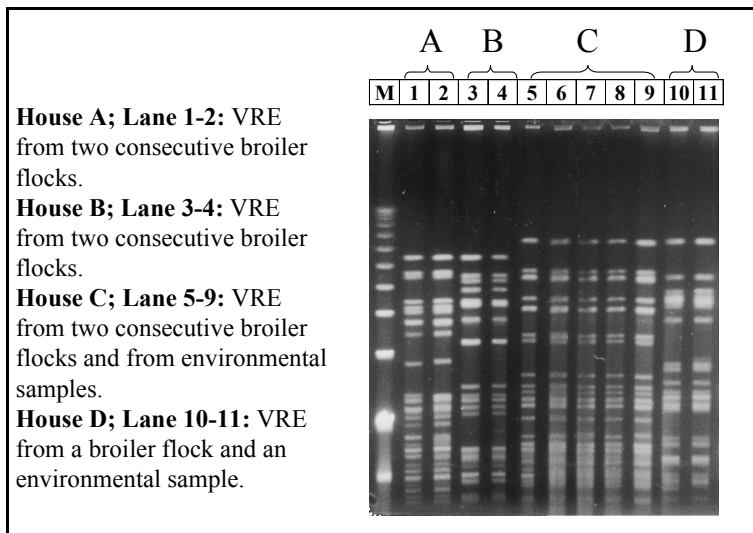
Figure 1. Examples of PFGE profiles

In conclusion this study demonstrated the persistence of VRE in broiler houses in the absence of the selective pressure exerted by avoparcin, and the presence of identical or closely related clones of VRE in epidemiologically related broiler flocks and broiler houses. The results indicate that VRE may be transmitted between consecutive broiler flocks by clones of VRE persisting in the broiler house environment despite cleaning and disinfection. This may explain the extensive occurrence of VRE in broiler flocks after the avoparcin ban. The results indicate that dissemination of vancomycin resistance determinants from poultry to humans should not be disregarded despite the avoparcin ban.

References

Aarestrup, F.M., Seyfarth, A.M., Emborg, H.D., Pedersen, K., Hendriksen, R.S. and Bager, F. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark. Antimicrob. Agents Chemother. 45, 2054-2059.

Anonymous. 2000. Beretning. Det Danske Fjerkræråd, 119-134.

Anonymous. 2001. DANMAP 2000 - Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark. ISSN-1600-2032, Copenhagen, Denmark.

Bager, F. 2000. DANMAP: monitoring antimicrobial resistance in Denmark. Int. J. Antimicrob. Agents 14, 271.

Bager, F., Aarestrup, F.M., Madsen, M., and Wegener, H.C. 1999. Glycopeptide resistance in *Enterococcus faecium* from broilers and pigs following discontinued use of avoparcin. Microb. Drug Resist. 5, 53-56.

Bager, F., Madsen, M., Christensen, J. and Aarestrup, F.M. 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant *Enterococcus faecium* on Danish poultry and pig farms. Prev. Vet. Med. 31, 95-112.

Borgen, K., Simonsen, G.S., Sundsfjord, A., Wasteson, Y., Olsvik, Ø. and Kruse, H. 2000. Continuing high prevalence of VanA-type vancomycin-resistant enterococci on Norwegian poultry farms three years after avoparcin was banned. J. Appl. Microbiol. 89, 478-485.

Borgen, K., Sørum, M., Kruse, H. and Wasteson, Y. 2000. Persistence of vancomycin-resistant enterococci (VRE) on Norwegian broiler farms. FEMS Microbiol. Lett. 191, 255-258.

Butaye, P., Devriese, L.A., Goossens, H., Ieven, M. and Haesebrouck, F. 1999a. Enterococci with acquired vancomycin resistance in pigs and chickens of different age groups. Antimicrob. Agents Chemother. 43, 365-366.

Butaye, P., Devriese, L.A. and Haesebrouck, F. 1999b. Comparison of direct and enrichment methods for the selective isolation of vancomycin-resistant enterococci from feces of pigs and poultry. Microb. Drug Resist. 5, 131-134.

Heuer, O.E. Occurrence, persistence and epidemiology of vancomycin resistant enterococci in Danish broiler production. Ph.D.- thesis. 2002. Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University and Danish Veterinary Institute. Copenhagen, Denmark.

Heuer, O.E., Pedersen, K., Andersen, J.S. and Madsen, M. 2002. Vancomycin resistant enterococci (VRE) in broiler flocks five years after the avoparcin ban. Microb. Drug Resist. 8, 133-138.

Hospital Infection Control Practices Advisory Committee. 1995. Recommendations for preventing the spread of vancomycin resistance. Hospital Infection Control Practices Advisory Committee (HICPAC). Infect. Control Hosp. Epidemiol. 16, 105-113.

Ieven, M., Vercauteren, E., Descheemaeker, P., van Laer, F. and H. Goossens. 1999. Comparison of direct plating and broth enrichment culture for the detection of intestinal colonization by glycopeptide-resistant enterococci among hospitalized patients. J. Clin. Microbiol. 37, 1436-1440.

Kruse, H., Johansen, B.K., Rorvik, L.M. and Schaller, G. 1999. The use of avoparcin as a growth promoter and the occurrence of vancomycin-resistant *Enterococcus* species in Norwegian poultry and swine production. Microb. Drug Resist. 5, 135-139.

Moellering, R.C.J. 1992. Emergence of *Enterococcus* as a significant pathogen. Clin. Infect. Dis. 14, 1173-1176.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H. and Swaminathan, B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. 33, 2233-2239.

van den Bogaard, A.E., Bruinsma, N. and Stobberingh, E.E. 2000. The effect of banning avoparcin on VRE carriage in The Netherlands. J. Antimicrob. Chemother. 46, 146-147.

6. Effects of the termination of antibiotic growth promoters use on presence of resistance genes in bacterial isolates from production animals

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Abstract

The genetic background for resistance to several growth promoters was investigated by molecular characterization of the genes and their location. This characterization has documented that the same genes encode resistance to both growth promoters and therapeutic antimicrobials used in humans. Thus, usage of growth promoters in animals will select for resistance, in animals, to therapeutic drugs of public health significance. Some classes of macrolide resistance genes were found only in a single bacterial species indicating that inter-species transfer of these classes of macrolide resistance does not occur frequently. In *Campylobacter coli* macrolide resistance was encoded by a point mutation, so macrolide resistance in campylobacter is only spread to humans by the spread of bacteria. Co-selection of structurally unrelated compounds was found due to physical linkage of resistance genes on mobile DNA elements. Co-selection was also indicated for usage of, and resistance to, copper, macrolides and vancomycin in *E. faecium* from pigs. Indistinguishable bacterial strains and resistance genes were found both in both animals and humans indicating that transfer of resistance between the animal and human reservoir occur. In the case of vancomycin resistance in *E. faecium* genetic characterization clearly indicated that the route of transmission for vancomycin resistance was from animals to humans.

Introduction

Monitoring of antimicrobial resistance is an important tool in understanding occurrence, prevalence and spread of antimicrobial resistance. By molecular characterization of the resistance genes and determination of their position on the chromosome or on mobile DNA elements important additional information is gained. Resistance genes may encode resistance to more than one antimicrobial. Normally resistance gene will encodes resistance to structurally related compounds. This is called *cross-resistance*. Examples of this are that the *van*A gene cluster encodes resistance to the glycopeptides avoparcin and vancomycin and the *erm*(B) gene encodes resistance to the macrolides erythromycin and tylosin.

Resistant bacteria are selected by usage of antimicrobial and a correlation exists between usage and prevalence of resistance under most circumstances. In some cases resistance persists long after usage has been terminated and no simple explanation exist for these discrepancies. Since resistance genes can be encoded by plasmids and plasmids can contain several resistance genes, usage of one antimicrobial would select for presence of the plasmid and hereby resistance to all compounds encoded by the resistance genes present on the plasmid. This is called *co-selection*.

By knowing which genes are present, spread of not only antimicrobial resistance but also spread of the resistance genes can be monitored. Resistance genes can spread with the resistance bacteria it self (called *vertical spread of resistance*) or by transfer to sensitive bacteria either of same species or to new species (called *horizontal spread of resistance*).

In the work on characterization of genes encoding resistance to growth promoter focus has been on prevalence of genes encoding cross-resistance between used growth promoters and antibiotics used for animal and human therapy. We have investigated three such cases. These are macrolides, streptogramins and glycopeptides (vancomycin). Result on genetic characterization of resistance genes to these growth promoters and possible co-selection of resistance to other therapeutic antimicrobials will be

presented here. The results obtained will be used to discuss the transfer of resistance from animals to humans and the possible impacts on resistance in bacteria of human origin.

Results

Macrolide resistance

Tylosin and erythromycin belongs to the macrolides. Macrolide resistance can be encoded either by resistance genes or by point mutations in the ribosomal genes. The *erm*-genes are the most prevalent genes for macrolide resistance (Roberts et al., 1999). Presence of these genes was investigated in macrolide resistant bacteria of animal and human origin. High prevalence of the *erm*-genes encoding resistance both to tylosin and erythromycin was found. As expected different *erm*-genes were found in enterococci and staphylococci but the same genes were dominant in isolates from animal and human origin (Table 1). This result indicates that transfer of macrolide resistance between the animal and human reservoir can occur both horizontally and vertically.

High prevalence of macrolide resistance had been detected in *Campylobacter coli* isolated from pigs (Anonymous, 2001). None of the above mentioned genes were found. Instead a well-described point mutation in the ribosomal DNA was detected (Jensen & Aarestrup, 2001). This result documents that macrolide resistance will not transfer from *C. coli* to sensitive *Campylobacter jejuni* neither in animals nor humans and that spread of macrolide resistance in campylobacter only will be vertical.

Streptogramin resistance

The streptogramin virginiamycin has for several years been used for growth promotion in Europe and is still used in United States. The streptogramin quinopristin/dalfopristin was licensed for human usage in 1999 (Johnson & Livermore, 1999). Streptogramins were thought to be the successor for glycopeptides since treatment failures using vancomycin were starting to occur. Streptogramin consists of two compounds, streptogramin A and streptogramin B. Streptogramin A resistance is encoded by either *vat*(D) or *vat*(E) in *E. faecium* while *E. faecalis* is natural resistance to streptogramins. Streptogramin B resistance is encoded by the macrolide resistance genes *erm*(B) giving cross-resistance to macrolide and lincosamide (Roberts et al., 1999).

The genetic background for streptogramin resistance was defined in streptogramin resistant *E. faecium* isolates of animal origin (Jensen et al., 2002). Results are presented in Table 2. In most isolates (73%) *vat*(D) or *vat*(E) genes were linked to the *erm*(B) gene (Hammerum et al., 2001; Jensen et al., 2000). In *E. faecium* of porcine origin the genetic background for streptogramin A could not be identified in most isolates. In most isolates the resistance genes were positioned in a mobile DNA element. The same genes and physical link have been found in *E. faecium* isolates from humans (Jensen et al., 2000) indicating a possible horizontal transfer of the mobile DNA element. Since this element contains the erm(B) gene co-resistance to macrolide exist.

Table 1. Prevalence of the *erm*-genes among bacteria of animal and human origin

Macrolide resistance genes in bacteria of animal and human origin								
		enterococci				staphylococci		
		number	erm(A)*	erm(B)*	erm(C)*	number	erm(A)*	erm(B)*	erm(C)*
animals	pigs	48	0	75	0				
						16	6	0	75
	poultry	157	2	87	0				
						20	5	0	95
humans		17	0	100	0				
						44	23	0	75

* All numbers indicated are in percentage

Table 2. Streptogramin resistance genes in resistant E. faecium of animal origin

Streptogramin resistance genes in resistant E. faecium from animals

	number of isolates	number of strains with (%)				
		vat(D)	vat(D)-erm(B)	vat(E)	vat(E)-erm(B)	erm(B)
Pigs	23	0	2(9)	1(4)	1(4)	19(83)
Poultry	138	3(2)	8(6)	18(13)	106(77)	3(2)
Total	161	3(2)	10(6)	19(12)	107(66)*	22(14)

* Three isolates contained both vat(D) and vat(E)

Vancomycin resistance

The study of spread of vancomycin resistance has been the key story in the banning of growth promoters in Europe. Avoparcin and vancomycin belongs to the glycopeptides. The glycopeptide avoparcin has for years been used in Europe for growth promotion (Aarestrup, 1999), while no avoparcin has been used in United States. Here high prevalence of glycopeptide resistance exists among enterococci isolated from humans. That avoparcin selects for vancomycin resistance has been shown (Bager et al., 1997). The most frequent identify resistance gene in human vancomycin resistant isolates has been the *vanA* gene cluster (Arthur & Courvalin, 1993).

Genetic typing by Pulse Field Gel Electrophoresis (PFGE) of animal vancomycin resistant enterococci (VRE) from Denmark showed that isolates from poultry were not related while porcine isolates were very clonal. This could be seen since the porcine isolates had identical PFGE patterns (Aarestrup, 2000). This indicates vertical spread of vancomycin resistance among porcine isolates while horizontal spread occurred among poultry isolates. Among the few human VRE an identical isolate to a porcine clone was found (Jensen et al., 1999). Since the person from whom the VRE were isolated did not have any contact with the farm environment, this indicates transfer of VRE via the food chain.

The genetic background for glycopeptide resistance was defined in glycopeptide resistant enterococci of animal and human origin. All ani-mal isolates contained the *vanA* gene cluster (Tn*1546*) and only resistant *E. faecium* isolates were found in animal (Jensen et al., 1998). Since vancomycin never has been used for animals, this result proves that usage of avoparcin as growth promoter has selected for the same resistance genes (*vanA*) as usage of vancomycin for human therapy. Furthermore the genetic characterization of the *vanA* gene cluster found small genetic variations, that could be used for tracking spread of resistance. Several genetic sub-types of the *vanA* were defined and presence of these among isolates of animal and human origin was investigated. Results are presented in Table 3 (Jensen et al., 1998). Identical sub-types of the *vanA* gene cluster were found among isolates of animal and human origin, indicating transfer of VRE between the animal and human reservoir.

Different sub-types of the *vanA* gene cluster were found among isolates from poultry and pigs respectively, while both the poultry and porcine sub-types were found among human VRE isolates. A unique point mutation in the *vanA* gene cluster separated the porcine and poultry sub-types. Presence of this unique point mutation was tested in non-related VRE isolates from animals and humans from different countries. Results are presented in Table 4. Since almost equal number of the porcine and poultry version were found among human isolates and these two version was only found in either poultry or porcine isolates this clearly indicates transfer of vancomycin resistance from animals to humans (Jensen, 1998).

VanA gene cluster
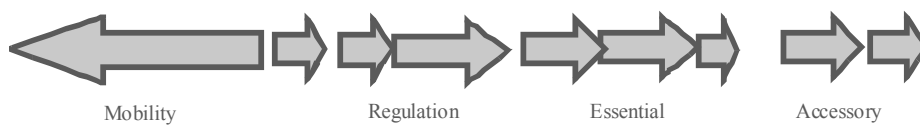
Figure 1. The *vanA* gene cluster (Tn*1546*). The gene cluster consist of 9 genes but only 3 genes are essential for glycopeptide resistance

Table 3. Genetic sub-types of the *vanA* gene cluster. Sub-types were defined due to variations. Variations found were point mutations and presence of IS-sequences

Genetic characterization of the vanA gene cluster in vancomycin resistant isolates from animals and humans

| | | number of isolates | Genetic sub-types of the vanA gene cluster | | | | | | |
			1	2	3	4	5	6	7
Animals	Pigs	8		8					
	Poultry	10	9						1
Humans		32	5	8	6	8	2	2	1
Total		50	14	16	6	8	2	2	2

Table 4. Distribution of a unique point mutation in VRE isolates of animal and human origin

Presence of a unique point mutation
in VRE of animal and human origin

| | in percentage | |
	T variant	G variant
Human	35	64*
Pigs	97	3
Poultry	0	100

Human isolates were collected from Denmark, Norway, United Kingdom, United States, The Netherlands, Germany, Saudi Arabia and France. Porcine isolates were from Denmark, Norway, Germany, United Kingdom and France. Poultry isolates were from Denmark, Norway, The Netherlands, Finland, United Kingdom and France * Among the human isolates 7 were from Norwegian poultry farmers and 5 from Saudi Arabia

Higher prevalence of vancomycin resistant enterococci was observed among isolates from pigs even after the ban of avoparcin. When these isolates were investigated they all contained a large plasmid of 175 kb. Present on this plasmid were both the *vanA* gene cluster and the

erm(B) gene. This physical linking explains why co-selection of vancomycin resistance by the use of macrolides has been observed among porcine isolates (Figure 2). Prevalence of vancomycin resistance prevailed at approximately 20 % until the voluntarily discontinuation of the usage of the macrolide tylosin in 1998. Further characterization of the 175 kb plasmid revealed the presence of copper resistance genes. To investigate possible co-selection of resistance to macrolides and vancomycin by copper the presence of the copper resistance genes among resistant isolate was investigated. Results are presented in Table 5 (Hasman & Aarestrup, 2002). Statistically significant indications for co-selection were found for both macrolide (erythromycin) and vancomycin resistance among isolates from pigs. Since copper is used in high concentrations in feed to pigs, presence of this concentration of copper may also co-select for both macrolide and vancomycin resistance. This is currently being investigated.
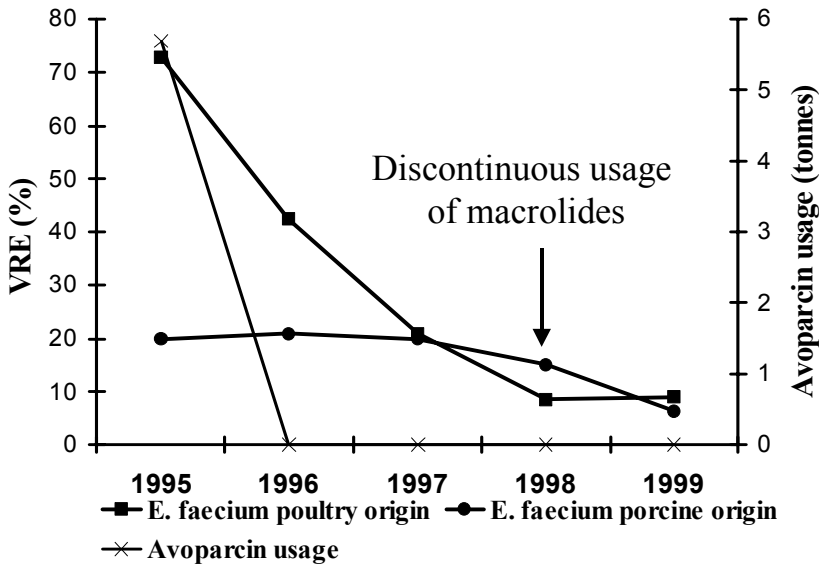
Figure 2. Prevalence of vancomycin resistance among isolates of poultry and porcine origin from Denmark. Usage of the antimicrobial growth promoter avoparcin is shown in tonnes

Table 5. Co-resistance of copper, macrolide and vancomycin among *E. faecium* isolates of animal and human origin. Numbers are given as percentage of the number of isolates

Enterococcus faecium isolates of animal and human origin

		Macrolide		Vancomycin	
		resistant	sensitive	resistant	sensitive
Pigs	Copper resistant	91	9	31	69
	Copper sensitive	0	100	0	100
Broilers	Copper resistant	50	50	30	70
	Copper sensitive	58	42	0	100
Humans	Copper resistant	33	67	0	100
	Copper sensitive	46	54	0	100

Discussion

Identical resistance genes were found by characterization of the genes encoding for resistance in bacteria of animal and human origin for all invested antimicrobials. This clearly indicates that the animal and human reservoirs are overlapping and that horizontal transfer of resistance can occur between the two reservoirs. Macrolide resistance was in most isolates encoded by an *erm*-gene, but specific genes predominate in the different bacterial species, indicating limited transfer of macrolide resistance between bacterial species. In *C. coli* resistance was encoded by a point mutation so macrolide resistance in campylobacter will only spread vertically.

In the study of vancomycin resistance more genetic variations were found in the *vanA* gene cluster encoding resistance and by characterization of especially one variation clear indication for transfer of vancomycin resistance from the animal reservoir to humans were found.

Not only have genetic characterization indicated the flow of resistance from animals to humans but have also given some suggestions to why resistance may persist in the absence of a specific selective pressure. When looking at the prevalence of vancomycin resistance among isolates of porcine origin discrepancies between usage and levels of resistance was discovered. High levels of vancomycin resistance persisted until the use of macrolides as growth promoters was terminated. By characterization of the vancomycin resistant isolates a large 175 kb plasmid was discovered. This plasmid not only encoded resistance to macrolide and vancomycin but also to copper suggesting that also the use of copper as a feed additive might co-select for those resistance determinants. Usage of macrolide has until the ban in 1998 upheld resistance to vancomycin and the continued usage of copper in animal feed may pose a selective pressure in favour of VRE, however this needs to be investigated further.

References

Aarestrup, F.M. 1999. Association between the consumption of antimicrobial agents in animal husbandry and the occurrence of resistant bacteria among food animals. Int. J. Antimicrob. Agents 12, 279-285.

Aarestrup, F.M. 2000. Characterization of glycopeptide-resistant enterococcus faecium (GRE) from broilers and pigs in Denmark: genetic evidence that persistence of GRE in pig herds is associated with co-selection by resistance to macrolides. J. Clin. Microbiol. 38, 2774-2777.

Anonymous. 2001. DANMAP 1998. Consumption of antimicrobial Agents and occurrence of antimicrobial resistance in bacteria from animals, food and humans in Denmark. Report Danish Zoonosis Center, Copenhagen.

Arthur, M. and Courvalin, P. 1993. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob. Agents Chemother. 37, 1563-1571.

Bager, F., Madsen, M., Christensen, J. and Aarestrup, F.M. 1997. Avoparcin used as a growth promoter is associated with the occurrence of vancomycin-resistant Enterococcus faecium on Danish poultry and pig farms. Prev. Vet. Med. 31, 95-112.

Hammerum, A.M., Flannagan, S.E., Clewell, D.B. and Jensen, L.B. 2001. Indication of transposition of a mobile DNA elements containing the vat(D) and erm(B) genes in Enterococcus faecium. Antimicrob. Agents Chemother. November 2001.

Hasman, H. and Aarestrup, F.M. 2002. tcrB, a gene conferring transferable copper resistance in Enterococcus faecium: occurrence, transferability, and linkage to macrolide and glycopeptide resistance. Antimicrob. Agents Chemother. 46, 1410-1416.

Jensen, L.B. 1998. Differences in the occurrence of two base pair variants of Tn1546 from vancomycin-resistant enterococci from humans, pigs, and poultry. Antimicrob. Agents Chemother. 42, 2463-2464.

Jensen, L.B. and Aarestrup, F.M. 2001. Macrolide resistance in Campylobacter coli of animal origin in Denmark. Antimicrob. Agents Chemother. 45, 371-372.

Jensen, L.B., Ahrens, P., Dons, L., Jones, R.N., Hammerum, A.M. and Aarestrup, F.M. 1998. Molecular analysis of Tn1546 in Enterococcus faecium isolated from animals and humans. J. Clin. Microbiol. 36, 437-442.

Jensen, L.B., Hammerum, A.M. and Aarestrup, F.M. 2000. Linkage of vat(E) and erm(B) in streptogamin-resistant Enterococcus faecium isolates from Europe. Antimicrob. Agents Chemother. 44, 2231-2232.

Jensen, L.B., Hammerum, A.M., Bager, F. and Aarestrup, F.M. (2002). Streptogramin resistance among *Enterococcus faecium* isolated from production animals in Denmark in 1997. Accepted in Microb. Drug Resist. (winter edition).

Jensen, L. B., A. M. Hammerum, R. L. Poulsen, and H. Westh. 1999. Vancomycin-resistant Enterococcus faecium strains with highly similar pulsed-field gel electrophoresis patterns containing similar Tn1546- like elements isolated from a hospitalized patient and pigs in Denmark. Antimicrob.Agents Chemother. 43, 724-725.

Johnson, A.P. and Livermore, D.M. 1999. Quinupristin/dalfopristin, a new addition to the antimicrobial arsenal. Lancet 354, 2012-2013.

Roberts, M.C., Sutcliffe, J., Courvalin, P., Jensen, L.B., Rood, J. and Seppala, H. 1999. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob. Agents Chemother. 43, 2823-2830.

7. Effects of termination of antimicrobial growth promoter use for broiler health and productivity

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Summary

On 15 February 1998, the Danish poultry industry voluntarily decided to discontinue the use of all antimicrobial growth promoters (AGPs). To investigate how the removal of AGPs influenced the broiler productivity and health in Denmark, data from 6,815 flocks collected from November 1995 to May 1999 by the Danish Poultry Council were analysed. The three flock parameters were: kg broilers produced per square meter (per rotation), feed-conversion ratio (total kg feed used per rotation/total kg live weight per rotation) and total percent dead broilers ((number of dead broilers during the rotation/number of broilers put in the house per rotation) x100). The analyses showed that kg broilers produced per square meter and percent dead broilers in total were not affected by the discontinued use of AGPs. However, the feed-conversion ratio increased marginally by 0.016 kg/kg. A follow up on the same 3 flock parameters from June 1999 until June 2002 indicated that kg broilers produced per square meter increased compared to the period before AGP withdrawal. In the same period the feed-conversion ratio decreased slightly, while percent dead broilers in total remained low or even a slight decrease has been the result.

Introduction

The practice of adding antimicrobials to broiler feed in sub-therapeutic doses was initiated in Denmark in the beginning of the 1960's. Subsequently, antimicrobial growth promoters (AGPs) have been used widely in the Danish poultry production since the beginning of the 1970's. AGPs were included in the feed because of both growth-promoting and antibacterial effects (especially on *Clostridium perfringens* –an agent of necrotic enteritis (Anonymous 1997)).

Some of the antimicrobial agents used for growth promotion, e.g. avoparcin and virginiamycin belong to the same classes of antimicrobials used for human therapy. Consequently, resistance towards the AGPs may result in resistance towards therapeutic drugs used for humans, e.g. vancomycin and quinopristin/dalfopristin. In May 1995, the Danish Ministry of Food, Agriculture and Fisheries banned the AGP avoparcin because *E. faecium* isolates resistant to vancomycin and avoparcin were found commonly in faeces from pigs and poultry (Aarestrup et al. 1996). Subsequently, avoparcin was banned in EU in 1997. In January 1998, the AGP virginiamycin also was banned in Denmark because of cross-resistance to quinupristin/dalfopristin (Wegener et al. 1999). Along with the bans of the two AGPs, consumers were increasingly concerned about the presence of resistant bacteria in food products. Accordingly, the Danish poultry industry investigated the possibility of a complete removal of all AGPs from poultry production. On 15 February 1998, the poultry industry decided to discontinue the use of all AGPs despite concerns that the removal would result in decreased productivity and increased morbidity and mortality.

This presentation was based on data from the productivity database established by the Danish Poultry Council. The aim was to investigate how the removal of AGPs influenced broiler productivity and health. Based on data from November 1995 to May 1999, we analysed the effect of the discontinued usage of AGPs on the flock parameters 1) kg broilers produced per square meter, 2) feed-conversion ratio and 3) percent dead broilers in total (mortality) (Emborg et al. 2001). In addition, the trend in the above mentioned flock parameters from June 1999 until June 2002 was evaluated.

Material and methods

The data were extracted from a productivity database administrated by the Danish Poultry Council. The Danish Poultry Council compiles statistics for each broiler flock produced thereby providing the producers with an overview of the production results of each flock and an opportunity to compare their results with the average flock produced in Denmark at the same time. The database was established in the middle of the 1970's and contains production data originating from different sources in the industry.

Productivity data from November 1995 to May 1999

The following flock informations were extracted from the broiler productivity database and included in the analyses: farm-identification number, house-identification number within farms, flock rotation number within the house, the broiler breed, age of parent stock hen, percent dead broilers the first week, percent dead broilers in total, feed supplier, percent wheat added to the feed, feed-conversion ratio, size of the broiler flock, stocking density, hatchery, date of hatching, date of slaughter, the average weight of a broiler at slaughter, age of broilers when slaughtered, kg broilers produced per square meter, percentage of condemned kg carcass. In addition, we defined the period before and after withdrawal of AGPs and 3 variables to model the seasonal variation within year.

From November 1995 to early May 1999, 14,057 flocks were slaughtered in Denmark. These flocks were produced by a total of 343 farms in 929 houses. In total 7,242 flocks were excluded from the analyses mainly because of missing data values and a few because of erroneous values. Subsequently, a total of 6,815 flocks representing 237 farms and 575 houses were included in the analyses.

The data had a strictly hierarchical structure with a 3-level hierarchy: farm → houses → flocks. Houses were clustered within the same farm and multiple measurements of flock productivity from the same house were clustered over time. Data were analysed using a mixed model. This analysis is capable of handling fixed and random effects in the same model.

The correlation structure AR(I) was included to describe the repeated measurement of flock productivity (SAS Institute Inc. 1996). More detailed information on data and the statistical analyses can be obtained from Emborg et al. (2001).

Productivity data from June 1999 to June 2002

The Danish Poultry council made additional data available from the productivity database for the time period June 1999 to June 2002. Trends in the 3 flock parameters 1) kg broilers produced per square meter, 2) feed-conversion ratio and 3) percent dead broilers in total (mortality) were compared with the results of the statistical analyses performed for the period November 1995 to May 1999. From June 1999 to June 2002, 13,368 flocks were slaughtered in Denmark. A total of 7,189 flocks were excluded because of missing values leaving data from 6,179 flocks to be evaluated.

Results

Kilogram broilers produced per square meter

The mean monthly kg broilers produced per square meter from November 1995 to June 2002 is shown in Figure 1 (flocks were grouped within month according to the day of hatching).

November 1995 to May 1999

The variable period before and after withdrawal of AGPs did not remain in the model meaning that no difference was observed in kg broilers produced per square meter before and after the AGP withdrawal. Figure 1 shows a seasonal variation in kg broilers produced per square meter with the lowest average production registered from May to July. The lower production in the summer was most likely due to a reduction in stocking density during the warm months, because of the fear of high mortality due to heat stress. The variables percent dead broilers the first week, stocking density and weight at slaughter had the largest impact on kilogram broilers produced per square meter. The analysis showed that more dead broilers the first week would decrease kg broilers produced per square meter while an increase in either weight at slaughter or in the stocking density would in-

crease kilogram broilers produced per square meter.

Figure 1 indicates that kg broilers produced per square meter from June 1999 to June 2002 was higher compared to the period before withdrawal of antimicrobial growth promoters.

Feed-conversion ratio

The mean monthly feed-conversion ratio from November 1995 to June 2002 is shown in Figure 2 (flocks were grouped within month according to the day of hatching).

November 1995 to May 1999

The average feed-conversion ratio was significantly lower (0.016 kg/kg ($P<0.001$)) in the period before than after the removal of the AGPs. The age of the broilers at the time of slaughter had the largest impact on the feed-conversion ratio and a positive estimate indicating that with increasing age, it takes more feed to produce one kg of bird. In addition, the feed-conversion ratio increased when condemned kg carcass or the percentage of wheat added to the feed increased.

June 1999 to June 2002

An increase in the feed-conversion ratio was observed after the AGP withdrawal. Data from June 1999 to June 2002 indicated that this initial increase was followed by a slight decrease in the feed-conversion ratio.

Percent dead broilers in total (mortality)

The mean monthly percent dead broilers in total (mortality) from November 1995 to June 2002 is shown in Figure 3 (the flocks were grouped within month according to the day of hatching).

November 1995 to May 1999

The variable period before and after withdrawal of AGPs did not remain in the model meaning that percent dead broilers in total did not differ before and after the AGP withdrawal. The variable percent dead broilers the first week had the largest impact on percent dead broilers in total. As expected, the result of the analysis suggested that an increase in percent dead broilers the first week will result in an increase in percent dead broilers in total. The model predicted that an increase in either age at the time of slaughter or percent condemned kg carcass will increase the percentage of dead broilers in total.
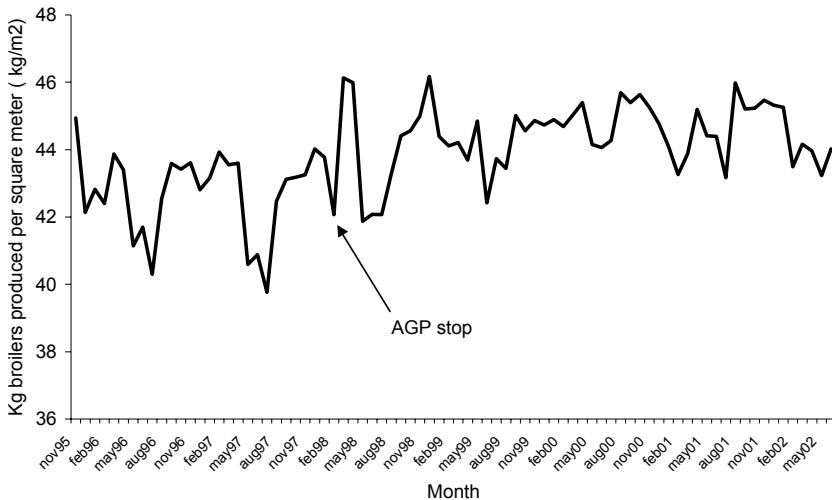
Figure 1. The mean monthly kilogram broilers produced per square meter from November 1995 to June 2002. Flocks were grouped within month according to the day of hatching
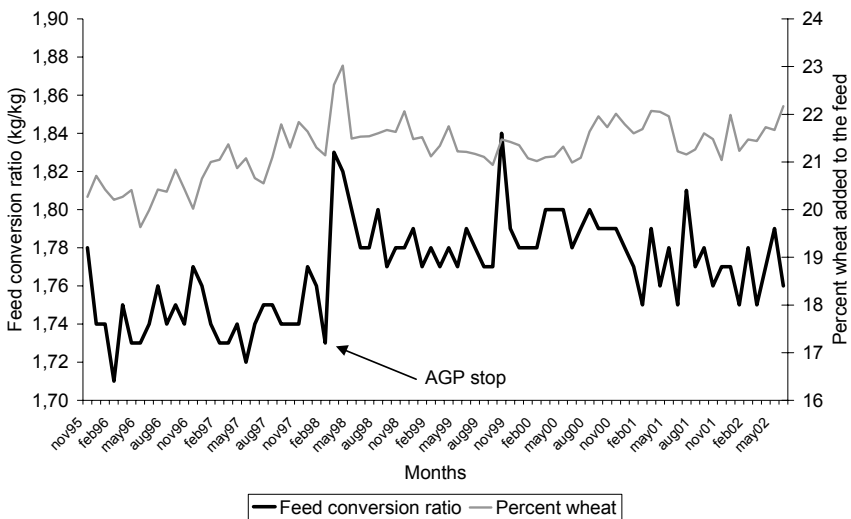
Figure 2. The mean monthly feed-conversion ratio and the mean monthly percent wheat added to the feed from November 1995 to June 2002. Flocks were grouped within month according to the day of hatching
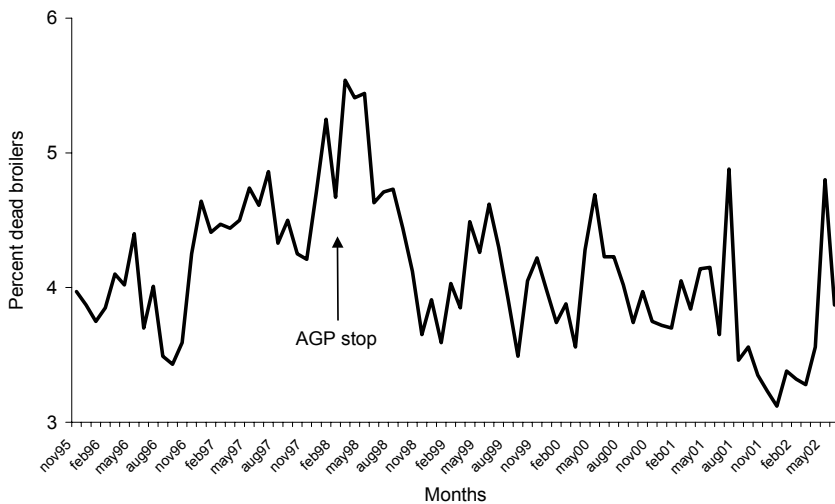
Figure 3. The mean monthly percent dead broilers in total from November 1995 to June 2002. Flocks were grouped within month according to the day of hatching

June 1999 to June 2002
Figure 3 indicates that from June 1999 to June 2002 percent dead broilers in total remained low and even a slight decrease seem to have occurred.

Discussion

November 1995 to May 1999
The variable period before and after withdrawal of AGPs did not remain in the two models for kg broilers produced per square meter and per-

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cent dead broilers in total. This indicated that these two production variables were not affected by the discontinued use of AGPs. The results from the analyses are in agreement with anecdotal evidence expressed by the producers, who did not observe a decrease in the number of kg broilers produced or increased mortality. But these results were unexpected to both the slaughterhouses and the producers, who expected economic losses due to a lower productivity and especially an increased mortality due to outbreaks of necrotic enteritis and chronic hepatitis.

During the first 2-3 months after the withdrawal of AGPs, simultaneous increases were observed in: kg broilers produced per square meter, feed-conversion ratio, percent wheat added to the feed and percent dead broilers in total (Figure 1-3). These increases coincided with a general strike in Denmark where also the broiler slaughterhouses were affected. Due to the strike, the producers were forced to keep the broiler flocks for a longer period than expected before they could send them to slaughter. Our analyses showed that an increase in the age or the weight of the broilers would increase kg broilers produced per square meter, feed-conversion ratio and percent dead broilers in total. The wheat content of the feed is increased with the age of the broilers. If the producers are forced to keep the broiler flocks longer than planned, the average percentages wheat included in the feed during the rotation are increased. Therefore, the increases in the 4 parameters observed immediately after the withdrawal of AGPs was most likely caused by the general strike and not by the AGP stop (Figure 1-3).

Improvements in breeding, feed and management in the study period are expected to have influenced the outcome variables in our analyses. However, the effect of these factors on the flock parameters studied could not be accounted for in the analysis.

The feed-conversion ratio increased by 0.016 kg/kg immediately after the withdrawal of AGPs (Figure 2). This increase in feed-conversion ratio was most likely associated with the withdrawal of the AGPs. With an average price of 155 DKr pr 100 kg broiler feed, an increase

of 16 gram feed per kg broiler correspond to an additional expense of 0.025 DKr per kg broiler produced. The estimated average price for AGPs were 0.027 DKr per kg broiler. Therefore the additional expenses to extra feed did not exceed the expenses to AGPs. According to three major Danish feed suppliers, other feed changes were introduced to optimise the feed simultaneously with the removal of AGPs. However, these reported feed changes were not uniform as some reported changes were in the feed composition, e.g. changes of the content of protein, synthetic amino acids and/or fat and some included probiotics.

In Denmark, it is common practice to mix wheat in the compound feed. Our analysis indicates that this practise increased the feed-conversion ratio. Feed trials done in Denmark showed that a 1% increase in wheat content increased the feed-conversion ratio with 0.0022 kg per kg broiler (Petersen 1999). Our model predicted a similar increase in the feed-conversion ratio. During the study period, the percentage wheat included in the feed tended to increase slightly (<1%). The increase was observed from the beginning of 1997 and on-ward (Figure 2). The slight increase in the percentage of wheat mixed in the feed would most likely have contributed to an increase in the feed-conversion ratio, but it could not explain the immediate increase observed after the withdrawal of AGPs. Because wheat is cheap compared to compound feed, it is still profitable to include wheat in spite of an increased feed-conversion ratio (Petersen 1999).

The analysis showed that an increase in stocking density would increase kg broilers produced per square meter. These findings are in accordance with production figures reported by Shanawany (1988) and Elwinger (1995) who found that profit increased when stocking density increased.

The analysis showed no increase in percent dead broilers in total and except for a few extra necrotic enteritis outbreaks veterinarians and producers reported no additional increase in morbidity. The amount of antimicrobials used to treat disease in broilers at the time of AGP withdrawal is not known. Figures from the Vet-Stat programme showed that in 2001, 161 kg

antimicrobial (56,764 animal standard dosages) were used to treat disease in 140 million broilers. The use of coccidiostats in Danish broiler production might be part of the explanation why no decrease in productivity and health was observed after the AGP withdrawal.

June 1999 to June 2002
There have been speculations about the long-term effect of withdrawing AGPs. One of the concerns were if the negative effects would slowly build-up and then become visible at a later point in time. An evaluation of the trends in the 3 flock parameters 1) kg broilers produced per square meter, 2) feed-conversion ratio and 3) percent dead broilers in total (mortality) up to more than 4 years after the AGP withdrawal did not identify negative effects, and could therefore not support these concerns. In contrast, kg broilers produced per square meter seem to have increased compared to the period before AGP withdrawal. After a minor increase (<1%) in the feed-conversion ratio immediately after the AGP withdrawal, data up to June 2002 indicates that the feed-conversion ratio decreased while percent dead broilers in total remained low or even decreased slightly after the AGP withdrawal.

References

Aarestrup, F.M., Ahrens, P., Madsen, M., Pallesen, L.V., Poulsen, R.L. and Westh, H. 1996. Glycopeptide susceptibility among Danish *Enterococcus faecium* and *Enterococcus faecalis* isolates of animal and human origin and PCR identification of genes within the VanA cluster. Antimicrob. Agents Chemother. 40, 1938-1940.

Anonymous 1997. Antimicrobial feed additives. Report from the commission on antimicrobial feed additives, Stockholm, Sweden.

Elwinger, K. 1995. Broiler production under varying population densities - A field study. Arch. Geflügelk. 59, 209-215.

Emborg, H.D., Ersboll, A.K., Heuer, O.E. and Wegener, H.C. 2001. The effect of discontinuing the use of antimicrobial growth promoters on the productivity in the Danish broiler production. Prev. Vet. Med. 50, 53-70.

Petersen, C.B. 1999. Foder- og foderkvalitet i relation til produktionsresultater i slagtekyllingeproduktionen i perioden 1975 til 1998 (in Danish). Landskontoret for Fjerkrærådgivning, Skejby, Denmark, 109 pp.

SAS. 1996. The MIXED Procedure. In SAS/STAT Software: Changes and Enhancements through Release 6.11 pp. 531-656. Cary, NC: SAS Institute Inc.

Shanawany, M.M. 1988. Broiler performance under high stocking densities. Br. Poult. Sci. 29, 43-52.

Wegener, H.C., Aarestrup, F.M., Gerner-Smidt, P. and Bager, F. 1999. Transfer of antibiotic resistant bacteria from animals to man. Acta Vet. Scand. Suppl 92, 51-57.

8. Effects of termination of AGP-use on pig welfare and productivity

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Summary

Together with the feedstuff industry, the National Committee for Pig Production initiated a voluntary ban on antibiotic growth promoters to finishers (weighing more than 35 kg) in the spring of 1998. This more than halved the use of antibiotic growth promoters for pigs by 1999. Experiences collected in 62 finisher herds showed that the majority of the herds (63%) did not experience any problems such as reduced growth or increased frequency of diarrhoea. 26% of the herds experienced temporary problems, while 11% experienced permanent problems. The result of this is confirmed by statements of the national average of the efficiency controls (Econtrol), where the development in daily gain and mortality was not particularly affected by the removal of growth promoters.

The application of antibiotic growth promoters for weaners (and all other livestock production in Denmark) ceased as of January 2000. This reduced the application of antibiotics as growth promoters to zero. Since then, the use of antibiotics for therapeutic treatment has increased, which reflects the increasing problems with diarrhoea seen in the weaner period (7-30 kg). The consequences are also reflected in the statements of the national average of the E-controls, where daily gain has decreased and mortality has increased since the removal of growth promoters from weaner feed.

Overall, the removal of antibiotic growth promoters has only had a significantly negative effect on production and health in the weaner period (7-30 kg).

Introduction to Danish pig production

Annually, 13,500 pig producers produce 22.5 million pigs in Denmark (=1.8 million tonnes of pork), and of these approx. 1.6 million are used for live export. More than 95% of all pig slaughterings in Denmark are made at the two farmer-owned co-operative slaughterhouses (Danish Crown and Tican). 80-85% of the pork are exported primarily to the EU, Japan and the USA. The remaining 15-20% are sold in the domestic market – making a total value of €4.3 billion. Table 1 presents the main key figures from Danish pig production.

The Danish pig producers delivering pigs for slaughter to one of the co-operative slaughterhouses pay a levy of approx. €1.90 per pig. The money is managed by the DANISH BACON & MEAT COUNCIL, and is spent on research and development (The National Committee for Pig Production, NCPP), marketing, information etc.

Table 1. The average production level in Denmark (April 2001 - April 2002)

Sows:	
Sows/herd	246
Weaned pigs/litter	10.5
Dead before weaning, %	13.2
Weaners (7-30 kg):	
Age at weaning, days	29.9
Weight at weaning, kg	7.3
Dead after weaning, %	3.5
Daily gain, g	415
Age at 30 kg, days	85.5
Finishers (30-100):	
Produced pigs/herd, pcs.	3,290
Daily gain, g	824
Kg feed/kg gain *	2.70
Average lean meat %	60.2
Dead and rejected, %	3.6
* with 13.2 MJ ME/kg	

Chain of events in Denmark

On January 1, 2000 antibiotic growth promoters (AGPs) were removed from animal production in Denmark. The reason for removing the anti-

biotic growth promoters from the feed was to minimize the consumption of antibiotics so that they are only used for therapeutic purposes. The chain of events leading up this decision is seen below:

1995	National ban on avoparcin.
1995	Voluntary agreement between NCPP and the feedstuff industry to minimize the use of AGPs.
Jan. 1998	National ban on virginiamycine.
Mar. 1998	Voluntary agreement not to use AGPs for pigs >35 kg (**finishers**). Control and penalty systems introduced.
Sep. 1998	National tax on AGPs.
Oct. 1998	NCPP announces action plan to phase out AGPs for weaners by January 1, 2000.
Jul. 1999	EU ban: tylosine, bacitracine, spiramycine & virginiamycine.
Sep. 1999	EU ban: olaquindox & carbadox
Jan. 2000	Voluntary agreement not to use AGPs for pig <35 kg (**weaners**). Control and penalty systems introduced.

Consequences

Finishers
NCPP collected experiences from 62 Danish finisher herds in the period after they stopped using APGs. The majority (63%) of the herds did not experience problems such as reduced daily gain or increased frequency of treatments for diarrhoea when AGPs were removed from the feed. 26% of the herds experienced a temporary decrease in the daily gain, while 11% experienced permanent problems, probably due to the removal of AGPs from the feed. Thus the removal of AGPs from the feed has been fairly unproblematic in the herds participating in this study. The result from the study is confirmed by statements of the national average of the production efficiency controls. The developments in daily gain and mortality reveal no significant changes in the 1998 statement (the first year without growth promoters for finishers) compared with the previous year (cf. table 2). It appears however that the increase in daily gain (from 1997/98 to 1998/99) was not quite as high as in the previous years, and mortality was marginally higher.

Weaners
The use of APGs for weaners ceased completely as of January 1, 2000. However, many pigs (approx. 50%) were fed without APGs already from mid-1999. Table 3 presents the developments in daily gain and mortality. The table illustrates the decrease in daily gain (20 g) and a corresponding increase in the pigs' age at 30 kg. Postweaning mortality has also increased (0.7% units) in the 1999 statement compared to 1998.

Thus, the removal of AGPs from feed for weaners has had significantly negative consequences in the form of reduced gain and higher mortality.

Table 2. National average for production efficiency control – finishers

April – April	With growth promoters				No growth promoters		
	1995 - 1996	**1996** - 1997	**1997** - 1998	**1998** - 1999	**1999** - 2000	**2000** - 2001	**2001** - 2002
Daily gain (annual increase)	744 -	762 (+18 g)	778 (+16 g)	786 (+8 g)	798 (+12 g)	817 (+19 g)	824 (+7 g)
Mortality	3.0	3.2	3.2	3.4	3.6	3.4	3.5

See graphs in figure 1 below.
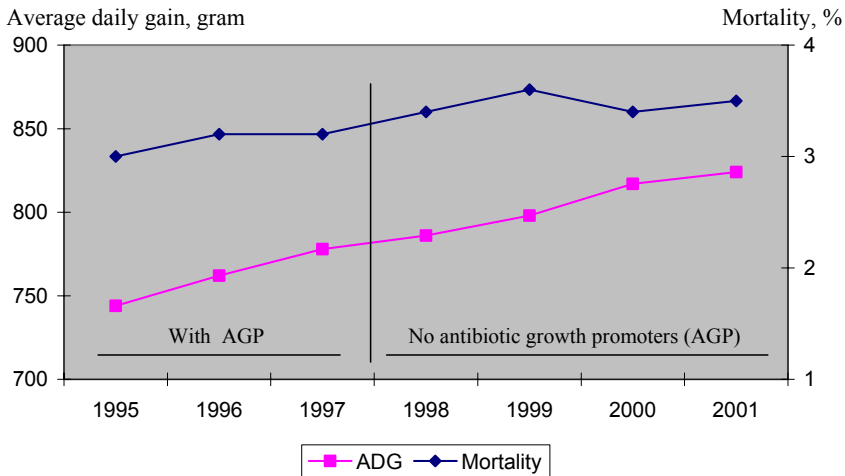
Figure 1. National average for production efficiency control – *finishers*. (See data in table 2)

Table 3. National average for production efficiency control – weaners

April - April	With AGP				Reduced AGP	No AGP	
	1995 - 1996	**1996** - 1997	**1997** - 1998	**1998** - 1999	**1999** - 2000	**2000** - 2001	**2001** - 2002
Daily gain	422	420	419	427	407 (-20 g)	411 (+ 4 g)	415 (+ 4 g)
Mortality	2.7	2.8	2.9	2.9	3.6 (+ 0.7)	3.5 (-0.1)	3.5 (0)
Age at 30 kg	82.6	82.6	82.8	82.9	85.3 (+ 2.4)	85.5 (+ 0.2)	85.5 (0)

See graphs in figure 2 below.
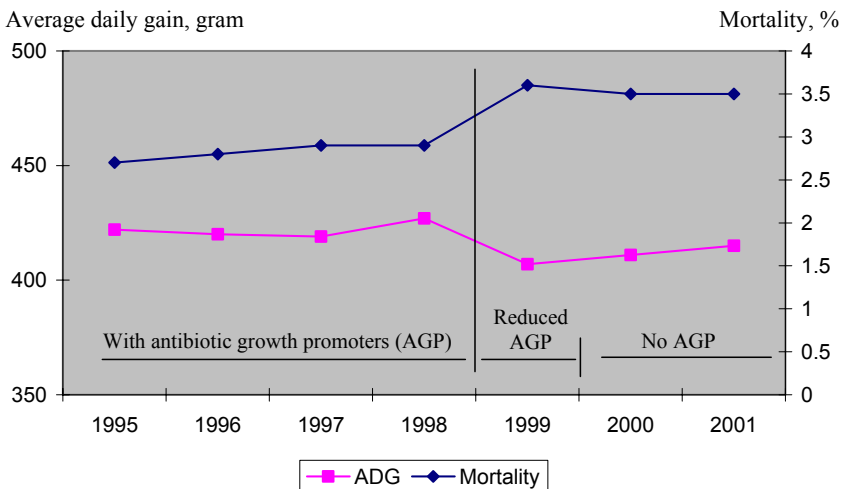
Figure 2. National average for production efficiency control – *weaners*. (See data in table 3)

The effect on the consumption of medicine

Denmark has some of the world's strictest legislation on medicine. This is one of the reasons why Denmark only uses approx. 1% of the EU consumption of medicine for pigs even though we produce 10% of the pigs in the EU.

A change in legislation in 1995 prohibited vets from profiting on sales of medicine. This resulted in a halving of the consumption of medicine in pig production from 90 tonnes in 1994 to 48 tonnes in 1996.

Changes were made in nutrition and management in order to fight the increase in outbreaks of diarrhoea after removing AGPs from the feed. Nevertheless, many herds find it necessary to apply medicine for treatment of diarrhoea in weaners and young stock. Figure 3 below illustrates the influence of this on the present consumption of antibiotics.

While the consumption of AGPs has decreased from 107 tonnes in 1997 to 0 tonnes 2001, the consumption of antibiotics – primarily macro-lides and tetracyclines – for treatment of diseases has increased from 56 tonnes in 1997 to 94 tonnes in 2001. Overall, the consumption of antibiotics was nearly halved in five years, but both politicians and consumers put great attention to the increasing use of medicine.

Due to the increased focus on the consumption of medicine and development in resistance, great efforts in both herds and on the research and development area are put into improving management and nutrition so that the problems may be reduced without using AGPs or increase the usage of medicine.

Conclusion

The removal of antibiotic growth promoters from the feed in Denmark has only had significant consequences for the weaner production (7-30 kg) in the form of increased mortality and reduced gain. These consequences are also reflected in the fact that the consumption of therapeutic antibiotics has increased.
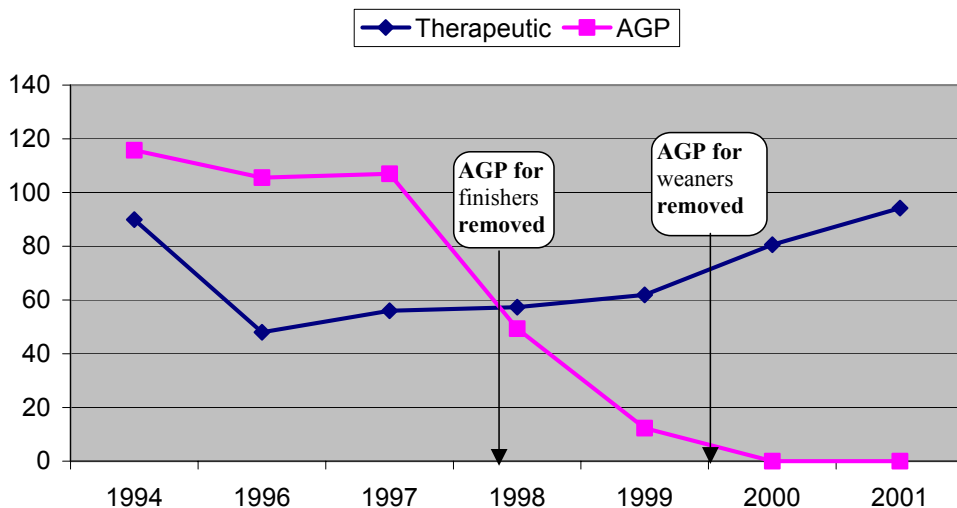
Figure 3. Consumption of antibiotics (therapeutic and AGP) in animal production in Denmark

9. Surveillance experiences from DANMAP and VetStat
- usage of antimicrobials in animals

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Abstract

For many years, in Denmark access to antimicrobials for therapy has been comparatively restrictive. This means that over the counter (OTC) sales of antimicrobials for systemic therapy are not permitted. This applies to antimicrobials for use in humans as well as in animals. Antimicrobials are available only through pharmacies (which must be licensed), irrespective of whether it is for use by an animal owner or for use in veterinary practice.

Consequently, the distribution pyramid is simple and transparent and lends itself to inexpensive and reliable monitoring of veterinary medicinal usage.

Data is available on veterinary antimicrobial usage since 1986. For the years 1986 to 1994, sales statistics have been collated bi-annually by the Danish Pharmacy Association and from 1996 to 2001 by the Danish Medical Agency. In both cases, usage in amount of active ingredient was available, however, usage in individual target animal species could only be 'guesstimated'. Since 2001, VetStat has provided prescription-based monitoring of usage in individual farms, including information about target animal species, age group of animal, reason for prescribing and other variables.

Data on usage of antimicrobial growth promoters and coccidiostats has been collected by the Danish Plant Directorate since 1990. The data have included information on the amount of active ingredient, but not routinely on target animal species which may, however, in some cases be estimated.

Since 1996 the DANMAP reports have published annual statistics and analyses of usage of antimicrobial therapeutics, antimicrobial growth promoters and coccidiostats.

Analysis of the data shows that total usage of therapeutics has varied very considerably, between highs of 90 and 94 tons of active compound in 1994 and 2001, respectively, and a low of 48 tons in 1996. The change in usage between 1994 and 1996 was not associated with any apparent change in animal health or with changes in use of antimicrobial growth promoters. The discontinued use of antimicrobial growth promoters has resulted in a significant decrease in total amount of antimicrobials used. There seems to be no straightforward association between the amounts used for therapy and for growth promotion.

Introduction

In Denmark, access to antimicrobials for therapy is comparatively restrictive. This means that over the counter (OTC) sales of antimicrobials for systemic therapy are not permitted. This applies to antimicrobials for use in humans as well as in animals. Antimicrobials are – with one exception only – available only on prescription through pharmacies (currently 283 in number, which must be licensed), irrespective of whether it is for use by an animal owner or for use in veterinary practice. The exception is antimicrobials that have special approval for use in feed medication. Such antimicrobials may be obtained by feed mills holding the appropriate license directly from medical wholesalers or importers, however, the mills will only sell medicated feed to a farmer on the basis of a written order, issued by a veterinarian.

In other words, there is no sale directly from pharmaceutical wholesalers or importers to veterinarians. The distribution pyramid is simple and transparent and lends itself to inexpensive and reliable monitoring of veterinary medicinal usage.

In 1995 the legal basis for sale of prescription medicines to farmers was changed. The supply of medicinal stocks to a farm for more than 5 day's use now required the signing of a standard contract by a farmer and his veterinary practitioner for animal health advisory service. These contracts stipulate monthly visits by the veterinarian, on the basis of which drugs to treat specific herd health problems during the following 35-day period can be sold or prescribed. Within this framework, the decision about when to treat as well as the extent of treatment is made by the farmer. In practise, however, the farmer will also use medicines supplied under the agreement to treat sporadic cases of acute illness, even when they are not part of the herd diagnosis made by the veterinarian.

Collection of usage data

Therapeutics

Prior to marketing, all veterinary medicines must given a product code, issued by the Danish Medical Agency. The code is a unique identifier which designates the type, formulation, and strength of the drug and the size of the pack, in addition to other information.

In 1994, veterinary antimicrobial usage data were collated retrospectively by the Danish Pharmacy Association (DPA) at bi-annual intervals from 1986 onwards. They were based on reports by all Danish pharmacies of the number of items sold for each product code. In 1996, the Danish Medical Agency (DMA) started collating veterinary usage statistics. In contrast to data from the DPA, the official statistics were based on annual returns by importers and manufacturers of veterinary medicines of the number of items sold for each product code and represents sales to pharmaceutical wholesalers, rather than sales to the end-user. In both cases - as most of the veterinary antimicrobials have multi-species claims – information about usage in individual animal species was not available.

During 2000, the VetStat usage monitoring programme was launched. In addition to the identity and quantity of medicine sold, the programme records the target animal species, the age group and the disease category as well as the identity of the farm where the animals are located and the identity of the prescriber. The information is extracted automatically during electronic processing of prescriptions at the pharmacy and reported to the register at monthly intervals. Veterinarians in large animal practise are required to provide the same type of information relating to their usage of prescription medicines in production animals. Input into VetStat is shown in Figure 1.
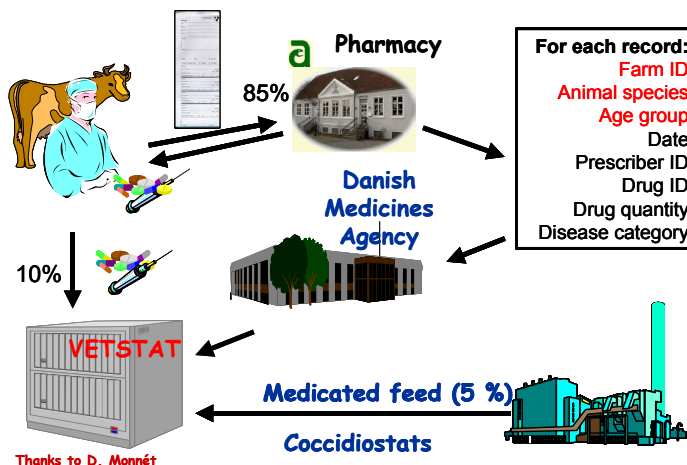
Figure 1. Data flow into VetStat

Feed mills with a license to manufacture and sell medicated feed are required to report the sales to VetStat, providing the information about farm identity and target species as for other therapeutics.

As VetStat records information about target animal species and age group it is possible to report usage in terms of animal daily dosages (ADD), permitting comparison of usage of antimicrobials of unequal potency. Farmers are mainly prescribed medicines following a monthly visit by a veterinarian to treat specific herd health problems that were identified. While there is little uncertainty that VetStat records correctly the farm where the medicine will be used, the farmer may use some of the medicines to treat sporadic cases of disease not diagnosed by the veterinarian at his visit. Therefore, information in VetStat about age group, disease category and possibly animal species may be associated with some inaccuracy.

By law, all treatments of mastitis in dairy cattle have to be initiated by a veterinarian. Therefore, in cattle virtually all antimicrobials are either used by the veterinarian personally, or sold by the veterinarian to the farmer for completion of a course of treatment. Information in VetStat about farm ID and target animals species of this part of usage became reliable only during 2002.

Feed additives
Usage of feed additives (antimicrobial growth promoters and coccidiostats) has been monitored by the Danish Plant Directorate between 1990 and 2001. Data pre-1990 are not available. The basis has been 6-monthly returns by feed manufacturers licensed to produce premixes containing the EU approved additives. The statistics have represented the quantities of active compound sold. As some of the additives have multi-species claims, information about use in individual animal species has not been available on a routine basis. Sales of feed containing coccidiostats or growth promoters is now monitored through VetStat.

Distribution of usage by animal species
With the implementation of VetStat, data on the distribution of usage by animal species became available from 2001. Table 1 shows distribution by main animal species, excluding cattle, according to information received from pharmacies and feed mills. As most antimicrobials in cattle are used by the veterinarian personally – or sold by the veterinarian for completion of a treatment course as described above – cattle usage is included under the heading 'Use in practise', representing pharmacy sales to veterinary practices. Only 1.6 % of total antimicrobial usage is sold directly from pharmacy to cattle farmers on the basis of veterinary prescriptions.

Table 1. Distribution of antimicrobial usage by animal species in 2001. Distribution is based on information from pharmacies and feed mills (for medicated feed). The quantities sold for use in practice is used mainly in cattle. Data: VetStat

	Kg	% of total
Pigs	71,247	74
Poultry	329	0,3
Aquaculture	3,056	3
Companion animals	455	0,5
Production animals, species not given	3,360	3
Use in practice	15,253	16
Other species	2,536	3
Total	6,235	100

Medicated feed is used almost exclusively in pigs (1763 kgs in 2001) and in aquaculture (2883 kgs). Less than 1 kg active compound (of a macrolide) was used in poultry and none in other species.

Trends in usage
Table 2 shows usage of therapeutics by antimicrobial group and Figure 2 the overall trends in all antimicrobial usage in animals.

Table 2. Usage of veterinary therapeutics by antimicrobial group. Data: DANMAP 2000

Compound	1986	1988	1990	1992	1994	1996	1998	2000
Tetracyclines	3,800	3,600	9,300	22,000	36,500	12,900	12,100	24,000
Penicillins	3,700	3,800	5,000	6,700	9,400	7,200	14,300	15,100
Semisyn. pen. etc.	850	1,000	1,200	2,500	4,400	5,800	6,700	7,300
Sulfonamides + trimethoprim [a]	2,500	2,200	3,800	7,900	9,500	4,800	7,700	7,000
Sulfonamides	22,300	24,200	8,700	5,900	5,600	2,100	1,000	1,000
Macrolides + lincosamides [b]	10,100	9,300	10,900	12,900	11,400	7,600	7,100	15,600
Aminoglycosides	7,800	7,400	7,700	8,500	8,600	7,100	7,800	10,400
Others [a]	13,800	6,900	6,700	6,800	4,400	600	650	300
Total	64,800	58,400	53,400	73,200	89,900	48,000	57,300	80,700

[a] Does not include consumption in aquaculture
[b] The macrolides include: spiramycin, tylosin, lincomycin and tiamulin

Examination of Table 2 shows that the use of sulfonamides (mainly sulfadimidine usage in pigs) declined markedly between 1988 and 1990, following the discovery that it was recirculated in the pens, leading to violation of residue levels. The decline in sulfonamide usage was not entirely reflected in overall usage, as usage of tetracycline increased by 160% during the same period. The following 4-year period saw a remarkable increase in the usage of oral tetracyclines, whereas, by comparison, there was little change in usage of other antibiotic groups. The significant decline in the group 'Others' in Table 2 from 1986 to 1988 was caused by the banning in 1987 of dimetridazole, which was used in significant amounts in pig production.

As is apparent from Figure 2, total usage declined by almost 50% between 1994 and 1996. This was a result of legislative changes implemented in 1995 with no apparent relation to changes in the health of the national pig herd. Following this, we saw a slow increase in usage until 1999, when the rate of increase accelerated, this time driven again by higher usage of tetracyclines as well as macrolides and lincosamides (mainly the former). As will be clear from Figure 3, the increased use in recent years has mainly been in compounds used for water or feed medication.

In contrast, usage of antimicrobial growth promoters has been less variable (Figure 2). Only antimicrobials approved by the EU for use as feed additives (mainly tylosin, avoparcin, olaquindox and bacitracin) have been used for growth promotion (see DANMAP 2000 for further details). Usage for growth promotion increased at the time that use of therapeutics went up in the early 90's and was almost unaffected by the reduction in therapeutic use from 1994 to 96. Usage of antimicrobial growth promoters fell drastically in 1998 with the industry decision to discontinue use in broilers and in pigs over 30 kg and in 1999 when use in weaner pigs was phased out during the second half of that year. This had little effect on the quantities of antimicrobial used for therapy – usage of therapeutics, however, went up in 2000 and 2001.

Discussion and conclusion

Reliable statistics on usage of antimicrobial therapeutics have been collected since 1986 and on antimicrobial growth promoters since 1990. Usage of antimicrobials for therapy has varied considerably from year to year, without any apparent relation to changes in the health status of the national pig herd. There is also no apparent relationship between amounts of antimicrobials used for therapy and for growth promotion. Comparing total usage of antimicrobials in 1994 (the year before the first intervention against growth promoters) with total usage in 2000 (the first year without growth promoters) has seen a reduction from 205 tonnes to 80.7 tonnes or 60.6%.
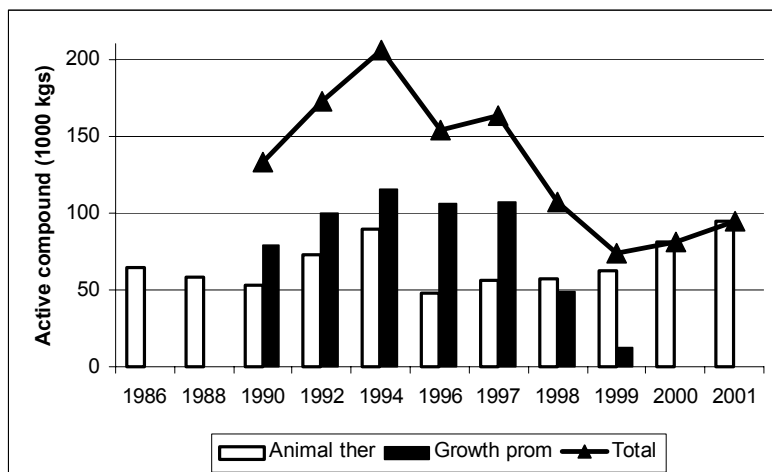
Figure 2. Trends in usage of antimicrobials for therapy and for growth promotion in Denmark. Animal ther: usage for treatment of disease; Growth prom: usage for growth promotion. Data: DANMAP
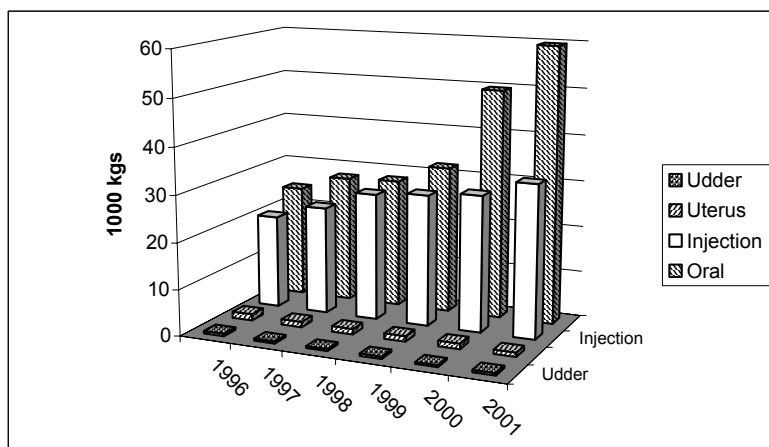
Figure 3. Trends in usage of therapeutics by route of application. Data: DANMAP

Okholm et al. (Danish Veterinary Journal, 2003, in press) studied usage of antimicrobial therapeutics in 96 Danish pig herds. Among a number of risk factors for high usage was, paradoxically, being a high health herd (Specific Pathogen Free herd, relative risk=2.9). Given that in pig herds the decision to treat is made by the farmer and may be based on less than rigid diagnostic criteria, and the fact that usage patterns in pigs have significant impact on overall usage, this may explain the variation over time in us-

age of therapeutics, and also the absence of apparent association between usage and major, widespread health problems in the Danish pig production.

References

DANMAP. DANMAP 2000 - Consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food ani-

mals, foods and humans in Denmark. ISSN 1600-2032.

Nielsen, E.O., Hassing, A., Wachmann, H., Bækbo, P., Pedersen, H.H. og Nielsen, J. P., 2002. Medicinforbrug i dansk slagtesvineproduktion. Risikofaktorer forbundet med høj frekvens af antibiotikabehandlinger. Dansk Veterinærtidskrift, 85, nr. 24, 6-13.

10. Consequences of termination of AGP use for pig health and usage of antimicrobials for therapy and prophylaxis

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Abstract

This paper reports the completion of a research project conducted by the Danish Veterinary Institute in the years 1998 – 2000. The purpose was to investigate the consequences of termination of AGP usage on animal welfare, drug usage, production/productivity data and antimicrobial resistance at a large number of farms. The data collection procedures and data material are described and the treatment-indication incidences and incidences of antimicrobial usage, standardised to Animal Daily Dosage, among post-weaning pigs are presented. A notable consequence of the terminated usage is a marked increase in diarrhoea-treatment incidences and concurrent increase in the usage indices for antibiotic preparations commonly used for diarrhoea treatment. Among weaner pigs, these incidences remain at this new high level one year after the terminated usage of AGP's, whereas the situation among grower/finisher pigs returns to the level before the terminated usage.

Introduction

Antimicrobials are used in food animal production to ensure a healthy production economy and animal welfare. Unfortunately, the consumption can lead to development of resistant bacteria. This fact constitutes a matter of concern, because it may contribute to antimicrobial resistance problems in both human and veterinary medicine. This concern has been the offset for research projects into resistance development in conjunction with growth promoting low-dosage administrations of the same compounds we would like to keep effective, when used in therapeutically dosage regimes to fight infections in humans. The produced evidence was enough to lead the EU countries to a ban of several antimicrobial compounds for growth promoting purposes. In Denmark the pork industry voluntarily offered to abandon the use of growth promoting antimicrobials (AGP's) in feed to slaughter pigs, 1998 and in weaners feed by the end of the year 1999.

The aim of a research project at the Danish Institute for Food and Veterinary Research (DFVF) was to investigate the consequences of ceased use of AGP's on animal welfare, drug usage, production/productivity data and antimicrobial resistance at a large number of farms. The project consisted of two parts, a cross-sectional study including all farms and a longitudinal study, involving only a limited number of farms, where groups of pigs were sampled frequently for antimicrobial resistance testing. The results from the longitudinal study are reported by another group of researchers, and will not be dealt with at this symposium. The outcome of the antimicrobial resistance investigation in the cross-sectional study is presented in another contribution to this symposium by Håkan Vigre, from the DFVF. Current paper describes the consequences on animal welfare and consumption of antimicrobials for therapeutic usage in the herds participating in the cross-sectional study.

Materials and methods

The collection of sufficient and valid data over a prolonged period of time was crucial to the success of the project. It was decided to work with a selected set of farms appointed by a selected set of vet practices. The course of any random selection procedure was thought to end up in an equally or otherwise selected material due failure by farmers or veterinarians to accommodate the high demands set for the data material.

Sixteen specialized swine practitioners were selected and asked to propose in all 150 farrow-tofinisher farm-units for project participation. The veterinarians were selected on the basis of

former research participation, personal acquaintance and geographic distribution of practice area. Their criteria for selection of the farm-units were thorough written recording of treatments and production-/productivity data and present or recent usage of AGP in weaners feed.

The vet-practitioners were paid their expenses and time consumption in the herds. They were offered a preset sum for participating and a sum per farm they enlisted in the project. The farmers were offered opportunity to submit samples/carcasses for further diagnosis and resistance testing free of charge within a preset but somewhat negotiable limit.

A written questionnaire of 42-questions was forwarded to the farm-veterinarian along with a manual elaborating on the interpretation of and background for some of the questions that could be subjected to misinterpretation. The purpose of the questionnaire was to record farmdata on management and feeding with an emphasis on AGP-usage. A draft questionnaire was tested by researchers and farm veterinarians in a couple of herds. This to ensure the comprehensibility of questions and in order to produce a questionnaire manual to the veterinarian. The pre-test resulted in only minor revisions of the questionnaire. In general the farm vet would administer the questionnaire and forward it to the Danish Veterinary Institute. One vet practice preferred to leave the questionnaire to be filled out by the farmer himself and a few questionnaires were undertaken by the researchers.

Included in the questionnaire was a statement the farmer could sign in order to allow free access for researchers to contact his local consultant office to obtain production data and the feedmills to obtain exact information of the composition of the feed. The Danish Bacon and Meat Council, National Committee for Pig Production produced the larger part of productivity/production data of the farms.

The pig producers were asked to record all antimicrobial treatments in the herd early year 1999 until September 1'st, year 2000. Please refer to Table 1-3 for farmer recordings per treatment.

Table 1. Age groups

Age group
Adults
Suckling piglets
Weaners
Growers
Finishers

Table 2. Indication for drug usage

Indication for drug usage
Diarrhoea
Respiratory disorder
Arthritis
MMA
Vaginal discharge after service
CNS disturbances
Skin disorders
Tale biting
Runting (unthriving)
Other

Table 3. Additional information per usage

Additional information
Trade name of drug preparation
Date of usage
Number of animals treated
Total amount of drug used
Initial usage/consecutive usage

The route of administration and concentration of the drug preparation were often obvious, given the commercial name, but had to be verified by phone in some instances. Prefabricated recording-schemes were forwarded to the vet-practices for photocopying and distribution among farmers. A number of farmers preferred to use their own recording schemes. This was accepted as long as there were no discrepancies in recording detail between the standard scheme and the farmers own forms. Through their vets the farmers were instructed to record the usage of antimicrobials but a majority found it easier to instruct their employees to report all drug usage including e.g. oxytocin to sows. A minority started out enthusiastically recording all vaccinations as well, but they soon gave up on this initiative.

The medicine recording schemes were collected at the farms by the vets at their regularly monthly visits and forwarded to the DFVF. A relational database application was established on an Oracle®-platform and data was entered into the application by a secretary and by vet students under close supervision by the author. Whenever the forms could be subjected to mis-interpretation the vet or the farmer was contacted by phone.

Since some degree of unintentional underreporting by the farmers on their drug usage was expected, a second point of data collection, the vet practice, was established. At each regular visit the farmer and his veterinarian are obliged to perform written account of the drug usage in crude amounts of given drug preparations to specific age groups since last visit. On this basis new prescriptions are issued, unused drugs are taken back or re-prescribed. These account forms were collected along with all written prescriptions one year before and one year after the individual farms stopped using AGP in weaners feed. Additional collected written information included treatment-regime hand-outs and visit reports. The data from the periodic account forms were also entered into the Oracle® application.

Calculations of treatment incidence
The farmer-recorded treatments were used when calculating treatment incidences. The frequencies of different indications for treatments within weaners and grower/finishers, respectively, were measured as the number of treatments a day per number of animals at risk a day – the treatment-incidence. The unit treatment indications per pig month was preferred for this presentation, one pig month being 30 pig days. The date specific indications for treatment incidence were summarised over 30-days periods for each farm, where the 30-days periods were related to the date were the farm stopped administering AGP's in weaners feed. If a farm had no recorded treatments during a particular 30-days period, this was assumed to be a result of missing recordings, and the farm was not included when calculating the average treatment-incidence in the farms of that particular 30-days period.

Calculation of antibiotic group-specific usage incidence
The monthly account-forms were used when calculating antibiotic usage incidences. To be able to compare the amounts of used antibiotics from different generic groups, the amount of drugs were standardised to doses using the principle of Defined Daily Dose (DDD) in human medicine. For any drug formulation an Animal Daily Dosage (ADD) was defined as the average daily dose as recommended by the drug company used for the major indication in weaners with a standard weight of 15 kilograms (kg), and in growing/finishing pigs with a standard weight of 50 kg. It can be interpreted as the antibiotic usage-intensity within the agegroup. If a farm had no recordings of antibiotic usage it was excluded from the calculations at that particular 30 day period.

The denominators used in the incidence-calculations were the number of weaners and grower/finishers housed, as stated in the questionnaire by the farmer.

Results
For this presentation of the study, it was decided to present data from 120 farms.

Profile of farms in this subset of study
The combined number of gilts and sows at the farms ranged between 34 and 725 (1st quartile: 127; median: 195; 3rd quartile 288). The number of weaners ranged between 75 and 2500 (1st quartile: 420; median: 700; 3rd quartile 1262) and the number of growers/finishers ranged from 45 to 4145 (1st quartile: 410; median: 700; 3rd quartile 1190). It should be mentioned, that some farms didn't raise all weaners, but sold off quite a few.

The date where AGP's no longer was found in rations to weaners and growers/finishers (AGP-Stop) could be determined within a range of 14 days. The farmers information from the questionnaires was validated through telephone interviews with the staff at the feedmills. For weaners this date ranged from the July 1'st, 1996 to February 25'th, 2000. 25% of the farms stopped using AGP before March 31'st, 1999,

50% of the farms before August 1'st, 1999 and 75% of the farms before the September 1'st, 1999.

For grower/finishing pigs the AGP-Stop-date ranged from July 1'st, 1994 to September 1'st, 1999. 50% of the farms stopped using AGP before January 1'st, 1997 and 75% before March 1'st, 1998.

Farmers registrations of indications for antibiotic usage

The farmers registrations of indications for treatments are displayed in Figure 1 and Figure 2. A marked increase in treatment incidence of diarrhoea among weaners after ceased use of AGP is obvious. The incidence remains high a year after the AGP-stop consistent with reports from farmers and veterinarians of severe difficulties controlling post weaning diarrhoea and later the infection with *Lawsonia intracellularis* in the grower pigs. The treatment incidence of diarrhoea among grower/finisher pigs likewise has marked increase after AGP stop but seems to reach its highest level after 6 months and return to the level before the AGP stop after a year. This return to normal could indicate better control of *Lawsonia* infection at the farms.

As mentioned earlier the dosage incidences are presented on the basis of another dataset than the treatment incidences. Nevertheless, due to the direct coherence between treatment and the consumption of antibiotics, a similar course is expected and found within both age groups. When observing the distribution between the different antibiotic compounds, the increase in the usage of macrolides (mainly tylosin) toweaners is remarkable. It might indicate a substitution of therapeutic prescribed tylosin over growth promoting tylosin.
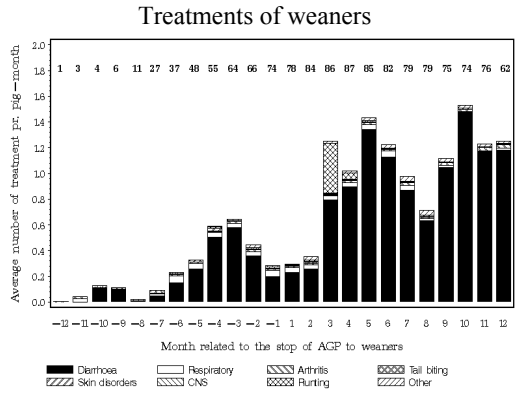
Figure 1. The relationship between the average frequency of indications for treatments among weaners per pig-month at risk and months before and after the ceased usage of AGP in the feed to weaners. At the top is listed the number of farms contributing data per month marker
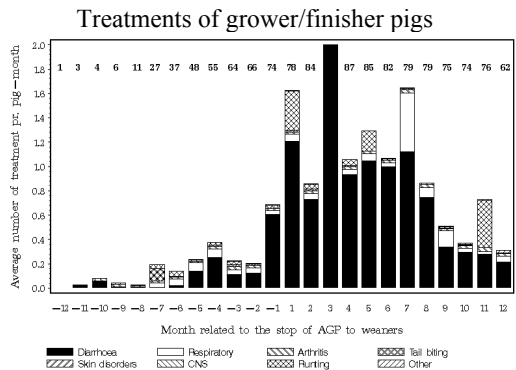
Figure 2. The relationship between the average frequency of indications for treatments among grower/finishers per pig-month at risk and the months before and after ceased usage of AGP in the feed to weaners. At the top is listed the number of farms contributing data per month marker

Animal daily dosage to weaners
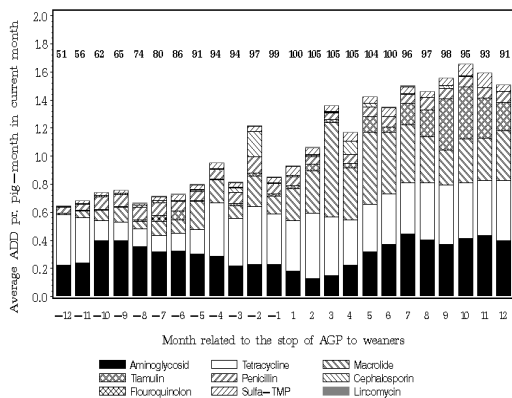
Figure 3. The relationship between the average ADD consumption to weaners per pig month at risk and the monthly time interval from the ceased usage of AGP in the feed to weaners. The ADD has been calculated using the manufacturers recommended dosage and if given as an interval, the mean dosage. At the top is listed the number of farms contributing data per month marker

Animal daily dosage to grower/finisher pigs
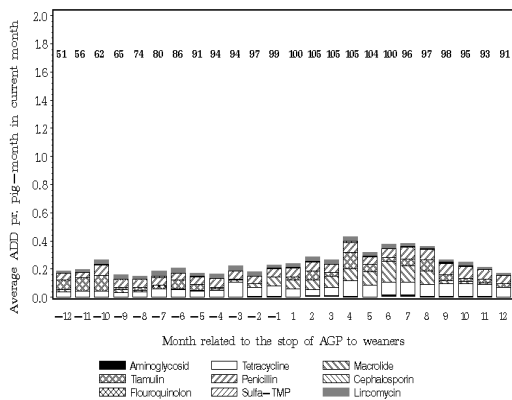
Figure 4. The relationship between the average ADD consumption to grower/finisher pigs per pig month at risk and the monthly time interval from the ceased usage of AGP in the feed to weaners. The ADD has been calculated using the manufacturers recommended dosage and if given as an interval, the mean dosage. At the top is listed the number of farms contributing data per month marker

Discussion

The study group of veterinarians and farms were highly selected in order to obtain valid data over a prolonged period of time. Nevertheless, the somewhat lacking tradition of *systematic* recording of disease, treatments, occurrences etc. in the herds, is obvious. Although mandatory, the recording of antimicrobial usage are not used for a purpose and therefore not given special attention, a 'catch22' situation. The mere size and complexity of the datasets yields though, a good basis for epidemiological analyses of the effects of terminating AGP usage in Denmark and solid 'baseline'-knowledge of treatment patterns at pig farms.

Given the increase in the treatment incidences is a consequence of more cases of disease and not altogether precautionary measures, the findings are consistent with reports from farmers and veterinarians of severe, lasting problems with containment of diarrhoea in the post weaning period. The data material would though benefit from corrections for seasonal variations in connection with clustering of AGP termination date. A range of other contributing factors to the diarrhoea incidence in the post weaning pigs can be thought of. These includes reduced supplements of zinc oxide, increased litter size, low pork prices etc. and should be taken into account before concluding on the consequences of the ban.

A ban of AGP usage should not be implemented overnight, but notice should be given way in advance in order for the farmers and their consultants to adjust the production means and facilities. An increase in the amounts of therapeutic usage seems inevitable based on the experiences in the Nordic countries. Guidelines for prudent therapeutic and prophylactic usage of antibiotics should be readily available and reliable.

11. Consequences of termination of AGP use for broiler health and usage of antimicrobials for therapy and prophylaxis

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Summary

For all broilers hatched after 15 February 1998 the Danish broiler industry adopted a voluntary ban on the use of antibiotic growth promoters (AGP).

In the first year without AGP Danpo slaughtered about 48 million broilers from about 1700 flocks. During this period necrotic enteritis (NE) was diagnosed in 25 flocks compared with 1-2 flocks only, when growth promoters were used. A total of about 24 kg amoxillin (active compound) was used to treat the outbreaks. Alternatively, without the voluntary stop, the Danpo flocks would have used approximately 1.500 kg active compound of antimicrobials for growth promotion.

For some months after the AGP-stop the growth rate seemed reduced, and the average mortality and condemnation rates increased in general in the Danish broiler production. After some months the average weight began to increase, and mortality and condemnations became like before. There has been no marked change in the health status in the Danish broilers in the last 4 years, and an increase in the use of therapeutic antibiotics has not been found. The total consumption of antibiotics has been reduced to some per cent of, what was used before the stop.

Without doubt the great efficiency in the Danish broiler production, the high hygiene level and veterinary status have been important key points in the success.

From an animal welfare, antibiotic politically and economically point of view it seems for the coming years only possible to produce broilers in Denmark, if it will still be allowed to use ionophores in the coccidiosis (and necrotic enteritis) prophylaxis.

Introduction

More than 130 million broilers are produced in Denmark every year. A typical Danish broiler house is about 1800 m^2 - central heated and well insulated. Feeding, temperature, humidity and light are automatically controlled. The "all in all out system" is used, and the houses are cleaned and disinfected thoroughly between every crop. A strong bio security system - tightened to make almost all flocks salmonella free - helps to avoid infections from the surroundings. The farmers are well educated and are continuously forced to optimize the production to be able to compete and survive in the broiler production. For all broilers hatched after 15 February 1998 the Danish broiler industry adopted a voluntary ban on the use of antibiotic growth promoters (AGP). Before that Danpo A/S, who slaughters and processes about 40 % of the Danish broilers, had done some trials and found that the consequences of an AGP-stop in the broiler feed seemed to be a higher feed conversion ratio, a minor growth rate reduction and a few mild attacks of necrotic enteritis (NE).

To compensate the flocks lower productivity, when the AGP was removed from the feed, the payment per Kilogram live weight had a rise of 0,07 Danish kroner. Furthermore Danpo formed a fund of 1 mill. Kroner, from which the Danpo-producers could get an economical compensation for the loses after NE. Meetings were arranged, where producers were told about symptoms and pathological signs of NE. To every broiler farm was sent a letter with this information plus addresses of pharmacies, where the veterinarian could order appropriate medicine day and night.

Results

During the first year without AGP Danpo slaughtered about 48 mill broilers from about

1700 flocks. During this period NE was diagnosed in 25 flocks compared with 1-2 flocks only, when growth promoters were used. A total of about 24 kg amoxillin (active compound) was used to treat the outbreaks. Alternatively, without the voluntary stop, the Danpo flocks would have used approximately 1.500 kg active compound of antimicrobials for growth promotion, assuming a mean inclusion rate in the feed of 10 ppm. There was paid less than 150.000 Kr in compensations for birds died or condemned as a consequence of NE and for veterinary expenses.

In Danpo we are quite sure we have been informed of at least the great majority, if not all of the NE cases by our broiler producers because of the information given and the possibility of economical compensation from the fund mentioned.

Among veterinarians in the broiler production it was feared that the clostridia level at some farms crop after crop should build up leading to outbreaks of NE or at least to a higher rate of liver condemnation. This has not been seen till now and sub clinical NE as described in some countries have had very little or no importance.

In general, in the spring and early summer 1998 the health status in the Danish broiler flocks was not quite as good as in the months before. As seen in the figures with data from the productivity database administrated by the Danish Poultry Council the mortality and condemnation rate increased slightly and the slaughter weight was as earlier mentioned also reduced in some months. The reason for this was obviously not only the exclusion of AGP in the feed. In that period avian leucosis was diagnosed in some parent flocks, and all broiler flocks were during one rotation vaccinated with an inter medium Gumboro disease vaccine, which in Denmark often reduces the growth rate and the litter quality.

Some months after the introduction of the voluntary ban the growth curve started to increase again, and this trend has continued during the past 4 years, as well as the mortality has decreased slightly and condemnation rates have fluctuated with a falling tendency.

As known it is only allowed to use antibiotics for therapy in broiler flocks in Denmark, when a veterinarian has made a diagnoses before he orders the medicine. Therefore prophylactic use of antibiotics is illegal.

Weight - 38 days

% dead + culled - total

month/year

% condemned

month/year
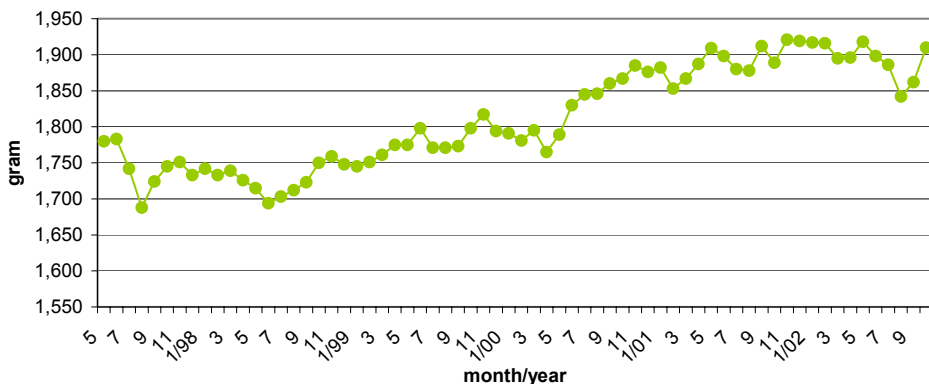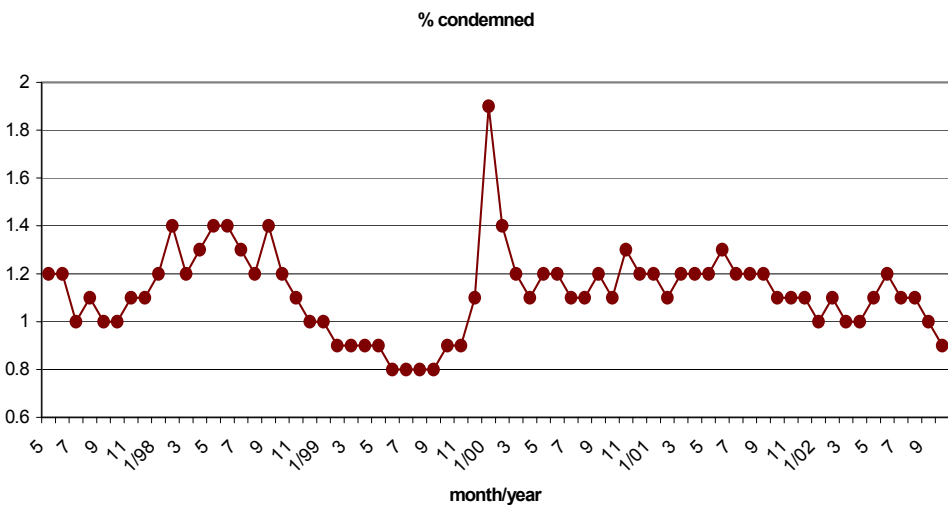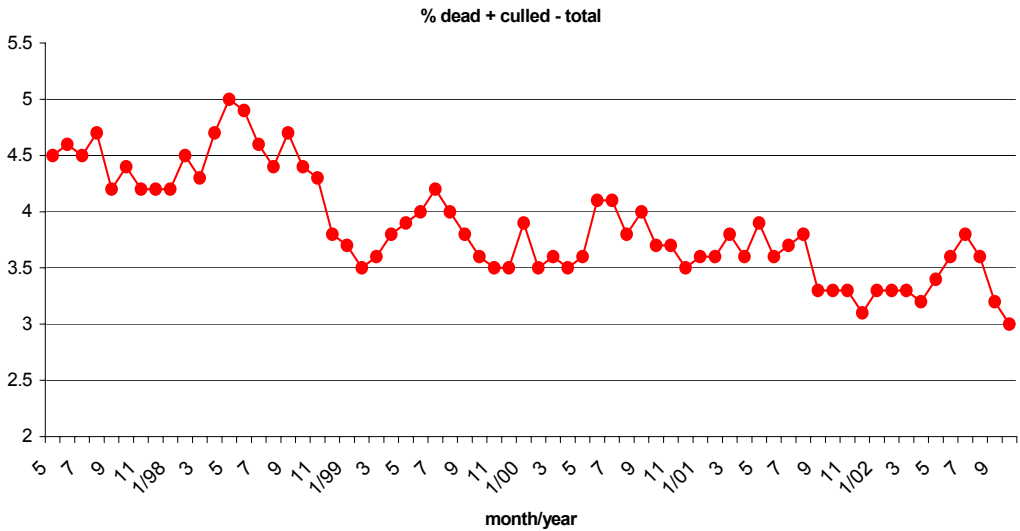
Source: The Danish Poultry Council

It is the general opinion among veterinarians working with poultry in Denmark that there has been no increase in the ordination of therapeutic antibiotics during the last more than 4½ years after the termination of AGP use in the broiler production. Vetstat under Danish Zoonosis Centre has since 1 January 2001 registered the consumption of therapeutic antibiotics in animals in Denmark inclusive in poultry for meat production (broilers, turkeys, gees, ducks and game birds). The use of active compound of penicillin with extended spectrum, which represent about 90% of the consumption in this group of poultry, and which is the drug of choice for NE, was 145 kg in 2001 and 82 kg in the first 6 month of 2002. This indicates that there has not been a marked increase in the treatment of broilers with antibiotics since the AGP-stop.

For many years the Danish broiler producers have learned the lesson to use ionophores in the feed during at least the first 18 – 20 days of the

chickens life to prevent NE. After the ban of AGP the ionophores have been the only - but effective - NE prophylaxis in broiler feed. Experiments with coccidiosis vaccines in Denmark and in other countries have unfortunately shown severe attacks of NE, when ionophores have been replaced by vaccines in the coccidiosis prophylaxis.

Conclusion

In the Danish broiler production it has been possible to terminate the use of AGP without marked consequences for the health and consumption of antibiotics for therapy. The number of flocks with acute NE and/or chronic hepatitis has became a little higher, than before the ban. For some months the growth rate seemed depressed and the average mortality and condemnation increased.

The total consumption of antimicrobials in Danish broiler flocks has been reduced to some per cent of the use before the ban.

Without doubt the great efficiency, the high hygiene level and veterinary status have been important key points in the success.

From an animal welfare, an antibiotic political and a financial point of view it seems only possible in the coming years to produce broilers in Denmark, if it will still be allowed to use ionophores in the coccidiosis (and necrotic enteritis) prophylaxis.

References

Emborg, H.D., Ersboll, A.K., Heuer, O.E. and Wegener, H.C. 2001. The effect of discontinuing the use of antimicrobial growth promoters on the productivity in the Danish broiler production. Prev. Vet. Med. 50, 53-70.

Kaldhusdal, M. 1998. Økende innsats for å forebygge nekrotiserende tarmbetennnelse. Dansk Erhvervsfjerkræ. 27, 62-63.

The Danish Poultry Council. Broiler Efficiency Control Databasis.

12. Sector and economy wide effects of terminating the use of antimicrobial growth promoters in Denmark[1]

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Abstract

In Denmark the poultry industry and the National Committee for Pig Production, together with the feedstuff industry, decided to voluntarily discontinue use of all Anti-microbial Growth Promoters (AGP). The poultry industry discontinued the use of AGP 15 February 1998 and the pig industry followed 1 March 1998 (for pigs over 35 kg) and 1 January 2000 (for pigs under 35 kg). To evaluate the effects of the discontinuance, data from both the poultry and pig industries were collected during a transition period for the removal of AGP from feedstuffs.

Utilizing these production data, this paper calculates economy-wide effects of the removal of AGP using the Agricultural Applied General Equilibrium (AAGE) model of the Danish economy.

The results show that the long-term effects are a moderate decline in the production and export of pig meat, and a positive indirect effect on other industries including poultry due to lower rental rates for primary factor inputs (land, labour and capital). Production of pig meat is projected to decline by 1.4%, and exports by 1.7%. In the case of poultry, production and exports increase by 0.4 and 0.5%. The overall implication is a small decline in real GDP of 0.03% (363 mill DKK at 1995 prices), and a consumption decline of 0.03% - equivalent to 45 DKK per capita per year.

Although the cost in terms of real GDP and consumption are small, such cost analyses could be compared with expected benefit of the removal of AGP. These benefits have not been a part of this analysis, but only if they are calculated or assumed to exceed the costs could AGP removal be said to be beneficial to society as a whole.

Introduction

In Denmark the poultry industry and the National Committee for Pig Production, together with the feedstuff industry, decided to voluntarily discontinue use of all Anti-microbial Growth Promoters (AGP). The poultry industry discontinued the use of AGP 15 February 1998 and the pig industry followed 1 March 1998 (for pigs over 35 kg) and 1 January 2000 (for pigs under 35 kg).

To evaluate the effects of the discontinuance, data from both the poultry and pig industries were collected during a transition period for the removal of AGP from feedstuffs. The objective of this paper is to utilize these production data to calculate the economy-wide effects of the removal of AGP, using an applied general equilibrium of the Danish economy.

Production data

The Danish Poultry Council investigated how the removal of AGP influenced broiler productivity in Denmark by analysing data from 6815 flocks during the period November 1995 to July 1999. It was found that broiler weight produced per square meter and percentage deaths were not affected but that the feed conversion ratio increased marginally (by about 1 %) (Emborg et al., 2001a).The National Committee for Pig Production has recorded, for many years, productivity data in a representative sample of Dan-

ish pig herds. Withdrawal of AGPs was found to have had no, or very limited, effect on finishers and growers. In the production of weaned pigs there were increased problems with post-weaning diarrhoea, a reduction in daily weight gain and increased post-weaning mortality (Callesen, 2000). Finn K. Udesen from the National Committee for Pigs, has estimated that these productivity loses incurred by removing AGP in the production of pigs has cost roughly 7.75 DDK per produced pig c.f. Table 1.

In Table 2 it can be seen that the total cost of pig keeping increases by roughly 1.0 % due to the abolishment of AGP when the increased cost of production (DKK 7.75 per produced pig) is compared to the total cost of pig keeping per sow in Denmark.

Due to the fact that the increased cost of production are best estimates made by Udesen, two sensitivity analyses are also undertaken where the cost of product is increased/decreased by 25 %.

In the case of poultry, the feed-conversion ratio increased by 0.016kg feed/kg broiler after the removal of AGP. In economic terms this amounts to 0.025 DKK/kg broiler using an average feed price of 1.55 DKK/kg. It has been estimate that the cost of adding AGP to broiler feeds is roughly 0.027 DKK/kg broiler wherefore the total cost of producing broilers in Denmark is not assumed to be affected by the AGP removal (Emborg et al., 2001b).

In the following the calculated percentage increases in costs of producing pigs (1.05 %) and poultry (0.0 %) are used to calculate the economy wide effects of removing AGP from feedstuffs in Denmark.

Table 1. Productivity reductions and cost per produced pig incurred by removing AGP

			DKK per pig produced
Excess mortality	0.6%	425 DKK/pig (20 kg)	2.55
Excess feeding days	1.6 days	1.10 DKK/day	1.75
Increased medication	25500 kg	value 53 mio. DKK	2.25
Increased workload	30 sec./pig	145 DKK/hour	1.20
Total cost			7.75

Source: Estimates made by Finn K. Udesen from the National Committee for Pigs

Table 2. Estimated total cost increase for pig keeping in Denmark, percent

	1997/98	Sensitivity analysis	
		-25%	+25%
Produced pigs per sow	20.0	20.0	20.0
Extra production cost per pig produced DKK..	7.75	5.81	9.69
Extra production cost per sow DKK	138	103	172
Total pig keeping cost per sow DKK	14832	14832	14832
Produced pigs per sow	1.05	0.78	1.31

Source: Economics of Agricultural Enterprises, Serie B. Economics of Agricultural Enterprises.
Danish Research Institute of Food Economics and own calculation

Model and scenario

There are five types of agents in the AAGE (**A**gricultural **A**pplied **G**eneral **E**quilibrium) model: industries; capital creators; households; governments and foreigners. The current database of the model identifies 68 industries producing 76 commodities (see appendix A). For each industry there is an associated capital creator. The capital creators each produce units of capital that are specific to the associated industry. There is a single representative household and a single government sector. Finally, there are foreigners, whose behaviour is summarised by export demand functions for Danish products, and by supply functions for imports to Denmark.

The nature of markets and prices

AAGE determines supplies and demands of commodities through the optimising behaviour of agents in competitive markets. Optimising behaviour also determines industries' demands for labour and capital.

The assumption of competitive markets implies equality between the producer price and the marginal cost in each industry. Demand is assumed to equal supply in all markets other than the labour market (where excess supply conditions can hold). The government intervenes in markets by imposing sales taxes on commodities. This places wedges between the prices paid by purchasers and prices received by the producers. The model recognises margin commodities (e.g. retail trade and freight) that are required for each market transaction (the movement of a commodity from the producer to the purchaser). The costs of the margins are included in purchasers' prices.

Demand for inputs to be used in the production of commodities

AAGE recognises two broad categories of inputs: intermediate inputs and primary factors. Firms in each industry are assumed to choose the mix of inputs, which minimises the costs of production for their level of output. They are constrained in their choice of inputs by nested production technologies (see appendix B). For the land-using industries (see appendix A), AAGE specifies nested substitutions between:

(a) capital, labour, energy and herbicides (CLEH);
(b) land, fertiliser and insecticides (LFI);
(c) CLEH and LFI (CLEHLFI); and
(d) CLEHLFI and an aggregate of remaining intermediate inputs

For non-land using industries substitution is allowed between capital, labour and energy (CLE) and between CLE and aggregate non-energy intermediate inputs.

Household demand

The representative household buys bundles of goods to maximise a utility function subject to a household expenditure constraint. Bundles are combinations of imported and domestic goods.

Demand for inputs to capital creation and the determination of investment

Capital creators for each industry combine inputs to form units of capital. In choosing these inputs they minimise costs, subject to technologies similar to that used for current production; the only difference being that they do not use primary factors. The use of primary factors in capital creation is recognised through inputs of the construction commodity.

Government demand for commodities

The government demands commodities. In AAGE, there are several ways of handling these demands, including: (i) endogenously, by a rule such as moving government expenditures with household consumption expenditure or with domestic absorption; (ii) endogenously, as an instrument which varies to accommodate an exogenously determined target such as a required level of government deficit; and (iii) exogenously. In this paper government demand changes follow household consumption expenditures.

Foreign demand (international exports)
Two categories of exports are defined: traditional, which are the main exported commodities; and non-traditional. Traditional export commodities face individual downward-sloping foreign demand curves. The commodity composition of aggregate non-traditional exports is treated as a Leontief aggregate. Total demand is related to the average price via a single downward-sloping foreign demand curve. Contrary to many conventional agricultural products, all organic products are assumed to be traditional export commodities.

Demand for foreign imports
For all industries, AAGE includes the standard Armington specification for imported and domestically produced inputs. This assumes that users of a given commodity regard the domestic and the imported varieties of this commodity as imperfect substitutes. The Armington assumption is also used in input demands for industry investment and in household demands for consumption.

Computing solutions for AAGE
AAGE is a system of non-linear equations. It is solved using GEMPACK, a suite of programs for implementing and solving economic models. A linear, differential version of the AAGE equation system is specified in syntax similar to ordinary algebra. GEMPACK then solves the system of non-linear equations as an Initial Value problem, using a standard method, such as Euler or midpoint. For details of the algorithms available in GEMPACK, see Harrison and Pearson (1996).

Scenarios and expected results
A baseline is constructed to introduce all ongoing policy developments and known shocks to the economy so as to ensure that the policy scenario is undertaken in an economy where all known developments and shocks are accounted for, with the exception of removing AGP. The Baseline takes the economy from the model's initial year (1995) to 2010, and the effects of removal of AGP are evaluated in the year 2010.

We construct the AGP scenarios as a change in the total factor productivity (TFP). This is because the model has no explicit treatment of AGPs. We use the calculated percentage increases in production costs (from Table 2) to reduce the TFP so that the unit cost of production increases by 1.05 % for pig production. Two sensitivity analyses are also undertaken where the cost per produced pig is increased/decreased by 25 % cf. table 2.

Expected results from the analysis
The removal of AGP increases the unit cost of pig production. A higher unit cost requires a higher product price if profits are to remain unchanged. Yet a higher product price invites lower demand. A decline in demand/production releases resources from the pig sector, which can then be used in other sectors of the economy. The increased supply of resources to other sectors in the economy lowers the price and required rent of these resources. A reduction in the required rental rates tends to favour those industries that are not affected by the removal of AGPs. As the production of pigs only accounts for a minor fraction of total national production, the effects on the rest of the economy are expected to be moderate. The expected negative impact on pig production is expected to lower the demand for cereal for feed purposes, exerting downward pressure on cereal prices. In turn, this is expected to benefit the cattle and poultry sectors that use cereals in this way. This should result in increased poultry production and a higher value of dairy quota (as the cattle sector is effectively constrained by the quota).

Results

This section presents results for production, exports and the macroeconomic performance of the calculated AGP scenario. The presentation focuses on the results for the primary agricultural and associated processing sectors[2].

Production and exports
The production of live and processed pigs falls by 1.4 %, cf. Table 3. This effect is due to the

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[2] A more thorough presentation of the Baseline scenario can be found in Jacobsen (2001).

AGP removal working as an increase in unit cost, which in the longer run requires higher product prices, lowering demand for the product. The increase in unit cost also affects the export possibilities for processed pig meat, which declines by 1.7 %. A large part of cereal production is used for feed purposes, and the reduced production of pigs also causes cereal production to decline by -0.1 %.

Table 3. Consequences of an AGP removal, percentage changes

	Production	Export
Agriculture:		
Cereal	-0.1	0.1
Oilseed	0.2	0.1
Potatoes	0.1	0.1
Sugar beet	0.0	
Roughage	0.0	
Cattle, live animals	0.0	
Milk	0.0	
Pig, live animals	-1.4	
Poultry and eggs	0.4	
Fur farming	0.1	
Horticulture	0.1	0.1
Manure	-0.6	
Processing:		
Cattle meat	0.0	0.1
Pig meat	-1.4	-1.7
Poultry meat	0.4	0.5
Dairy	0.0	0.1
Sugar refineries	0.0	0.1
Other (selected):		
Processed fruit and vegetables	0.0	0.1
Bread, grain mill and cakes	0.0	0.1
Bakery shops	0.0	0.0
Beverage and Tobacco	0.0	0.1
Agricultural services, forestry and fisheries	0.0	0.1
Textile, wood, paper and publishing	0.0	0.1
Basic chemicals	0.1	0.1
Construction incl. Supply	0.0	0.1
Metals products	0.1	0.1
Public services and utilities	0.0	0.1
Retail and wholesale margins	0.0	0.1
Private services	0.0	0.1

Even though the Baseline is not a subject of this paper it worth noting that the production of pigs is expected to grow by 30.5 % from 1995 to 2010 (Jacobsen 2001), but the removal of AGP will reduce this growth to 28.7 %.

The reduction in the production and processing of pigs leads to a lower demand for labour and new capital goods in these industries, resulting in a minor reduction In the wage rate and the price of new capital goods, cf. Table 4. This effect favours other industries not affected by the removal of the AGP´s since lower factor prices reduce unit costs causing production and exports to increase for these industries. Lower factor prices and lower price of cereals benefits poultry production, which is seen to increase by 0.4 % while the processing of poultry meats increases by 0.4 % and export volume increases by 0.5 %.

Macroeconomic results

The macroeconomic consequences of AGP removal are moderate. Real GDP falls by 0.03 % or 363 mill DKK at 1995 prices. This is the net result of the reduced production of pigs and cereals on the one hand, and the increased production in most other industries due to lower rental rates for primary factor inputs on the other hand.

Lower rental rates also affect real private and public consumption[3] falling by -0.03 %. This corresponds to a lower real value of private consumption of 45 DKK per capita per year.

The resulting reallocation of primary factor inputs results in an economic state where all factor input are a little less productive in the aggregate. All factors of production receive lower rental rates and the aggregate capital stock has somewhat declined, reflecting an economic state where production potential has decreased slightly.

Abolishing the use of AGP's also leads to a slightly lower (-1.37 %) price of agricultural land. The mechanism for this is the reduced demand for fodder reducing profitability in the cereals sector.

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[3] The two consumption categories are equalised in the so-called mode closure.

Table 4. Macroeconomic consequences of the AGP removal

	2010-Level[1] Billion 1995-DKK	AGP removal	
		Million DKK	Percent
Real GDP	1426.1	-363	-0.03
Real private consumption	694.7	-234	-0.03
Real public consumption	353.8	-119	-0.03
Real investments	253.1	-35	-0.01
Real stocks	39.3	0	0.00
Real exports	412.6	17	0.00
Real imports	319.0	-38	-0.01
Real capital stock			-0.02
GDP deflator			-0.03
Consumer price index			-0.02
Price of investment goods			-0.03
Terms of Trade			-0.00
Nominal wage rate			-0.06
Price of agricultural land			-1.37

[1] 1995-DKK in the year 2010

Sensitivity analysis
The results of the sensitivity analysis show that changes to pig production, real GDP and land prices vary with plus minus 25 % in accordance with the higher/lower estimated cost of removing AGP.[4] Therefore the results presented in this paper are sensitive to the initial estimation of increased cost due to the abolishment of AGP. The results of the sensitivity analysis are shown in appendix C.

Conclusion

This paper has analysed the economy wide implication of the unilateral Danish removal of Antimicrobial Growth Promoters in the production of pigs and poultry. The analysis shows that the long-term effects are a moderate decline in the production and export of pig meat, and positive indirect effects on other industries due to lower rental rates. Interestingly, positive indirect effects mostly impact the poultry sector, which also removed the AGPs from production. The overall implication is a small decrease in real GDP and consumption.

The decrease in the production of pig meat should be seen in the light of the baseline where pig production is expected to increase by 30.5 % over the 15 year period or 1.8 % per year on average. Removing AGPs from pig production reduces this growth in production to 28.7 % offsetting the ongoing expansion of the pig sector by approximately one year.

The sensitivity analysis undertaken in this paper show, that the results are sensitive to the initial estimation of the increased costs of abolishing AGP. Even though the cost in terms of real GDP and consumption are small, cost analysis such as the one presented could be compared with expected benefit of the removal of AGP. These benefits have not been a part of this analysis and only if the benefits are calculated or assumed to exceed the cost could such a removal be said to be beneficial to society as a whole.

Naturally, the results found should be evaluated in light of the assumptions employed. Compared with other, partial equilibrium, economic analysis the present analysis takes into account the economic linkages between the individual agricultural sectors and between the agricultural sectors and the industrial sectors, and consumer preference or willingness to pay. Furthermore,

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[4] Even though the model is non-linear the shock to the economy are so small that second round effects and non-linearity only plays a minor role to model results.

the analysis has taken into account the derived cost and price effects and the implications of explicitly representing the overall macroeconomic budgetary restrictions. The simulations have also been undertaken with a national AGE model assuming unilateral Danish policy initiatives, and it is assumed that the removal of the AGPs does not affect consumer preferences domestically or on the export markets for Danish pig and poultry meat.

References

Callesen, J., 2000. Vækstfremmerophørets effekt på produktiviteten i svineproduktionen. In Ophør med vækstfremmere: Sundhed, produktion, medicinforbrug og resistens i dansk svine- og slagtekyllingeproduktion. Intern rapport november 2000, nr. 136, from Danmarks JordbrugsForskning.

Emborg, H.-D., Ersbøll, A.K., Heuer, O.E. and Wegner, H.C., 2001a. The effect of discontinuing the use of antimicrobial growth promoters on the productivity in the Danish broiler production. Prev. Vet. Med. 50, 53-70.

Emborg, H.-D., Ersbøll, A.K., Heuer, O.E. and Wegner, H.C., 2001b. Effekten af ophør med brug af antibiotiske vækstfremmere på produktiviteten i den danske slagtekyllingeproduktion. Dansk Veterinærtidsskrift 2001, 84,22, 15/11, 6-10.

Harrison W.J. and Pearson, K.R., 1996. „Computing solutions for Large General Equilibrium Models Using GEMPACK", Computational Economics, Vol 9, 83-127.

Jacobsen, L.-B., 2001. Potentialet for økologisk jordbrug – Sektor- og samfundsøkonomiske beregninger. Rapport nr. 121. Statens Jordbrugs og Fiskeriøkonomiske Institut.

Appendix A
Table A.1 Industries and commodities in Organic-AAGE

		Industries				Commodities
*#	1-2	Cereal		*	1-2	Cereal
*#	3-4	Oil seeds		*	3-4	Oil seeds
*#	5-6	Potatoes		*	5-6	Potatoes
*#	7-8	Sugerbeets		*	7-8	Sugerbeets
*#	9-10	Roughage		*	9-10	Roughage
*	11-12	Meat cattle and milk producers		*	11-12	Meat cattle
*	13-14	Pigs		*	13-14	Milk
*	15-16	Poultry		*	15-16	Pigs
	17	Hunting and fur farming, etc.		*	17-18	Poultry
*#	18-19	Horticulture			19	Hunting and fur farming, etc.
	20	Agricultural services, etc.		*	20-21	Horticulture
	21	Forestry			22	Agricultural services, etc.
	22	Fishing			23	Forestry
	23	Extraction of coal, oil and gas			24	Fishing
*	24-25	Cattle-meat products			25	Extraction of coal, oil and gas
*	26-27	Pig-meat products		*	26-27	Cattle-meat products
*	28-29	Poultry-meat products		*	28-29	Pig-meat products
	30	Fish products		*	30-31	Poultry-meat products
*	31-32	Processed fruit and vegetables			32	Fish products
	33	Processed oils and fats		*	23-34	Processed fruit and vegetables
*	34-35	Dairy products			35	Processed oils and fats
*	36-37	Starch, chocolate products, etc.		*	36-37	Dairy products
*	38-39	Bread, grain mill and cakes		*	38-39	Starch, chocolate products, etc.
*	40-41	Bakery shops		*	40-41	Bread, grain mill and cakes
*	42-43	Sugar factories and refineries		*	42-43	Bakery shops
	44	Beverage production		*	44-45	Sugar factories and refineries
	45	Tobacco manufacture		*	46-47	Beverage production
	46	Textile, wearing apparel and leather			48	Tobacco manufacture
	47	Manufactured wood and glass products			49	Textile, wearing apparel and leather
	48	Paper products and publishing			50	Manufactured wood and glass products
	49	Oil refinery products			51	Paper products and publishing
	50	Basic chemicals			52	Oil refinery products
	51	Fertiliser			53	Basic chemicals
	52	Agricultural chemicals nec			54	Fertiliser
	53	Non-metallic building material			55	Agricultural chemicals nec
	54	Metal products			56	Non-metallic building material
	55	Machinery and non-transport equipment			57	Metal products
	56	Transport equipment			58	Machinery and non-transport equipment
	57	Electricity			59	Transport equipment
	58	Gas			60	Electricity
	59	Steam and hot water			61	Gas
	60	Construction			62	Steam and hot water
	61	Motor vehicles service			63	Construction
	62	Wholesale trade			64	Motor vehicles service
	63	Retail trade			65	Wholesale trade
	64	Freight transport			66	Retail trade
	65	Financial and property services			67	Freight transport
	66	Transport and communication services			68	Financial and property services
	67	Public services			69	Transport and communication services
	68	Dwelling ownership			70	Public services
					71	Dwelling ownership
					72	Coal imports
					73	Manure
					74	Fungicide
					75	Insecticides
					76	Herbicide

* Both conventional and organic product/production.   # Land using industries

Appendix B Nesting structure
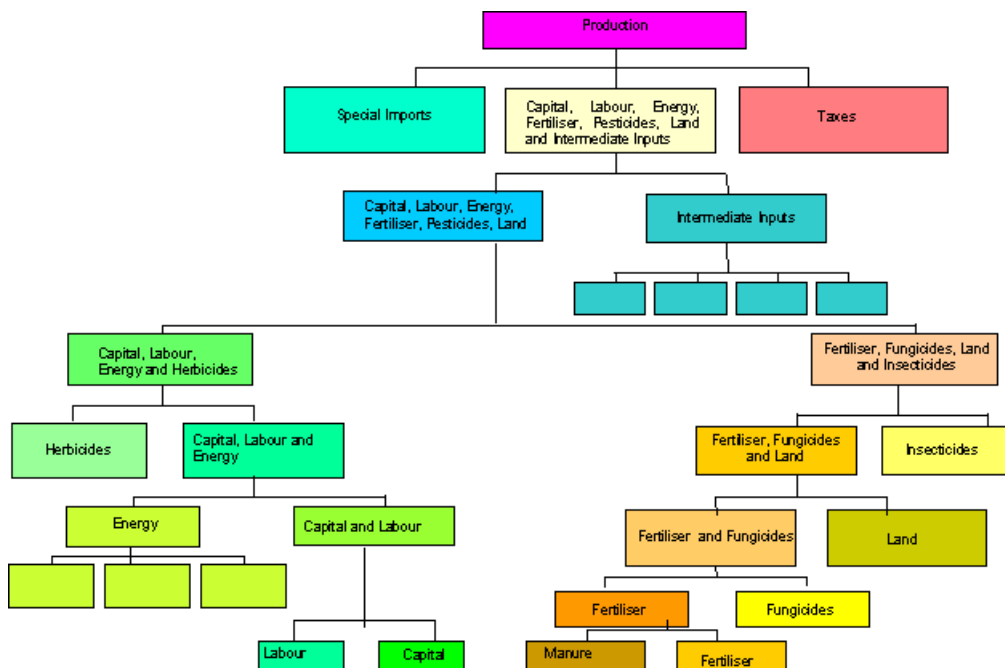
Appendix C, Result of sensitivity analysis.
Table C.1 Consequences of an AGP removal, percentage changes

	Production			Export		
	AGP -25%	**AGP**	AGP +25%	AGP -25%	**AGP**	AGP +25%
Agriculture:						
Cereal	-0.088	**-0.118**	-0.148	0.081	**0.109**	0.135
Oilseed	0.115	**0.155**	0.193	0.081	**0.109**	0.135
Potatoes	0.077	**0.104**	0.129	0.081	**0.109**	0.135
Sugar beet	0.000	**0.000**	0.000			
Roughage	0.015	**0.020**	0.025			
Cattle, live animals	0.006	**0.008**	0.010			
Milk	0.006	**0.008**	0.009			
Pig, live animals	-1.038	**-1.394**	-1.734			
Poultry and eggs	0.269	**0.362**	0.451			
Fur farming	0.064	**0.086**	0.107			
Horticulture	0.064	**0.086**	0.107	0.081	**0.109**	0.135
Manure	-0.481	**-0.646**	-0.804			
Processing:						
Cattle meat	0.008	**0.010**	0.013	0.081	**0.109**	0.135
Pig meat	-1.032	**-1.385**	-1.723	-1.299	**-1.743**	-2.169
Poultry meat	0.273	**0.367**	0.457	0.338	**0.454**	0.566
Dairy	0.009	**0.013**	0.016	0.081	**0.109**	0.135
Sugar refineries	0.010	**0.014**	0.017	0.081	**0.109**	0.135
Other (selected):						
Processed fruit and vegetables	0.019	**0.026**	0.032	0.081	**0.109**	0.135
Bread, grain mill and cakes	0.028	**0.038**	0.047	0.081	**0.109**	0.135
Bakery shops	-0.011	**-0.015**	-0.019	0.000	**0.000**	0.000
Beverage and Tobacco	0.036	**0.049**	0.061	0.081	**0.109**	0.135
Agricultural services, forestry and fisheries	0.019	**-0.025**	-0.031	0.081	**0.109**	0.135
Textile, wood, paper and publishing	0.034	**0.045**	0.056	0.081	**0.109**	0.135
Basic chemicals	0.056	**0.075**	0.094	0.081	**0.109**	0.135
Construction incl. Supply	-0.006	**-0.008**	-0.010	0.081	**0.109**	0.135
Metals products	0.054	**0.072**	0.090	0.081	**0.109**	0.135
Public services and utilities	-0.022	**-0.029**	-0.037	0.081	**0.109**	0.135
Retail and wholesale margins	0.004	**0.006**	0.007	0.081	**0.109**	0.135
Private services	0.000	**0.000**	0.000	0.081	**0.109**	0.135

Table C.2. Macroeconomic consequences of the AGP removal

		AGP-25%		**AGP**		AGP+25%	
	Billion 1995-DKK	Million DKK	Percent	**Million DKK**	**Percent**	Million DKK	Percent
Real GDP	1426.1	-270	-0.019	**-363**	**-0.025**	-452	-0.032
Real private consumption	694.7	-174	-0.025	**-234**	**-0.034**	-291	-0.042
Real public consumption	353.8	-89	-0.025	**-119**	**-0.034**	-148	-0.042
Real investments	253.1	-26	-0.010	**-35**	**-0.014**	-44	-0.017
Real stocks	39.3	0	0.000	**0**	**0.000**	0	0.000
Real exports	412.6	13	0.003	**17**	**0.004**	21	0.005
Real imports	319.0	-29	-0.009	**-38**	**-0.012**	-48	-0.015
Real capital stock			-0.013		**-0.017**		-0.021
GDP deflator			-0.022		**-0.029**		-0.036
Consumer price index			-0.017		**-0.023**		-0.028
Price of investment goods			-0.022		**-0.029**		-0.036
Terms of Trade			-0.001		**-0.001**		-0.001
Nominal wage rate			-0.042		**-0.057**		-0.071
Price of agricultural land			-1.021		**-1.371**		-1.706

13. Consequences of terminating the use of AGP in Denmark for competitiveness in the international market place

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Summary

Denmark is the world largest exporter of pig meat. Followed by Canada and USA Denmark has an export of more than 1,5 mill tonnes of pig meat to more the 130 different countries. As a result of consumer concern Denmark did terminate the use of antibiotic growth promoters for finishers in March 1998 and for weaners in January 2000. This voluntary ban has not shown a direct effect on sales volume, but it has given Denmark some competitive advantages with regard to meeting consumers concerns on human health. The ban may also have kept the market access to some valuable markets or product segments, even though antibiotic growth promoters only rank as number 3 or 4 on the list of sales parameters.

Background

Agriculture is a vitally important part of the Danish national economy. The industry employs a total of around 240,000 people in all sectors (app. 8,5% of the labour force). Sixty per cent of total agricultural production is exported, representing almost 20 % of the total Danish exports. Pig meat exports represent almost half of the agricultural exports with a value of more than 3 billion US$.

The cooperative system plays a significant role in Danish agriculture and especially in the Danish pig meat sector. The cooperative system was introduced in Denmark more than hundred years ago, and is the main reason for the current strength of the Danish Agricultural sector, and it is securing the influence of each individual farmer.

More than 95 % of Danish pigs are slaughtered, processed and marketed by the cooperative companies, owned and managed by the pig producers.

The number of pig producers in Denmark has fallen drastically over the last 20 years. While the total number of producers was 69.000 in 1980 and 33.000 in 1990, it has now dropped to about 13.000 pig producers in the country. The number of slaughter pigs produced, on the other hand, has increased considerably over the years. From a production of 13 million pigs in 1980, the production has increased to the present level of more than 23 million pigs.

A further reduction in the number of pig producers and an increase in production are to be expected.

Many important elements associated with pig meat production in Denmark are controlled and managed by the cooperative companies. A central organization -DANSKE SLAGTERIER - the Danish Bacon & Meat Council - links together the cooperative companies. This ensures that joint strategies and actions can be followed in important aspects such as breeding, research and development, marketing and improved productivity in primary production.

The unique Danish cooperative system results in a fully integrated production that gives the Danish exporters stable supplies of raw material of a very uniform and consistent quality to the benefit of our customers in different markets worldwide.

Danish farmers produce 23 million pigs per year. 85 % of the production is exported, and this makes Denmark the largest exporter of pig meat in the world. Being an exporter it is very important to be competitive on both price and quality. The Danish pig meat sector has therefore always put an effort into reducing cost and improving quality at the same time.
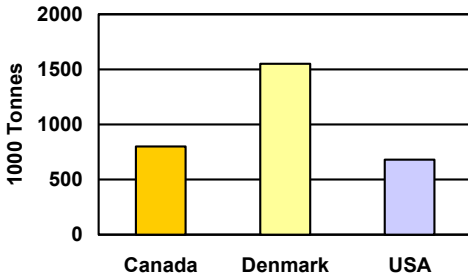
Figure 1. The 3 largest exporters of pig meat in the world (Danish Statistics, 2001)

The most important markets for Danish exports of pig meat

Pig meat from Denmark are exported to more than 130 countries around the world, and the most important markets are shown in figure 2.

As the figure shows Germany, UK and Japan are the most important markets for the export of Danish pig meat.
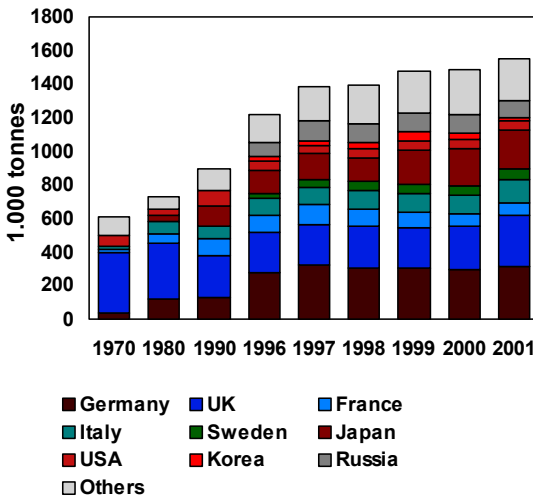
Figure 2. The most important markets for export of Danish pig meat (Danish Statistics, 2001)

Quality parameters

Pig meat is today sold on a lot of different quality parameters. These parameters do tend to differ in priority from market to market, but in general the list of priority has these parameters:

1. Price
2. Quality (Lean, pH, PSE, Size)
3. Food safety (Residues, Salmonella, Veterinarian medicine)
4. Food safety II (GMO, Animal feed, diseases, Antibiotic Growth Promoters)
5. Traceability
6. "Soft" parameters (Animal welfare, environment)
7. Independent audit – Certification

As shown in the list price and quality are still the most important sales parameters, where as the antibiotic growth promoters are found below residues, salmonella and veterinary medicine. This list of priority changes from market to market, and in some markets, for example the Danish home market, antibiotic growth promoters would be found together with residues, Salmonella and veterinary medicine.

Market consequence of terminating the use of AGP in Denmark

As a case we have been looking at 3 of the markets where the terminating of the use of AGP's where expected to have a positive effect on sales. Figure 3 shows the sales volume on the Swedish, Japanese, and the British markets from the first quarter of 1995 to the second quarter of 2002. The voluntary ban on the use of antibiotic growth promoters in Denmark was carried out in to steps. The fist step was the voluntary ban regarding finishers by March 1998 followed by the voluntary ban regarding weaners by January 2000.

As shown in figure 3, the termination of the use of antibiotic growth promoters has not given any direct effect on sales volumes to 3 of the markets where we would have expected an effect.
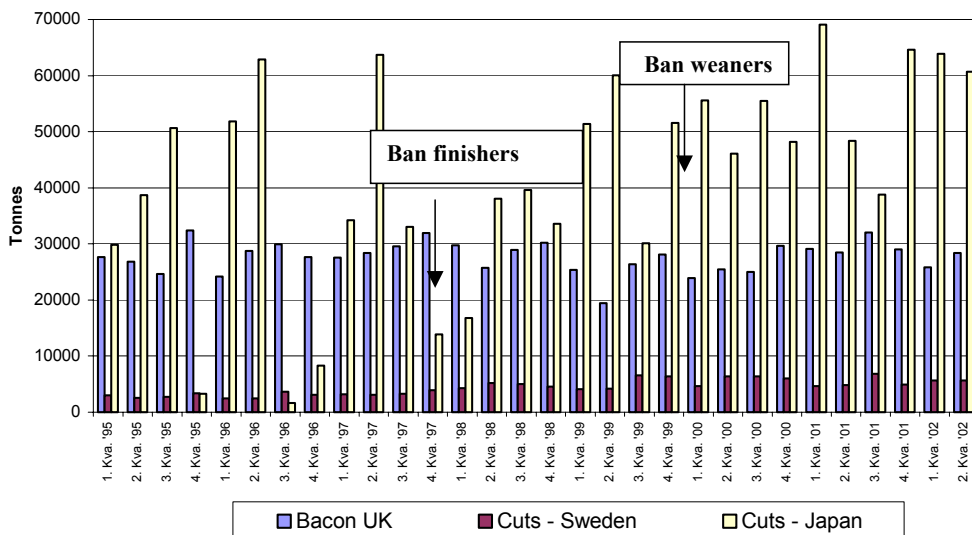
Figure 3. Sales of bacon to UK and cuts to Sweden and Japan from 1995 to 2002

The variations could be explained by import regulations in Japan (safe guard) and by swine fever in 1999 in UK and Foot and mouth disease in UK in 2001 and by the BSE foot scare in both Europe and Japan. What is not shown in the figure is of course the risk of loosing market shares or the risk of the products changing to low price segments, if Denmark had not terminated the use of antibiotic growth promoters.

Consumer trends
Looking at consumer trends Meiselman (2001) describes the human health as being the most important within the next 10 years in relation to the buying of foods. By terminating the use of antibiotic growth promoters we do meet the consumers concerns and we are able to build in a higher trust in the product.

Conclusion
By terminating the use of antibiotic growth promoters Denmark has some competitive advantages in comparison with our competitors. We have not been able to demonstrate higher market shares after the voluntary ban, but we are able to meet consumer concerns and by that

build in higher trust in the Danish production of pig meat.

References

Danish Statistics 2001. Udenrigshandel fordelt på varer og lande statistik service 2001:1; 2001;2; 2001:3; 2001:4, Danmarks Statistik 2001.

14. Environmental effects of the termination of AGP use in Denmark – in relation to zinc and copper

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Abstract

The termination of AGP use in Denmark increased the need for other feed additives to control the outbreak of diarrhoea particularly in piglets during the first days after weaning. Dietary inclusions of high levels of copper up to 175 ppm have for many decades been known to reduce scouring and increase the performance of pigs. Although this growth stimulating effect has mainly been manifested in young pigs, it is allowed to add up to 175 ppm copper for young pigs (up to 16 weeks of age)[1]. Since the very late 1980es, many experiments conducted with young weaned piglets have shown that a dietary content of 2000 to 3000 ppm zinc fed as zinc oxide significantly improves growth and reduces the incidence and severity of diarrhoea. The exact mechanism behind the effects of zinc and copper is still not clear, although the overall physiology of the piglets is affected indicating a greater demand in newly weaned piglets. New results indicate that weaned piglets may develop transient zinc deficiency. Furthermore, zinc and copper can stabilise the intestinal microflora. However, the current legislation dictates that dietary levels above 250 ppm zinc are not allowed in pig diets[1]. This means that it is not accepted to use the addition of extraordinary high levels of zinc as a simple feed additive for young newly weaned piglets in order to control diarrhoea. The reason for this is mainly due to environmental concern because zinc and copper are essential nutrients but also heavy metals. These may disturb the soil ecosystem and crop yield when agricultural soil is supplied with manure containing too much heavy metals.

With reference to the Danish soil quality criteria, it can be calculated how many years it will take to reach a critical zinc or copper level in soil when supplied with manure from pigs fed different dietary levels of zinc or copper. The usage of 2500 ppm zinc for two weeks after weaning results in a zinc concentration in manure that are 3 to 4 times bigger compared with manure from pigs (7.2 to 30 kg) fed officially permitted levels. When copper is fed at the legally accepted 175 ppm for piglets (from weaning to 30 kg), the copper concentration in manure is increased almost 20 times compared with piglets fed 10 ppm. In worst case, the above mentioned critical levels of zinc and copper may be reached within 50 years when zinc or copper are added in high amounts. This situation can occur when soil is supplied with manure solely produced by piglets, where this manure is not mixed with manure from sows or finishing pigs.

In conclusion, the nutrients zinc and copper can to some extend succeed AGP in young pigs. However, there may be contradictions between the responsibility paid to the health and growth of the animal and to the environment.

Introduction

Zinc and copper are essential trace elements and they are important nutrients that affect the health and productivity of animals. Furthermore, zinc and copper may have particular importance for the young pig in a short period after weaning. Young piglets have to adapt to many changes at weaning, and one of these is caused by dietary changes that impose an urgent need for quick intestinal adaptations to differences in composition of the substrates. This may call for

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[1] From January 26, 2004 the maximum allowed amount will be 170 ppm copper and 150 ppm zinc for piglets.

special attention to the need for zinc and copper and a better understanding of how we can fulfil their need.

However, zinc and copper are also classified as heavy metals and the use of large doses of zinc and copper may impose an accelerated accumulation of zinc and copper in soil applied with pig manure (Poulsen, 1998). This may in the future affect soil fertility and crop yield. As such, both zinc and copper may be candidates to become pollutants. This seems to impose a dilemma between livestock production and environmental interests. Nevertheless, this will request a balanced approach that on the one hand does not compromise the physiologic need to ensure health of the pigs and on the other hand do not create an environmental problem.

Zinc

Zinc is an abundant trace element and is present to a lesser or greater extent in all tissues of the body. It is known to be a component of more than 200 enzyme systems modulating carbohydrate, lipid, protein, and nucleic acid metabolism. Consequently, zinc is particularly important to active growth and metabolism, immune and inflammatory responses and repair following injury. Zinc also helps to maintain the integrity of cellular and organelle membranes, growth and carbohydrate tolerance. Recent studies have shown that zinc also plays an important role in gene expression.

Zinc deficiency results in e.g. growth retardation, anemia, skin disease and impaired wound healing (Lansdown, 1995). Hypozincemia with pathophysiological consequences has also been reported in a variety of diseases including diarrhoea, anorexia, bacterial and parasitic infections, endocrine disorders etc. Acquired zinc deficiency in humans is well documented and it has been clearly recognized that foodstuffs containing e.g. phytate bind zinc and greatly impair its absorption inducing severe growth retardation, skin diseases, impaired wound healing etc. (Prasad, 1995). However, zinc supplementation can eliminate these symptoms. Oral zinc therapy has also been shown to be beneficial in the treatment of wounds in otherwise healthy humans, resulting in a much quicker healing up of

wounds (Lansdown, 1995). The effect was greatest during the epithelialization phase indicating that in the early post-wound phase there is a local depletion of zinc through losses from the damaged tissue. In contrast, there is at the same time an increased local need for zinc in RNA and DNA polymerases and other zinc-dependent enzymes in the regenerating tissue. As such, there is a demand for either increased intestinal absorption (dietary oral supplementation) or mobilisation of body reserves (Lansdown, 1995).

As mentioned, zinc stimulates growth, and growth retardation is typically seen in relation to zinc deficiency in young animals of all species. Furthermore, cyclic changes in feed intake are often seen, especially in severe zinc deficiency. This means that the growth stimulating effect of zinc results partly from increased feed intake (appetite) but also from improved feed utilisation (Hambidge et al., 1986). Loss of appetite (voluntary feed intake) and poor growth may be the only overt sign of mild zinc deficiency, but they can be overcome by supplementing dietary zinc. In particular, young animals are susceptible to zinc deficiency because of dietary insufficiency, disturbances in the gastrointestinal tract (diarrhoea), excessive dietary levels of phytate, plant fibre, etc. and excessive dietary calcium.

Zinc and young weaned piglets
Since the very late 1980es several studies have shown that zinc fed as zinc oxide at dosages of 2,000 to 3,000 ppm fed for two weeks after weaning reduces the incidence and severity of scouring in weaned piglets (Holm, 1988; Poulsen, 1989, 1995; Hahn & Baker, 1993; Carlson et al., 1999). Many studies have also shown that in general the same dosages of zinc stimulate feed intake and growth of the piglets whether they were suffering from diarrhoea or not. Some studies have revealed no or small effects but the overall picture is that high dietary levels of zinc fed as zinc oxide may reduce the problems associated with weaning of young piglets. However, the exact mechanisms behind this beneficial effect have so far not been fully established but there might be several contributing factors.

For instance, piglets are subjected to many changes at weaning. The dietary change is associated with the shift from sow milk to a diet mainly composed of cereals supplemented with protein sources as soybean meal, skim-milk powder and/or fish meal. These diets are normally supplemented with minerals and vitamins according to the current recommendations.

Weaning is commonly accompanied by changes in the intestinal morphology reducing the absorptive capacity (e.g. Hampson, 1986). Many studies have shown a lower feed intake after weaning and recently, McCracken et al., 1999 have shown that inadequate feed intake during the immediate postweaning period may contribute to intestinal inflammation and thus compromise villus-crypt structure and function. A direct consequence of the very low feed intake after weaning is that the general nutrient need is not met. As such, piglets after weaning may transiently suffer from malnutrition. This may have serious effects on the health and functioning of the newly weaned pig. Addressing zinc, it is known that the labile pool is very small and that the need is big in the young animal. As such, it may be hypothesised that piglets may develop marginal transient zinc deficiency during the first days after weaning because of the very limited feed intake, and that extra zinc supplementation as zinc oxide may alleviate this. The question is whether this can explain the beneficial effect of high levels of zinc oxide after weaning.

For many years it has been recognized that diarrhoea is one of the gastrointestinal symptoms of zinc deficiency in humans (Okada et al., 1976), especially in malnourished children in the developing countries (Golden & Golden, 1985). Furthermore, in zinc deficiency the organism is more susceptible to toxin producing bacteria or enteroviral pathogens that stimulates chloride secretion. This may result in diarrhoea and impaired absorptive capacity, thus exacerbating an already compromised zinc status (Wapnir, 2000). Accordingly, it has become more and more apparent that zinc deficiency, malnutrition and diarrhoea are closely linked in humans, especially in children (Wapnir, 2000). Several reports have shown that bacterically induced endotoxins may result in damage of the intestinal

tissue which may be mediated through the actions of nitric oxide (Hansen & Skadhauge, 1997). Administration of interleukin-1a (a cytokine that can induce (like the endotoxins) the enzyme inducible nitric oxide synthase whereby nitric oxide is produced) showed that zinc deficient rats responded more severely than zinc sufficient rats, resulting in a greater incidence of diarrhoea in zinc deficient rats (Cui et al., 1997). This may explain the anti-inflammatory effect of zinc (Abou-Mohamed et al., 1997). In a study with guinea pigs, Rodriguez et al. (1996) found that protein deficient diets increased intestinal paracellular permeability to small molecules and that this increase was fully prevented by high doses of dietary zinc. Darmon et al. (1997) confirmed these results and in addition they found that the intestinal secretory response to serotonin was reduced when malnourished guinea pigs were fed high levels of dietary zinc. They concluded that zinc is able to prevent intestinal dysfunction caused by malnutrition and suggested the protective effect to be antianaphylactic and antisecretory. Lately, it was found that the secretory response to serotonin was reduced in weaned piglets fed high concentrations of dietary zinc (Carlson et al., 2004). It is now believed that the gastrointestinal tract may be one of the first target areas where zinc insufficiency may be manifested (Wapnir, 2000). Interestingly, the beneficial effect of high dietary zinc levels on the incidence and severity of diarrhoea in weaned piglets is associated with an increase in plasma zinc concentration (e.g. Poulsen, 1989, 1995; Baker and Hahn, 1993). This may indicate that the effect is a systemic effect. However, studies on the effect of zinc applied to incisional wounds showed that zinc oxide advanced healing (Lansdown, 1995) which may indicate a contributing local effect. The same study revealed no beneficial effect of the more acidic zinc chloride application. The mechanism is not known but it is assumed that zinc oxide becomes hydrolyzed and absorbed by the epithelial cells at the wound margin, becoming available for uptake and ultimately incorporation in enzyme systems (Lansdown, 1995). It can be speculated if this may be the actual situation in newly weaned piglets where the absorptive area of the intestinal tract is injured by rupture of the villi. Maybe zinc ions that are slowly released from zinc oxide can stimulate the re-

pairing of the epithelial cells. In vitro studies with a small intestinal cell line have shown that zinc may promote intestinal epithelial wound healing by enhancement of epithelial cell restitution which is the initial step of epithelial wound healing (Cario et. al. 2000). The same study showed that excessive amounts of zinc might cause tissue injury and impair epithelial wound healing. This is in agreement with the finding that excessive dietary zinc (above 4000 ppm zinc as zinc oxide) results in reduced feed intake and growth in weaned piglets (Poulsen, 1989, 1995; Poulsen and Danielsen, in preparation). However, 11,000 ppm fed for 4 weeks after weaning did not result in the death of any piglets but did affect the overall physiology of the piglets (Poulsen and Danielsen, in preparation).

Salts of zinc have mild antiseptic properties and have been used to a limited extent for this purpose. In general, they have been used more for their actions against fungi than against bacteria. It has been suggested that the successful effects of zinc (as zinc oxide) might be due to a bactericidal effect of zinc on e.g. *Eschericia coli*. However, Jensen-Waern et al., 1998 found no effect on the number of *E. coli* and enterococci. This is in agreement with findings of Li et al., 2001 reporting no major effect of zinc (as zinc oxide) on the bacterial populations in ileal digesta and faeces. This is also in agreement with the findings in a recent experiment showing no major effects but a stabilising effect of zinc on the changing gastrointestinal microbial ecosystem adapting to the differences in the composition of the substrates (Højberg et al., submitted).

The bioavailability of zinc in phytate rich diets is rather low. The bioavailbility of zinc in zinc oxide has been determined to be about 20% (Poulsen & Larsen, 1995) and a recent study revealed that the zinc bioavailability was almost the same for zinc sulphate and zinc acetate (Poulsen & Carlson, 2001). In humans, the bioavailability was about 50% in breast milk (Abrams et al., 1997). This means that corrected to total amounts, the daily need will increase quite dramatically after weaning.

Concluding remarks about zinc

There is developing evidence that young weaned piglets may have a higher need for zinc for some few days after weaning. The situation may be parallel to the recognition that children having diarrhoea frequently suffer from zinc deficiency (especially in the developing countries) and that this may be caused by dietary modifications including the intake of cereals and frequent use of soy milk in stead of breast milk (Folwaczny, 1997). In addition, diarrhoea leads to excess zinc losses. Zinc supplementation results in accelerated regeneration of the mucosa, increased brush-border enzymes and enhanced immune capacity etc. (Folwaczny, 1997). These conclusions are supported by several controlled clinical trials providing evidence that zinc supplementation results in significant reductions in the risk of continued diarrhoea in children. There seems to be a comparable physiological cascade of reactions in the newly weaned piglet adapting to the new feeding conditions and substrates. Feeding situation is the same with an abrupt shift from milk to a diet mainly based on cereals and soybean meal with a high content of phytate rendering zinc less bioavailable. In addition, feed intake is very limited rendering the intestinal tissue more susceptible to bacterial endoxins. All these factors may contribute to the disrupture of the pre-weaning gastrointestinal function of the piglet after weaning.

In conclusion, the above mentioned statements and evidence from recent studies call for a revision of the way we are feeding zinc to newly weaned piglets. There seems to be a growing understanding that piglets have a higher need than the current recommendations say. We believe that we should keep in mind that the daily feed intake is very low (< 100 g) just after weaning. As the daily zinc requirement is rather big in young individuals, we have to think in daily amounts. Furthermore, we should keep in mind that generally the bioavailability of zinc is very low (< 20%). To compensate for this, a high dietary concentration is needed, but fortunately this high concentration is necessary for not more than two weeks.

Copper

Copper is associated with the function of a number of enzymes especially oxygenases including cytochrome c oxidase, the terminal component of the electron transport chain (Keen & Graham, 1989). A major copper enzyme is copper-zinc superoxide dismutase (CuZnSOD), which catalyses the dismutation of the superoxide anion to hydrogen peroxide. Another major function of copper is ceruloplasmin (ferroxidase I), a putative copper transport protein that has weak oxidizing properties and may be required for the incorporation of iron from the liver into transferrin for its transport to extrahepatic tissue (Keen and Graham, 1989). Furthermore, ceruloplasmin can serve as a scavenger of superoxide radicals (Cousins, 1985). An additional key copper enzyme is lysyl oxidase catalysing the biosynthesis of elastin and collagen, a step that is critical in the cross-linking of connective tissue needed for the build-up, maintenance and repair of connective tissue. High dietary levels of copper have also been shown to stimulate intestinal lipase and phospholipase A_2 activities, leading to an improvement of dietary fat digestibility in weaning pigs (Luo et al., 1996) and to increased protein utilisation, probably through activation of pepsin (Kirchgessner et al., 1976).

Many details in the overall metabolism of copper are still poorly understood. In general, information on copper requirements of animals is scarce and poor. However, it is common to add copper sources to diets for pigs. Copper absorption is affected by dosage and chemical appearance in the diet. Additionally, the absorption of copper is regulated and affected by the physiological state of the animal. Lönnerdal et al., 1985 report that copper absorption is higher in neonates than in adults indicating a higher physiological copper need in young individuals compared with adults. Phytate, ascorbic acid, fibre etc. appear to complex with copper and limit its absorption (Cousins, 1985). As such, the release of copper for absorption is affected by the enzyme phytase that increases the apparent bioavailability of copper (Pallauf et al., 1992; Adeola et al., 1995).

Copper and young pigs

For many decades, lots of investigations have shown that the addition of copper to the diets increases the growth of the pigs, but the mode of actions behind this growth promoting effect remain not well understood. The copper effect has been attributed to either a growth promoting effect through improved feed intake and/or feed conversion ratio or as a prophylactic barrier against gastrointestinal disorders e.g. acute diarrhoea in neonatal pigs. However, the pathogenesis of neonatal enteric infections is complex, often involving nutritional and environmental factors as well as infectious agents, such as enteropathogenic strains of *E. coli* and other viral or bacterial pathogens. Although its action has been attributed to its antimicrobial activity, only a few studies have focused on the effect of copper in the gastrointestinal tract of pigs (Jensen, 1998; Poulsen, 1998). Salts of copper have mild antiseptic properties and it has for a long time been the general saying that the effect of copper might be caused through its antimicrobial quality.

Fuller et al. (1960) observed in faeces from pigs fed high dietary copper during 100 days after weaning that the numbers of *Lactobacillus* spp. and *E. coli* were unaffected whereas the number of *Streptococcus* spp. was markedly lower. The changes in the microflora of the pigs did not correlate with the growth stimulating effect of copper because the same modifications of the microflora were seen in pigs showing a growth response or no growth response. Hawbaker et al. (1961) found in young pigs (between 42 and 107 days) that copper sulphate added to the diet significantly decreased the populations of *Lactobacillus* spp., *Streptococcus* spp., total aerobes and total anaerobes in the faeces. Complementary, Bunch et al. (1961) found that *Lactobacillus* spp., total aerobic and total anaerobic micro organisms in piglets were significantly lowered by addition of copper sulphate for 6 weeks. In pigs receiving copper oxide, only the coliforms were significantly lowered.

On the contrary, Smith and Jones (1963) found no obvious differences between the numbers of

Lactobacillus spp., *Streptococcus* spp., *E. coli*, *Clostridium perfringens, Bacteroides* spp. and yeasts in different parts of the gut of 5 month old pigs fed a diet containing 250 ppm copper sulphate. Observations that copper sulphate reduces the number of *Streptococcus* spp. and ureolytic micro-organisms in the gut of pigs was also confirmed by Varel et al. (1987). The results concerning the reduction of the *Streptococcus* spp. populations in the gut are in agreement with the observations of Dunning *et al.* (1998) that streptococci are susceptible to copper under anaerobic conditions while most lactobacilli and *E. coli* are insensitive. However, Jensen (1998) reports that copper sulphate affects the microbial populations of *Lactobacillus* spp. and *E. coli* in the gut of pigs. Evidence that copper may have a growth promoting effect through the actions on the microbial flora is supported by the results of Shurson *et al.* (1990), who observed a positive effect of high dietary copper on daily growth rate and feed conversion rate in conventional pigs and a negative effect in germ-free pigs. It should be noticed that a recent study has indicated that high dietary copper may result in microbial resistance (Hasman & Aarestrup, 2002).

Though it has been expected that the prophylactic effects of copper are exerted on the microbial ecosystem in the gut in one way or another, no data has so far definitely linked the growth stimulating effect of copper to the effects on the microflora. However, there might be another understanding of the growth promoting effect of copper, because recently, it has been claimed that the growth promoting effect of copper in piglets might be a systemic effect rather than an antimicrobial effect in the intestinal tract (Shurson et al., 1990; Radecki et al., 1992).

In addition, elevation of blood copper is associated with various diseases, e.g. inflammation and infection (Sorensen, 1987). A central question is whether this elevation is a cause of the disease or a response to the disease. Generally, it is interpreted as a cause of the disease, but it may also be plausible that the elevation is a response to the disease and thus a normal pharmacological reaction and activity in individuals suffering from these diseases (Sorensen, 1987). In chickens, *Salmonella* infection increased

plasma ceruloplasmin synthesis. Furthermore, *E. coli* toxin administration to hamsters also increased plasma ceruloplasmin (Cousins, 1985). After endotoxin injection, an initial and immediate release of hepatic ceruloplasmin was followed by *de novo* synthesis (Cousins, 1985). Maybe these findings could be extended to the prophylactic effect of copper on diarrhoea in young pigs but it has not been investigated so far.

If it can be established that elevation of plasma copper is a general physiologic response to disease states, this will facilitate a physiologic nutritional approach to disease prevention and treatment according to Sorensen, 1987. This statement or hypothesis may also be relevant to young pigs and may then draw attention to the suggestion that young piglets might have a higher physiologically based copper need than generally assumed for many decades. In that context, one should also bear in mind that pigs are generally weaned much earlier and more abruptly and therefore less physiologically mature than decades ago. Furthermore, the feed intake is very limited during the first postweaning period.

Concluding remarks about copper
Copper is a very important nutrient for the very young piglet in controlling outbreak of diarrhoea and stimulating growth, especially during the first period after weaning. High dietary levels of copper sulphate can quantitatively modify some gram positive bacterial populations of the gut as demonstrated for *Streptococcus* spp. and *Lactobacillus* spp. Favourable consequences for the pig through these gut modifications are possible but not yet clearly demonstrated. Recently, it has furthermore been emphasised that the growth promoting effect of copper in piglets might be a systemic effect rather than an antimicrobial effect in the intestinal tract. As such, young piglets might have a higher physiologically based copper need than generally assumed for many decades.

Interactions between zinc and copper
Classically there is an antagonistic interaction between copper and zinc and between copper

and iron (Davis & Mertz, 1987). For instance high dietary zinc may induce copper deficiency and vice versa because a high dietary dosage of one of the minerals suppresses the absorption of the other. Davis & Mertz, 1987 suggest that the discrepancies in the reported responses to high copper levels in pigs relate primarily to differences in zinc and iron levels of the basal rations. In weaned piglets, the interaction between copper and zinc results in reduced plasma copper when the diet is heavily supplemented with zinc oxide (Poulsen, 1995). Furthermore, the prophylactic effect of high dietary zinc inclusions interacted with the dietary copper content indicating that the effects of one specific mineral may be very complex because of interactions (Poulsen, 1995). Carlson et al. (in preparation) found that piglets fed high dietary copper had an increased plasma zinc concentration. We believe that this may indicate that the classical growth stimulating effect of copper might be explained by an improved zinc status. The site of action of the interaction between copper and zinc may be in the intestinal lumen, for instance through bindings to e.g. phytate, fibre etc.. Experiments with phytase confirm that copper bioavailability is affected by the dietary content of zinc in an antagonistic way (Pallauf et al., 1992; Adeola et al., 1995). The interaction between copper and zinc may also be due to competition at the brush border membrane and/or during the transfer of the metals within the mucosal cell (Cousins, 1985). Interestingly, high calcium intake is reported to reduce zinc availability, whereby the risk of copper toxicity in pigs fed high copper diets is enhanced (Davis & Mertz, 1987).

Environmental aspects
In Denmark, there are restrictions on the amount of manure that can be applied to the agricultural soil. This amount is defined by the amount of nitrogen that is excreted from the pigs of different categories (piglets (7.2 to 30 kg), finishers (30 to 100 kg), sows (incl. piglets to weaning)). The nitrogen excretion is calculated based on the standard values for feed intake, productivity, nutrient content of the feed and retention in the pig body (Poulsen et al., 2001). Similarly, the excretion of zinc and copper can be calculated for different scenarios. This has been done for all categories of pigs when assuming different

dietary contents of either zinc or copper (Poulsen, 1998). The obtained values indicate that the accumulation of zinc or copper depend to a large extent on the dietary concentration. Furthermore, it was calculated how many years would pass before the upper soil quality criteria is reached in a standard soil. The critical value for soil zinc and copper is laid down by the Danish Environmental Protection Agency for sludge and is based on the EU Directive 86/278/EØF (Larsen et al., 1996). It can be calculated that under Danish conditions, the critical values may in worst case be reached within about 50 years of constant application of manure solely excreted by young piglets fed either 175 ppm copper or 2,500 ppm zinc. Under normal conditions, manure from different categories of pigs will be mixed whereby the content of zinc and copper will be diluted. Nevertheless, the exact environmental effect of a higher content of zinc and copper in the soil remains to be fully exposed.

Conclusions

In conclusion, zinc and copper are very important nutrients, especially for young piglets. Recent experiments emphasise that the current recommendations on zinc and copper to weaned piglets need to be revised. Experiences from human nutrition support the hypothesis that young weaned piglets may develop transient zinc deficiency. Furthermore, a quite high dietary concentration of zinc may be required to ensure their physiological daily zinc need because their feed intake is very limited the first few days after weaning. However, a balanced approach is needed because zinc and copper are heavy metal and may call for environmental concern.

References

Abou-Mohamed, G., Papapetropoulos, A., Catravas, J.D., Caldwell, R.W., 1998. Zn^{2+} inhibits nitric oxide formation in response to lipopolysaccharides: Implication in its anti-inflammatory activity. Europ. J. Phamacol. 341, 265-272.

Adeola, O., Lawrence, B.V., Sutton, A.L., Cline, T.R., 1995. Phytase-induced changes in mineral utilization in zinc-supplemented diets for pigs. J. Anim. Sci. 73, 3384-3391.

Bunch, R.J., Speer, V.C., Hays, V.W., Hawbaker, J.H., Catron, D.V., 1961. Effects of copper sulphate, copper oxide and chlortetracycline on baby pig performance. J. Anim. Sci. 20, 723-726.

Cario, E., Jung, S., d`Heureuse, J.H., Schulte, C., Sturm, A., Wiedenmann, B. Goebell, H., Dignass, A.U., 2000. Effects of exogenous zinc supplementation on intestinal epithelial repair in vitro. European J. Clin. Invest. 30, 419-428.

Carlson, D., Poulsen, H.D., Sehested, J. 2004. The influence of weaning and the effect of post weaning dietary zinc and copper on the electrophysiological response to glucose, theophylline and 5-HT in piglet small intestinal mucosa. Comparative Biochemistry and Physiology, Part A (accepted).

Carlson, M.S., Hill, G.M., Link, J.E., 1999. Early- and traditionally weaned nursery pigs benefit from phase-feeding pharmacological concentrations of zinc oxide: effect on metallothionein and mineral concentrations. J. Anim. Sci. 77, 1199-1207.

Cousins, R.J., 1985. Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol. Rev. 65, 238-309.

Cui, L., Takagi, Y., Wasa, M., Iiboshi, Y., Khan, J., Nezu, R., Okada, A., 1997. Induction of nitric oxide synthase in rat intestine by interleukin-1 alpha may explain diarrhea associated with zinc deficiency. J. Nutr. 127, 1729-1736.

Darmon, N., Pelissier, M.A., Candalh, C., Chappuis, P., Blaton, M.A., Albrecht, R., Desjeux, J.F., Heyman, M., 1997. Zinc and intestinal anaphylaxis to cow's milk proteins in malnourished guinea pigs. Pediatr. Res. 42, 208-213.

Davis, G.K., Mertz, W. 1987. Copper. In: Trace elements in human and animal nutrition - Fifth edition, vol. 1. Ed.: W. Mertz, Beltsville Human Nutrition Center, Beltsville, Maryland. Academic Press, Inc., 301-364.

Dunning, J.C., Marquis, R.E. 1998. Anaerobic killing of streptococci by reduced, transition metal cations. Appl. Environ. Microbiol. 64, 27-33.

Folwaczny, C., 1997. Zinc and diarrhea in infants. J. Trace Elem. Med. Biol. 11, 116-122.

Fuller, R., Newland, L.G.M., Briggs, C.A.E., Braude, R., Mitchell, K.G., 1960. The normal intestinal flora of the pig. IV. The effect of dietary supplements of penicillin, chlortetracycline or copper sulfate on the feacal flora. J. Appl. Bacterol. 23, 195-205.

Golden, B.E., Golden, M.H.N., 1985. Zinc, sodium, and potassium losses in the diarrhoeas of malnutrition and zinc deficiency. In: Trace elements in man and animals - TEMA 5. Eds. Mills, C.F., Bremner, I., Chesters, J.K. Rowett Research Institute, Bucksburn, Aberdeen, Scotland. 228-232.

Hahn, J.D., Baker, D.H., 1993. Growth and plasma zinc responses of young pigs fed pharmacological levels of zinc. J. Anim. Sci. 71, 3020-3024.

Hambridge, K.M., 1986. Zinc deficiency in the weanling- How important ? Acta. Pædiatr. Scand. 323, 52-58.

Hampson, D.J., 1986. Alterations in piglet small intestinal structure at weaning. Res. Vet. Sci. 40, 32-40.

Hansen, M.B., Skadhauge, E., 1997. Signal transduction pathways for serotonin as an intestinal secretagogue. Comp. Biochem. Physiol. 118A, 283-290.

Hasman, H. & Aarestrup, F.M., 2002. *tcrB*, a gene conferring transferable copper resistance in *Enterococcus faecium*: Occurrence, transferability, and linkage to macrolide and glycopeptide resistance. Antimicrob. Agents Chemoter. 46, 1410-1416.

Hawbaker, J.A., Speer, V.C., Hays, V.W., Catron, D.V., 1961. Effect of copper sulfate and other chemotherapeutics in growing swine rations. J. Anim. Sci. 20, 163-167.

Holm, A. 1988. Escherichia coli-betinget fravænningsdiarré hos grise,-zinkoxid tilsat foderet som antibacterielt princip. Dansk Veterinær Tidsskrift 71, 1118-1126.

Jensen-Waern, M., Melin, L., Lindberg, R., Johannisson, A., Petersson, L., Wallgren, P., 1998. Dietary zinc oxide in weaned pigs - effect on performance, tissue concentrations, morphology, neutrophil functions and faecal microflora. Res. Vet. Sci. 64, 225-231.

Jensen, B.B., 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J. Anim Feed Sci. 7, 45-64.

Keen, C.L., Graham, T.W. 1989. Trace Elements. In: Clinical Biochemistry of Domestic Animals, Fourth Edition. Ed.: J.J. Kaneko. Academic Press, Inc.753-795.

Kirchgessner, M., Beyer, M.G., Steinhart, H., 1976. Activation of pepsin (EC 3.4.4.1) by heavy-metal ions including a contribution to the mode of action of copper sulphate in pig nutrition. Br. J. Nutr. 36, 15-23.

Lansdown, A.B.G., 1995. Physiological and toxicological changes in the skin resulting from the action and interaction of metal ions. Critical Reviews in Toxicology. 25, 397-462.

Larsen, M.M.. Bak, J., Scott-Fordsman, J.. 1996. Monitering af danske dyrknings- og naturjorder. Prøvetagning 1992/93. Faglig rapport nr. 157, Danmarks Miljøundersøgelser.

Li, B.T., Van Kessel, A.G., Caine, W.R., Huang, S.X., Kirkwood, R.N., 2001. Small intestinal morphology and bacterial populations in ileal digesta and feces of newly weaned pigs receiving a high dietary level of zinc oxide. Can. J. Anim. Sci. 81, 511-516.

Lou, X. G. and Dove, C. R., 1996. Effect of dietary copper and fat on digestive enzyme activities, nutrient digestibility and tissue mineral levels. J. Anim. Sci. 74 [suppl.1], 181-181.

Lönnerdal, B., Bell, J.G., Keen, C.L. 1985. Am. J. Clin. Nutr. 42, 836.

McCracken, B.A., Spurlock, M.E., Roos, M.A., Zuckermann, F.A., askins, H.R., 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. J. Nutr. 129, 613-619.

Okada, A., Takagi, Y., Itakura, T., 1976. Skin lessions during intravenours hyperalimentation: Zink deficiency. Surgery. 80, 629.

Pallauf, J., Hohler, D., Rimbach, G., 1992. Effect of Microbial Phytase Supplementation to a Maize-Soya-Diet on the Apparent Absorption of Mg, Fe, Cu, Mn and Zn and Parameters of Zn-Status in Piglets. J. Anim. Physiol. Anim. Nutr. 68, 1-9.

Poulsen, H.D., 1989. Zinkoxid til grise i fravænningsperioden. Statens Husdyrbrugsforsøg Meddelelse nr. 746, 4 pp.

Poulsen, H.D., Larsen, T., 1995. Zinc excretion and retention in growing pigs fed increasing levels of zinc oxide. Livest. Prod. Sci. 43, 235-242.

Poulsen, H.D., 1995. Zinc oxide for weanling piglets. Acta. Agric. Scand. 45, 159-167.

Poulsen, H.D., 1998. Zinc and copper as feed additives, growth factors or unwanted environmental factors. J. Anim. Feed. Sci. 7, 135-142.

Poulsen, H.D., Børsting, C.F., Rom, H.B., Sommer, S.G. 2001. Kvælstof, fosfor og kalium i husdyrgødning - normtal 2000. DJF rapport, Husdyrbrug nr. 36, 152 pp.

Poulsen, H.D. & Carlson, D., 2001. Bioavailability of zinc from different zinc sources. Book of abstracts, EAAP - 52nd Annual Meeting, Budapest, 26-29 August 2001, p 123.

Prasad, A.S., 1995. Zinc: An overview. Supplement to Nutrition. 93-99.

Radecki, S.V., Ku, P.K., Bennink, M.R., Yokoyama, M.T., Miller, E.R., 1992. Effect of dietary copper on intestinal mucosa enzyme activity, morphology, and turnover rates in weanling pigs. J. Anim. Sci. 70, 1424-1431.

Rodriguez, P., Darmon, N., Chappuis, P., Candalh, C., Blaton, M.A., Bouchaud, C., Heyman, M., 1996. Intestinal paracellular permeability during malnuttition in guinea pigs: effect of high dietary zinc. Gut 39, 416-422.

Shurson, G.C., Ku, P.K., Waxler, G.L., Yokoyama, M.T., Miller, E.R., 1990. Physiological relationships between microbiological status and dietary copper levels in the pig. J. Anim. Sci. 68, 1061-1071.

Smith H.W., Jones J.E.T., 1963. The effect of the addition of copper sulphate to the diet on the bacterial flora of the alimentary tract of the pig. J. Appl. Bacteriol., 26, 262-265.

Sorenson, J.R.J. 1987. A physiological basis for pharmacological activities of copper complexes: an hypothesis. In: Biology of Copper Complexes. Ed.: J.R.J. Sorenson, University of Arkansas, Little Rock, Arkansas.

Varel W.H., Robinson J.M., Pond W.G., 1987. Efect of dietary copper sulfate, Aureo SP250, or clinoptilotite on ureolytic bacteria found in the pig large intestine. Appl. Environ. Microbiol., 53, 2009-2012.

Wapnir, R.A., 2000. Zinc deficiency, malnutrition and the gastrointestinal tract. J. Nutr. 130: 1388S-1392S.

15. Antimicrobial use in food-producing animals in the UK

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Introduction

Food-producing animals in the United Kingdom comprise populations of ruminants and monogastric animals which include poultry and fish. The actual species are listed in Table 1 which includes the major edible products of the species.

The UK is not self-sufficient in many foods of animal origin and imports considerable amounts of specialist food of animal origin such as Parma ham and commodities which are available in the Community and Third Countries at a lower price than in the UK (43% self-sufficient in bacon, 76% in fresh pork). The country exports food products of animal origin, notably Scottish salmon, game, horsemeat and specialist or regional products such as cheeses as well as commodity products such as poultry meat, eggs and red meat when conditions of trade are favourable or as part of world trade patterns, subject to disease restrictions.

Table 1. Species used for food production in the United Kingdom

Animal species	Major product	Subsidiary products	Numbers 2001 (where available)
Cattle			**11,133,000**
	Milk (dairy cows)		2,336,000
	Beef (cows)		1,842,000
Sheep and lambs			**42,261,000**
	Meat (Lambs under one year)		20,855,000
	(Ewes and gimmers)	Milk	20,447,000
Goats	Milk	Meat	
Deer	Meat		
Pigs			**6,482,000**
	(Sows and gilts)	Meat	610,000
	Meat (Other pigs)		5,872,000
Horses	Leisure	Meat	
Rabbits	Leisure	Meat	
Total fowls			**155,082,000**
Hens and pullets	Eggs	Meat	38,147,000
Broilers etc	Meat		105,688,000
Ducks	Meat	Eggs	
Turkeys	Meat		
Game (reared)	Meat		
Game (wild)	Meat		
Ostriches	Meat		
Salmon	Meat		
Trout	Meat		
Other seafish	Meat		
Shellfish			

Farming systems in the UK

The farming industry consists of large commercial farms with multiple sites, family or tenanted farms deriving all or most of their income from farming and smallholdings and crofts where the main income is rarely provided by farming. This pattern of ownership is repeated in the egg industry, but most broiler production is on farms belonging to integrated producing and packing firms. The same applies in fish farming, where most salmon production is by large companies but trout and shellfish production is often on a smaller scale.

Special features of the production of foods of animal origin in the UK include the widespread use of grazing and extensive husbandry systems at some stage of production of beef, lamb and some pig meat. There is a trend towards the production of 'organic' foods with a minimum of inputs including antimicrobial treatments in animal husbandry. Unlike many continental countries, there are relatively limited sales of foods of animal origin direct to the consumer , although these are increasing. Animal feeds are often supplied by sophisticated feed companies able to meet the complex nutritional requirements of recently-weaned pigs and calves. Feeds for older animals may be produced on farm using home-produced ingredients, but supplements are provided and the ration balanced by the feed companies. There is little or no white veal production, largely for reasons of perceived animal welfare and national sentiment and no sows are tethered. A number of farm assurance schemes exist to provide quality assurance for the livestock sector.

The UK market for foods of animal origin is dominated by the few large supermarket chains. They have production protocols and inspect production at every stage. Their buying policies have influenced major suppliers such as the poultry producers to adopt welfare and management practices of a standard not yet required by legislation and to abandon the use of antimicrobial growth promoters.

A special feature of UK animal husbandry is the fate of culled cows. No bovine animal aged over 30 months can enter the food chain and all are slaughtered and rendered. This outlet for such animals means that they rarely enter trade, are not treated for disease to the same extent and represent a dead end from the point of view of disease-producing organisms.

Overall, the number of farmed livestock is declining in the UK following two major disease outbreaks and the adverse exchange rate which has resulted in poor profitability. This decline in livestock farming incomes has resulted in reduced capital expenditure and may have reduced adoption of disease control strategies.

Antimicrobial use in the UK

Antimicrobials are licensed for use by the European Medicines Agency or by the UK Veterinary Medicines Directorate as in other European countries. Therapeutic antimicrobials are prescribed by veterinary surgeons to animals under their care for treatment and disease management. Individual treatment is the norm for cattle, sheep, breeding and sucking pigs and horses, but it may be administered in the drinking water or feed where these methods provide the best route of administration or where there are pharmacological reasons for doing so (e.g. valnemulin). Growing pigs and poultry are routinely treated by these routes. Occasional groups of calves may be treated in feed or water, as may be members of other species for specific purposes and where the antimicrobial is compatible with use in that species.

A withdrawal period is observed for all food producing animals to which antimicrobial has been administered. When veterinary surgeons use a licensed antimicrobial (or other medicine) which is not licensed for use in a particular food-producing animal and has no specified withdrawal period for that species or route of administration (for example in a trout or a pheasant), then a standard withdrawal period is observed. This process of using a medicine for an unlicensed use can only be carried out when no licensed medicine is available for the purpose required and is known as the 'cascade'.

The process of prescribing and administering an antimicrobial in the UK is as follows: The vet-

erinary surgeon examines the animal or group of animals concerned and reaches a provisional diagnosis.

If medication is required, an appropriate treatment is selected and the veterinary surgeon administers the first treatment personally from her/his supply. The treatment is recorded in the Treatment Record Book with details of the animals treated, date and name of the person administering the medicine.

The veterinary surgeon may return to complete the treatment, but often provides sufficient medicine for the farm staff (appropriately trained) to complete the treatment and any subsequent cleaning and disinfection. Where the disease does not resolve or occurs in successive groups of animals (as on pig and broiler farms), samples may be taken for confirmation of diagnosis and determination of the antimicrobial sensitivity of the agents present.

Decisions are then taken about longer term disease management. In some circumstances, a group of animals may require medicated water or feed. Feed medication is supplied by the veterinary surgeon and mixed on farm if the feed mixing equipment there is licensed, but is generally prepared by a feed company upon receipt of a prescription valid for the batch of animals and a maximum of one month. The feed company employs a pharmacist to dispense the product and detailed records are kept by all three parties.

Disease management in the longer term may be by treatment of individual affected animals (cases of mastitis in dairy cows) with treatment administered by the farm staff without further reference to the veterinary surgeon. Treatments are recorded and withdrawal periods observed. Further supplies of antimicrobial are only available on prescription from the veterinary surgeon who usually supplies the prescription unless it is for medicated feed. Where possible, vaccines are used and hygiene regimes or, even, in the case of pig or poultry enterprises, depopulation of buildings or entire farms may be used to manage or eradicate disease. The actual methods used depend upon the disease, the economics of the procedure and on the practicality of the management measures (availability of vaccines, ease of administering medication by a specific route etc).

It is important to realise that zinc oxide can be legally supplied on prescription and mixed in feed in the UK for the prevention of post weaning diarrhoea in pigs.

Antimicrobial growth promoters are still used in the UK. The four products still licensed in the EU are all available. Little or no antimicrobial growth promoter is used in table poultry as the supermarket buyers have indicated that their suppliers should not use them. Salinomycin and monensin may still be used as part of the anticoccidial control programme, but they are not used directly as growth promoters. In cattle, monensin may still be used, but the major use of these products is in pigs where avilamycin and salinomycin are commonly used. Once again, those with contracts from the large supermarkets may decide not to use them following discussion with the purchaser. They are always supplied by the feed compounder under licence.

Factors affecting antimicrobial use in the UK

Antimicrobial use is not uniform across the food producing species in the UK. Some species harvested for food receive no antimicrobial (wild game, extensive sheep) and others receive antimicrobial only at specific times in the production cycle. Examples of these uses are the treatment of pheasant chicks and poults for disease prior to release, young lambs during housing, ewes at lambing or with mastitis and cows with mastitis. Antimicrobial use in broilers, pigs and salmon is not uniform and may be completely absent from some sites, flocks and herds. Antimicrobial use is most common in pigs during the sucking and post-weaning periods when a number of bacterial diseases occur. Treatment may be administered to these animals parenterally, in drinking water or in feed.

Factors tending to reduce use of antimicrobial in the UK

Cost of treatment is a factor in antimicrobial use as the margins over costs are low in many parts of UK agriculture. Disease and treatment may

make batches of broilers uneconomic and all possible measures are taken to reduce and prevent disease, thus reducing incidentally the use of antimicrobial. Where the costs of disease have led to complete depopulation and repopulation of pig herds, the new stock are usually of higher health status and require fewer treatments. There may be hesitation before choosing an expensive antimicrobial for certain farms, thus reducing the development of resistance to new products. Costs also affect the treatment of bovine mastitis: once an animal is chronically affected, culling often follows the development of disease as the margin on milk production is currently so low.

The 'organic' movement with its minimal use of antimicrobial and proscription of the use of growth promoters is increasing in size and will eventually reduce the total use of antimicrobial in food animals in the UK.

Health policies have been introduced by cattle, sheep and pig farmers as part of their quality assurance schemes and their operation reduces disease and the consequent need for treatment.

New vaccines have been introduced for a number of diseases and the diseases concerned no longer require treatment. Examples are the introduction of the vaccines for enzootic pneumonia, Glasser's Disease and pleuropneumonia in pigs, the upgrading of the *Mannheimia haemolytica* vaccines for sheep and cattle and the introduction of a vaccine for colisepticaemia in chickens.

Guidelines for the use of antimicrobial use have been prepared by industry, Government and veterinarians in order to reduce the amount of antimicrobial prescribed and used in the UK in both food and companion animals and distributed to all veterinarians. Requests have been made of the veterinary schools to emphasise to students that they should use antimicrobials responsibly when they graduate and the subject is given considerable exposure at professional meetings.

Factors tending to increase use of antimicrobial in the UK food animal population

Increases in animal disease are important reasons for increases in the prescription of antimicrobial. The best example of this comes from the pig industry in which the recently-recognised Postweaning Multisystemic Wasting Syndrome (PMWS) has now spread to most parts of the UK. PMWS is a virus disease, but its consequences include a reduction in resistance to pathogens, a possible association with the use of vaccines, a reduction in growth rate and consequent overcrowding and pressure on hygiene. There has been a surge in antimicrobial use as veterinarians attempt to manage the syndrome and to treat the bacterial diseases which have emerged. Similarly, the restrictions on movement consequent upon the recent outbreak of Foot and Mouth Disease appear to have increased disease and led to local increases in antimicrobial use.

The introduction of new diagnostic tests has allowed veterinarians to confirm the existence of diseases such as Proliferative Enteropathy of pigs (*Lawsonia intracellularis* infection) much more accurately than before. This has led to specific attempts to manage and reduce the levels of infection in pig herds.

Reductions in the use of antimicrobial growth promoters have had some effect already on antimicrobial use. In the broiler industry, disease such as necrotic enteritis has been much more obvious and has been treated with therapeutic antimicrobial. The costs of the disease and subsequent treatment have, however, led to strenuous efforts to reduce the occurrence of the disease and to minimise its effects and the overall effect may be neutral in some cases. The pig herds which have discontinued the use of antimicrobial growth promoters have seen a reduction in growth rate in some cases which has resulted in overcrowding. Once management adjusts to the changes, herds can be grown satisfactorily in the absence of their use, with little or no more disease or antimicrobial use than before.

Illegal imports and the illegal use of antimicrobials occur in the UK and are identified by Government inspectors. The source of the antimicrobials used varies, but the products often derive from other Community countries within the euro zone. They are not registered for use in the UK, but are registered in the country of origin. They are imported illegally, sold and used by farmers without veterinary prescription because of the strength of sterling against the euro. Products from third countries may also follow the same channels Importers and farmers involved are prosecuted if the evidence is sufficiently strong. The effects of these imports on antimicrobial use are not fully known.

The introduction and marketing of new antimicrobials has an effect on the sales and use of the class of compound concerned, although the effect may not be great in terms of the total value of antimicrobial sales or the total use of that class of antimicrobial. Substitution of products within a class may occur. One effect is that the older antimicrobials and the generic products are not supported in this way and their use decreases. A case in point is the treatment of Proliferative Enteropathy in the pig where tetracyclines are the gold standard for treatment, cheap and easy to administer, and have been used effectively for 30 years. Most tetracycline products, however, have no specific claim for the disease and there is a tendency to use products with label claims for treatment. Thus tylosin, lincomycin and tiamulin are used in preference to the older product.

Antimicrobial resistance may increase the use of antimicrobial, but usually only results in a shift in class. Increases in use generally stop once the diagnosis is reconfirmed and an appropriate antimicrobial is prescribed. This can, of course, result in an increase in resistance to the new product.

Monitoring of antimicrobial use and resistance

Antimicrobial use is monitored by the pharmaceutical industry who report to Government annually. Sales figures are published but the tonnages may remain confidential. In the field, inspection of records takes place during Government welfare inspections and during Quality Assurance visits. Residues of antimicrobials in meat and animal products are sought by the Veterinary Medicines Directorate and other bodies and reported. Occurrences are followed up and breaches of the Medicines legislation result in prosecution. Few residues are found. Figures for September 2002 show tetracycline residues in 3 of 2,500 red meat kidneys, sulphonamide residues in 5 of 544 pig kidneys, 1 monensin residue in 162 sheep kidneys, 8 tetracycline residues in 292 turkey kidneys and 1 sulphonamide residue in 181 turkey kidneys. Eggs, milk and salmon were completely free from antimicrobial residues.

Antimicrobial resistance has been monitored systematically and the resistance recorded in salmonellae in the UK since 1975 by Government laboratories. The records for 2001 show that a relative decline in *S. enterica* subsp *enterica* Typhimurium has reduced the percentage of salmonellae resistant to antimicrobials in cattle and in particular, a decline in the proportion of DT104, a multiresistant strain, has reduced the degree of resistance within the Typhimurium isolates. The figures show a decrease in sensitivity to fluoroquinolones (measured using nalidixic acid) in these isolates. Antimicrobial sensitivity testing is carried out on other isolates of clinically-relevant bacteria, but systematic collection of the data from Government sources is only beginning. No collation is yet possible from private or University laboratories. These data are supplemented from time to time by surveys of antimicrobial resistance in normal flora such as enterococci and organisms of zoonotic importance such as campylobacters and in populations such as wildlife.

Conclusions

1. Antimicrobials are used in the treatment of food animal disease in the UK
2. They are administered parenterally, by the intramammary route, in drinking water and in feed.
3. They are prescribed by veterinary surgeons and supplied by veterinary surgeons, licensed feed compounders and a few pharmacists.

4. Their use is recorded, records are inspected and the observation of withdrawal times is monitored.
5. Abuse is investigated and illegal activity prosecuted
6. The veterinary profession and Government are actively involved in responsible use
7. Antimicrobial growth promoters licensed by the EU are used, but only in some ruminants and some pigs.
8. Antimicrobial use is generally level unless a major outbreak of disease occurs.
9. Antimicrobial resistance is monitored in the UK for salmonellae but not on a country-wide basis for other organisms except in surveys.

16. Use of antimicrobial growth promoters in the United States – patterns of use and public health concerns

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Introduction

The United States has a population of approximately 270 million persons. The size of the food animal industries in the United States can be judged by the number of animals slaughtered; approximately 35 million pigs, 100 million cattle, and 7,800 million broiler chickens are slaughtered each year in the United States. For comparison, Denmark, a country with 5 million inhabitants, slaughtered approximately 23 million pigs and 135 million broiler chickens in 2001.

An important barrier to the comparing the patterns of use of antimicrobial growth promoters in United States with the pattern of use elsewhere, including Europe, are the definitions used to describe how antibiotics are used in the United States. The United States Food and Drug Administration defined the low dose, long duration addition of antibiotics to animal feed as „subtherapeutic" antibiotic use (dose of >200 grams per ton of feed for >14 days). Antibiotics that are approved for subtherapeutic use have label indications for „growth promotion," and „feed efficiency." The United States Food and Drug Administration has also approved uses of antibiotics in animal feed for „disease prevention" (e.g., prevention of liver abscesses in feedlot cattle) with dosage regiments that are high dose (>200 grams per ton of feed), long duration (>14 days). „Disease prevention" antibiotic use is intended to be the use of antibiotics for the prevention of disease prior to the occurrence of disease; such uses, therefore, occur in the absence of disease. In contrast, „disease control" uses occur in the presence of disease. The „disease prevention" term creates confusion because the Food and Drug Administration has also approved „disease prevention" uses of antibiotics,

usually by injection (e.g., prevention of early chick mortality in broiler chickens), with high dose, short duration regiments. Therefore, antibiotics approved for „disease prevention" have two possible dosage regiments (high dose, long duration; or high dose, short duration).

The American Veterinary Medical Association defined therapeutic use of antibiotics as uses for therapy, disease control, and disease prevention; all „disease prevention" uses of antibiotics, even if used for long periods of time in animal feed, are considered to be therapeutic uses by the American Veterinary Medical Association. Therefore, „disease prevention" use makes the comparison of patterns of antibiotic use in Europe and the United States difficult. In Europe, all long duration uses of antibiotics in animal feed are „antimicrobial growth promoters". There are, therefore, some uses of antibiotics in the United States that the American Veterinary Medical Association considers to be therapeutic uses, but in Europe are considered growth promotion uses; long duration disease prevention uses in the United States would be considered growth promotion uses in Europe.

An alternative term that has been proposed is „nontherapeutic" antibiotic use. Nontherapeutic uses are uses of antibiotics in the absence of disease. Nontherapeutic uses, therefore, includes all subtherapeutic antibiotic uses and all disease prevention uses. Classifying antibiotic uses as nontherapeutic or therapeutic enables a more direct comparison of the patterns of antibiotic use between the United States and Europe because „nontherapeutic" antibiotic use includes all long duration uses of antibiotics in animal feed (subtherapeutic uses and disease prevention uses). To make a direct comparison, all that is neces-

sary is to exclude the high dose, short duration disease prevention uses in the summary of the patterns of antibiotic use in the United States.

Patterns of use

The Food and Drug Administration publishes a listing of antibiotics approved for use in the United States, with the label indications, in the United States Code of Federal Regulation. The Food and Drug Administration also maintains a listing of approved antibiotics in the „Green Book", which is available on the Food and Drug Administration website (http://www.fda.gov/-cvm). The most useful listing of what antibiotics are used animal feed is available by subscribing to the *Feed Compendium*; this compendium, which is updated monthly, provides a catalog of antibiotics currently marketed in the United States. There are at lease seventeen classes of antibiotics approved for nontherapeutic uses in animal feed. These classes include several classes of antibiotics that are commonly used in human medicine including lincomycin (human analog is clindamcin), macrolides (tylosin in animals, human analog is erythromycin), penicillin, sulfonamides, tetracyclines, and streptograms (virginiamycin in animals, human analog is quinupristin/dalfopristin). Antibiotics that are commonly used in human medicine are not allowed to be used as antimicrobial growth promoters in Europe.

There is no validated antibiotic use reporting system in the United States, nor are there been validated estimates of quantities of antibiotics used in food animals in United States. Establishing such a reporting system is a high priority action item in the United States Interagency Action Plan for combating antimicrobial resistance.

Two groups have provided recent estimates of the quantities of antibiotics used in food animals. The Animal Health Institute, which represents approximately 80% of the manufacturers of animal drugs in the United States, has published a summary of the quantities of antibiotics used in 1999 based on a survey of their member companies (http://www.ahi.org). This summary is laudable because it represents the first report by animal drug companies on the quantities of antibiotics used in the United States and suggests that gathering of animal drug use information in the United States is neither burdensome nor impractical. Unfortunately, no details are provided on the survey methodology; no information is provided on the number of companies surveyed, the number of respondents, or the questions included in the survey. Furthermore, few details are provided in the survey summary; the data are not stratified by animal species, intended uses, types of antibiotics, etc. Results of the survey are difficult to interpret for several reasons. The Animal Health Institute follows the American Veterinary Medical Association definition for therapeutic antibiotic use; including all disease prevention uses in therapeutic use. Therefore, although the survey reports the quantity of antibiotics used for „growth promotion," in the United States in 1999, this quantity cannot be compared to quantities of antibiotics used for growth promotion reported in Europe because the definition of growth promotion use in Europe is broader than the definition used by the Animal Health Institute. In September 2002, the Animal Health Institute issued a press release describing the summary results from a additional membership survey of the quantity of antibiotics, this one conducted used in 2000; few details are provided (http://www.ahi.org). The new survey reports a small but important decline in the use of antibiotics in food animals compared to 1999. A thorough independent review of these findings would be very useful, particularly because several food animal companies have announced voluntary reductions in nontherapeutic antibiotic use.

The Union of Concerned Scientists has published an estimate of the quantities of nontherapeutic antibiotics used in the United States in a recent publication entitled „*Hogging it*" (http://www.ucsusa.org); to enable comparison to other countries, including those in Europe, short duration disease prevention uses are not included in their estimates. This report provides detailed estimates of the quantity of nontherapeutic antibiotics used in the United States in the cattle, swine, and broiler chicken industries in 1999, and a compares these estimates for the quantities used in 1985. Although the estimates

have not been validated, the report provides a detailed explanation of the methods used to derive estimates.

The Union of Concerned Scientists derived the estimates by using a combination of available data from the United Stated Department of Agriculture on the number of animals on farms and slaughtered each year in the United States, and published reports of surveys conducted of cattle and swine farms describing how nontherapeutic antibiotics are used. Estimates of which nontherapeutic antibiotics are used on chicken farms is derived from expert opinion. The report divides that number of animals on farms into several commonly used stages (i.e., starter, feeder, finisher) of production using the United States Department of Agriculture data and allocates the animals, using the survey and expert opinion, to various nontherapeutic antibiotic use patterns; not all animals are assumed to receive nontherapeutic antibiotics. The dose of nontherapeutic antibiotics used in the animal feed during each production stages is derived from the label indications of the antibiotics approved by the United Stated Food and Drug Administration. Again using United States Department of Agriculture data, the number of days an animal stays in each production stage and the amount of feed consumed is determined. Estimates of the quantity of nontherapeutic antibiotics used are derived for the animals on each nontherapeutic use regiment by multiplying the number of days in each production stage, by the amount of feed consumed and the dose of nontherpeutic antibiotic in the feed. A final estimate of the quantity of nontherpeutic antibiotics used in each industry is derived by summing the uses for each nontheraputic use regiment.

There are approximately 35 million cattle slaughtered each year in the United States, of which approximately 30 million cattle are fed in feedlots prior to slaughter. Cattle are given limited amounts of antibiotics prior to arrival in feedlots. Depending on the age and size of cattle when purchased, cattle spend various amounts of time in feedlots. Cattle typically spend approximately 145 days in a feedlot, but may spend up 235 days in feedlot. While in a feedlot, most cattle are feed nontherapeutic antibiotics for growth promotion and disease prevention

(e.g., prevention of liver abscesses). Antibiotics commonly used nontherapeutically in cattle in feedlots in the United States include monesin, lasalocid, clorotetracycline, oxytetracycline, and tylosin; chlorotetracycline and oxytetracycline, and an analog of tylosin (erythromycin) are commonly used in human medicine. The Union of Concerned Scientists estimated that approximately 1,600,000 kilograms of antibiotics were used nontherapeutically in cattle in the United States in 1999, of which 40% were antibiotics commonly used in human medicine. For comparison, an estimated 1,200,000 kilograms of antibiotics were used nontherapeutically in cattle in the United States in 1985.

There are approximately 100 million swine slaughtered each year in the United States. Piglets are commonly weaned at 14 to 21 days in the United States, and spend approximately 40 days as „weaner" pigs, 40 days as „feeder" pigs, and 90 days as „finisher" pig; pigs are commonly slaughtered at approximately 185 days of age. Antibiotics commonly used nontherapeutically in swine in the United States include bacitracin, chorotetracycline, oxytetracycline, and tylosin; each of these antibiotics are commonly used in human medicine. The Union of Concerned Scientists estimated that approximately 4,800,000 kilograms of antibiotics were used nontherapeutically in swine in the United States in 1999; of which 90% were antibiotics commonly used in human medicine. For comparison, an estimated 5,000,000 kilograms of antibiotics were used nontherapeutically in swine in the United States in 1985.

There are approximately 7,800 million broiler chickens slaughtered each year in the United States. Chickens are commonly slaughtered at approximately 42 days of age. Antibiotics commonly used nontherapeutically in chicken in the United States include virginiamycin, monesin, roxarsone, bambermycin, lasalocid and lincomycin; an analog of virginiamycin (dalfopristin/quinupristin) and lincomycin (clindamycin) are commonly used in human medicine. The Union of Concerned Scientists estimated that approximately 4,700,000 kilograms of antibiotics were used nontherapeutically in chicken in the United States in 1999, of which 10% were antibiotics commonly used in human medicine.

For comparison, and estimated 1,600,000 kilograms of antibiotics were used nontherapeutically in chicken in the United States in 1985; the remarkable increase in nontherapeutic antibiotic use in chicken reflects the large growth of the industry during this period.

The Union of Concerned Scientist also estimated that 1,300,000 kilograms of antibiotics were used nontherapeutically in food animals other than cattle, swine or chickens (e.g., turkeys, sheep) in the United States in 1999. Taken together, an estimated 12,300,000 kilograms of antibiotics were used nontherapeutically in food animals in the United States in 1999. Furthermore, the Union of Concerned Scientists estimated that approximately 700,000 kilograms of antibiotics were therapeutically used in food animals in the United States in 1999. Therefore, the Union of Concerned Scientists estimated that approximately 13,000,000 kilograms of antibiotics were used in food animals in the United States in 1999. Alternatively, the Animal Health Institute estimated that 9,000,000 kilograms of antibiotics were used in food animals in the United States in 1999.

Despite the variations in the estimates by the Union of Concerned Scientists and the Animal Health Institute, it is clear that the quantity of antibiotics used in food animals is vast. Furthermore, the Union of Concerned Scientists estimates that over half of the nontherapeutic antibiotics used in food animals in the United States are antibiotics commonly used in human medicine; antibiotics used in human medicine are not allowed to be used for growth promotion in Europe. The Union of Concerned Scientists also provides evidence that the quantity of antibiotics used nontherapeutically in chickens has remarkably increased in the past two decades. To combat increasing antibiotic resistance, the misuse and overuse of antibiotics needs to be reduced. Restrictions on certain antibiotics uses are scientifically justified and will protect the public health.

There are several strategies aimed at reducing the misuse and overuse of antibiotics used in food animals. Recently, several companies in the United States, citing consumers concerns about antibiotic resistance, have voluntarily announced their intention to only purchase chicken raised without the nontherapeutic use of antibiotics that are used in human medicine. These companies include Kentucky Fried Chicken, Subway, Hardees, Dominos, and Dairy Queen; Kentucky Fried Chicken is the single largest purchaser of chicken in the United States. Furthermore, several poultry companies have also announced their intention to raise chickens without the nontherapeutic use of antibiotics that are used in human medicine. These companies include Foster Farms, Perdue and Tysons. In addition to voluntary actions, the United States Food and Drug Administration has initiated a new process for evaluating and ensuring the human safety of antibiotics, including growth promoters, for use in food animals. Each antibiotic will be evaluated on an individual basis, taking into account its importance in human medicine and the extent of human exposure that would result from the proposed animal use. The proposed process for conducting this evaluation is complex and relies in part on pre-approval studies and post-marketing surveillance to evaluate potential and documented human health hazards. This process, if implemented in an appropriate and timely manner, has the potential to offer equivalent public health protection as the current European ban on the use human antibiotics as growth promoters. In addition to these voluntary and regulatory actions, there are also currently bills in the United States House of Representatives and Senate that, if enacted, would amend the Federal Food, Drug, and Cosmetic Act to prohibit the nontherapeutic use of human antibiotics in food animals. Such action would ensure that the nontherapeutic use of antibiotics in food animals does not compromise human health by contributing to the development of antibiotic resistance.

There is also a need to conduct research to determine the consequences of nontherapeutic antibiotic use in food animals. Conducting such research is a high priority item in the United States Interagency Action Plan to combat antibiotic resistance. The Centers for Disease Control is collaborating with several partners to foster applied public health research in this arena; currently two Cooperative Agreements have been established with Schools of Veterinary

Medicine to compare the consequences of raising pigs without nontherapeutic antibiotics in condition of modern husbandry. The National Pork Board has also recently funded research on alternatives to antimicrobial growth promoters. Among the important new research is a report of a large clinical trial conducted by Dritz et. al (*JAVMA*2002;220(11)1690-1695). Results from this research suggest that use of antibiotics for growth promotion should be limited to the nursery phrase (i.e., early period in the weaner stage) in pig production; use during the finishing pigs did not improve growth. According to the Union of Concerned Scientists report, the majority of nontherapeutic antibiotics used in swine in the United States are used in finishing pigs. There have also been several recent economic analyses as to what would be the consequence if the use of growth promoter antibiotics were to be discontinued in the United States. These studies have not been highly useful for several reasons. The recent studies overestimate the average daily gain for each pig, often using a rate of 5%; it is not clear that pigs or poultry will achieve such increases in weight gain in conditions of modern husbandry. Further research is clearly needed on the consequences of no longer using medically important antibiotics to promote the growth of food animals .

Conclusion

Compelling scientific evidence indicates that the use of antibiotics in food producing animals can lead to adverse public health consequences due to emergence of resistant bacteria that can be transmitted to humans through the food supply or direct contact with animals. An area of particular public health concern has been the prolonged feeding of antibiotics in low doses to animals as growth promotants. Comparing the patterns of antibiotic use in various countries or regions would be useful to understand the epidemiology of antibiotic resistance. An important barrier to the comparison of the patterns of use of antimicrobial growth promoters in United States with the pattern of use in elsewhere, including Europe, are the definitions used to describe how antibiotics are used in the United States. An additional important barrier is the absence of validated antibiotic use information; absence of such data makes interpretation of

human surveillance data complex and requires a more conservative approach to antibiotic use in food animals. The two recently available estimates of the patterns of antibiotics used in food animals in the United States demonstrate that the quantity of antibiotics used in food animals in the United States is vast. There is a need for a more precise antibiotic use reporting system in the United States for antibiotics used in food animals. Precise antibiotic use data is vital for monitoring voluntary and non-voluntary actions aimed at reducing the misuse and overuse of antibiotics, and thereby combating increasing antibiotic resistance.

17. Use of antimicrobial growth promoters – does it play a role in combating malnutrition in the developing world?

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Abstract

Daily protein intake remains inadequate in many countries. It has been argued that because antibiotics increase meat production when routinely added to animal feed, this will therefore help alleviate protein malnutrition. In developing countries any increase in meat production however is very unlikely to lead to an increase in protein intake by most of those who are malnourished. The poor currently consume little or no "industrialized" meats. Unless their incomes rise substantially they are also unlikely to have access to these meats in the future because relatively "vegetals" will continue to be much cheaper as a calorie and protein source. In addition most of the poor live in rural areas with poor infrastructure, which means they will also not be able to access these "industrialised" meats.

Many assume that antibiotics produce a 10% growth improvement in "industrialised" meat production. Therefore the expected effect on the dietary intake of protein of a population if growth promoters were not used can be estimated. If we assume that antibiotics are used in all poultry and pork production, then their cessation would result in a fall in protein intake of 0.5 g/d per person when all developing countries were grouped together (or 0.7% of total protein intake; 1997 FAO intake figures). Average daily intake would have been 66.5 g/d (well above the WHO/FAO recommended minimum of 50 g/d). It is also likely in developing countries that the weight gain benefits in animals receiving antibiotics are 2% or less, and that all poultry and pork do not receive them. Therefore any resultant decrease in dietary protein intake would be much less than 0.5 g/d.

In developing countries those who are relatively more affluent purchase and eat "industrialised" meats. The intake of "industrialized" meats is associated with an increased fat intake. This is likely to contribute to obesity, which is the current major nutritional problem in most developing countries rather than malnutrition.

In conclusion there is no evidence that the use of antibiotics as growth promoters in food production animals leads to improved health in the poor (or the malnourished) by increased dietary protein intake in these people or helps them economically. This practice however does increase the exposure of the human population to antibiotic resistant bacteria.

Improved meat production in developing countries; does it help malnutrition?

In developing countries there remain large numbers of people who are malnourished and suffer from protein/energy deficiency. Animal products (meats and milk) have been regarded as one of the best ways of overcoming this problem because meat has a much higher protein content compared to vegetal products. Therefore practices that promote meat production (such as antimicrobial growth promoters) are believed to improve protein production and therefore alleviate malnutrition in developing countries.

Poultry and pork are the main livestock in which continuous in-feed antibiotics are used (for growth promotion and for prophylaxis). Most cattle and sheep in developing countries are raised on grasslands and so do not receive antibiotics as in-feed additives. Antibiotics are used in aquaculture but very limited data is

available. Therefore in evaluating the potential value of continuous in-feed use of antibiotics in developing countries as growth promoters, this report has focused on poultry and pork production. Pork and chicken meats have had the largest increases in both meat production and consumption in the last 40 years in developing countries (over 500% increase in supply per capita since 1960).

Do antibiotics still work when used as growth promoters?

There is an assumption that significant weight gains in animals will result from the in-feed use of antibiotics, especially in developing countries and it is often assumed to be about 10%. However it remains unclear how much (if any) decrease in production of meats will result if antibiotics are not used routinely as in-feed additives in "industrialized" meat production. There have never been any double-blind, placebo controlled trails on the use of continuous in-feed antibiotics for growth promotion even in developed countries. The benefits of antibiotics remain based primarily on a belief of efficacy rather than on any recent data. Published data from developed countries often shows no benefit. Data from pharmaceutical companies on their own products show weight gain benefits of 2% or less (JETACAR 1999) and the benefits from competitors' products usually only show minimal or very small increase in weight gain compared to controls (e.g. 0.2%).

In Denmark all poultry manufacturers voluntarily ceased all in-feed use of antibiotics in February 1998. Chicken production has not suffered economically as a result. Meat production and chicken weights were maintained, as was output of chicken meat per square meter of pen size (Emborg et al, 2001). The only economic parameter that worsened without antibiotic use was the very small amount of extra feed that was used. The FCE increased by 0.9% (0.016 kg/kg). Antibiotics also do not usually improve mortality in developed countries (e.g. mortality rate 3.9% in controls compared to 4.8% in broilers on virginiamycin; JETACAR 1999). In Denmark there was no increase in mortality when in-feed antibiotics were no longer used in poultry (Emborg et al, 2001).

Data from developing countries is much more limited. Because animals may be under greater stress (poor food supplies or heat), it is often assumed that weight gain benefits will be much greater in these countries. Data was submitted from some developing countries to WHO following the "call for data" which preceded the Oslo meeting in 2001. Unpublished studies involving over 5 million chickens by poultry producers in Brazil suggests that the weight gains are variable and at most about 2%. In one study no weight gain with antibiotics occurred compared to controls (-1.9% wt gain) although there was a slight improvement in feed efficiency (0.5%) and mortality (0.6%). One small study from India suggests that a 9% weight gain can occur with antibiotic use (Mohan et al 1996). However in this latter study the final weigh of the chickens at 6 weeks on antibiotics (1141 g) was only about half the weight of chickens reared without continuous antibiotics in developed countries (e.g. Denmark) or in Brazil.

WHO/FAO recommendations for daily protein requirements

In the developing world calorie intake has increased markedly in the last 30 years and now exceeds recommended minimum intakes for an individual (2,100 calories per day) in most countries. Protein intake however is still below recommended levels in many countries. The average protein intake for adults in a population should exceed 50 g/day (or >0.8 g/kg/day; WHO 1997).

Mal-distribution of foods

In most developing countries there is an uneven distribution of foods between different segments of the population. Those living in rural areas are more likely to be malnourished than those living in cities. Rural areas are where most of the poor and malnourished in developing countries live. Generally those who live in cities are more affluent and generally better feed. Indeed in almost all developing countries there are already substantial numbers of the population who are obese and obesity levels are rapidly rising (both adults and children).

WHO and others have now identified obesity as one of the major health problems in most developing countries (WHO 1990 "Diet, Nutrition and Prevention of Chronic Diseases"). Relatively small increases in income are associated with large increases in animal product consumption and obesity. Increased intakes of sugars and saturated fats are major factors in these rising obesity rates (and the health problems associated with obesity - cardiac disease, diabetes and some cancers). In the WHO report on this issue important points that were made include:

- food of animal origin are no longer viewed as dominant items in an optimal healthy diet
- both saturated fat and dietary cholesterol are abundant in fatty products of animal origin
- that plant food provides protein as well as numerous vitamins and minerals.

"Authorities in developing countries are cautioned not to imitate agriculture, farming, the food production and promotion policies that were designed to emphasis the production of animal products and are based on nutritional knowledge long since outmoded. The difficulty of altering such policies now apparent in many wealthy nations, serve as a further warning against their introduction."

The poor do not eat meat produced with continuous in-feed antibiotics

70% of world's poor live in rural areas. These areas usually have poor infrastructure, food distribution networks and refrigeration. This means that little "industrialized" meats will be distributed and available for sale where these people live. They therefore do not have access to these types of meats. The malnourished and poor in these countries eat and consume very little (an in most cases none) of the "industrialized" meats that are produced in those countries. What chicken and pork the poor do eat in rural areas is usually locally produced from animals that eat by scavenging. Continuous in-feed antibiotics are not used in the production of these animals.

Most if not all "industrialized" meats that are consumed within developing countries are sold and eaten in or near cities. The vast majority of these "industrialized" meats are being eaten by the relatively affluent in those countries whose major nutrition problem is not malnutrition but rather obesity. Therefore even if the continuous in-feed use of antibiotics in these countries was banned and there was a decrease in production by as much as 10% of "industrialized" meats, then this is not very likely to cause malnutrition in any segment of the population.

The poor are "sellers" of meats rather than "buyers" or consumers. Most cereals are about 15% of the price of meat for the same calorie value. Therefore the poor will buy cereals rather than meats as foods while money remains in short supply. Meats are regarded as "luxury" items. The poor therefore do not have access to these meats because of income constraints. In developing countries the consumption of meat is "demand" driven and income dependent. Significant quantities of meat are not purchased and consumed until people have enough money to afford them. This does not occur until people have already passed minimum protein and calorie intakes through the intake of cereal and other vegetal products. Improved access to meats is dependent on increasing the income of the poor rather than increasing the supply of meat.

The contribution of "industrialized" meats to improved nutrition in a nation is negligible

Poultry and pork are the main "industrialized' meats produced in developing countries. FAO (Food and Agriculture Organization of the United Nations) data can be used to evaluate the contribution of each of these meats to a nation's nutrition (FAO 1997 data). The effects that removing the use of antibiotics as growth promoters may have in developing countries can be estimated using the FAO nutrition database. For the purposes of these calculations we will make an assumption that all poultry and pork produced receive in-feed antimicrobials and the growth promotion effect of this is 10%.

If these antimicrobials were not used, then total daily protein intake of the population would fall by 0.5 g/d if production fell by 10% (and thus the intake of pork and poultry) when all developing countries are combined. This however represents only a 0.7% reduction in daily pro-

tein intake to 66.5 g/d. Fat intake would also fall, but by a larger amount (1.1 g per day or 1.8%).

In no developing country would removing 10% of "industrialized" meat production shift the protein intake down to levels that would be deleterious a population's nutrition (see table 1 and figure 1). In those countries where protein intake was already above 50 g/d, then the per capita protein intake remained above this level. For those countries with a protein intake below 50g/day, then pork and poultry intakes are usually very small. A 10% drop in this pork and poultry intake therefore makes almost no change to the population's average daily protein intake. The largest effects were seen in coun-

tries where there are already high average daily protein intakes (e.g. China and Argentina).

It is more likely that in these countries the weight gains benefits in animals from in-feed antibiotic use are 2% or less rather than the 10% assumed and also that not all pigs and poultry will have received these antibiotics. Therefore the resultant loss of protein intake if antibiotics were not used would be much less than even these modest decreases in intake estimated above. If only a 2% weight gain occurs with antibiotic use and only half the animals received antibiotics, then for "all developed countries" combined the fall in protein intake would have been only 0.05 g/d (or 0.07% of daily protein intake).
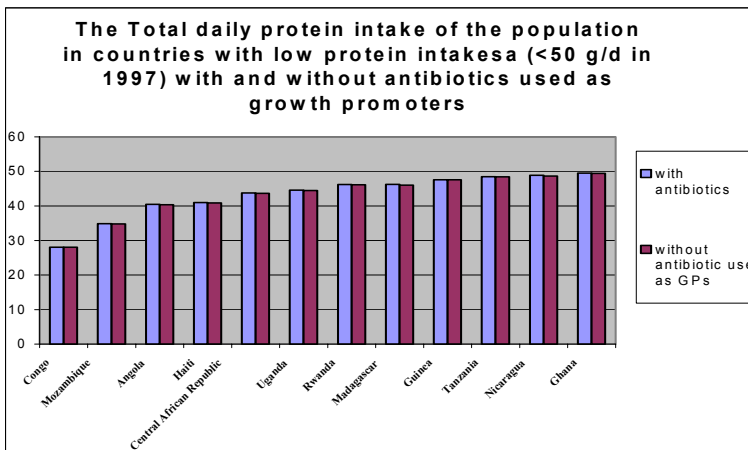
Figure 1.

Table 1. Meat Supply changes in developing countries if antibiotics were not used (1997)

1997 DAILY PROTEIN INTAKE	Pig meat	Poultry Meat	Grand Total	Pig meat plus poultry	10% of pig/pork	Grand Total if no Antibiotics used	% fall in protein intake
Angola	0.8	0.4	40.5	1.2	0.12	40.38	0.3
Brazil	2.3	7.6	76.4	9.9	0.99	75.41	1.3
China	8.6	2.8	77.6	11.4	1.14	76.46	1.5
India	0.2	0.2	59.2	0.4	0.04	59.16	0.1
Indonesia	0.9	1.7	66.5	2.6	0.26	66.24	0.4
Mexico	3.1	5.3	82.5	8.4	0.84	81.66	1
Thailand	1.9	5.3	53.6	7.2	0.72	52.88	1.3

The use of in-feed antibiotics may contribute to an adverse effect on nutrition in a country. The segment of the population that is currently eating most of this industrial meat production (and their high content of saturated fats) has a problem with obesity rather than protein-energy malnutrition. Decreasing the supply of "industrialized" meat by 10% should lead to a decrease in overall consumption of meat by a similar amount in the population. If pork is a major component of this industrialized meat production, then a 10% decrease in meat intake will be associated with a much greater decrease in fat intake compared to the fall in protein intake. In China in 1997 there was a 26.6 g daily intake of fat from pork compared to an 8.6 g intake of protein. Therefore every 1 g of protein intake from pig meat is associated with a much larger intake of fat (3.1 g).

The use of in-feed antibiotics may harm the poor economically

The use of in-feed antibiotics is likely to harm the poor economically in developing countries because they will likely receive lower prices for their livestock, they may pay higher prices for cereals and there may be less employment in the agricultural sector.

The poor are usually "sellers" of meats rather than "buyers". Large-scale industrial meat production leads to an increased supply of meats in a country. When supply significantly increases, then prices will usually fall. This means that overall the poor are likely to receive lower prices for their animals.

The price of meat will however not fall low enough so that the price now becomes comparable to cereals as a source of calorie intake in their diet. Most cereals are about 15% of the price of meat for the same calorie value. Therefore the poor will buy cereals rather than meats as foods while money remains in short supply or unless the price of meat dropped by about 85%. Lack of income by the poor therefore will still usually preclude meats as a significant dietary source of protein.

In the market place there is not a "level playing field". From a financial perspective, tax breaks are often given to the industrialized sector as well as subsidies. Also infrastructure support (e.g. roads, water etc) are usually built and paid by government. This means that the animals produced by the "industrialized" sector can reach markets more easily and be sold for lower prices in the market place than would be the case without these subsidies. These lower prices are of benefit to consumers. However it also means that lower prices are received for the animals the poor sell in the same market. The poor however do not usually receive any of these subsidies.

The poor however may have to pay higher prices for cereals, which are their main nutritional intake. Poultry and pork in "industrialized" production need a predictable supply and good quality cereals for feed. Many of these cereals however can be the same foods as the poor eat. The poor are "buyers" of cereals. Because those in the "industrialized" meat sector are also buyers of cereals, there will therefore be more "demand". Higher prices are likely to result, unless "supply" also increases.

Increased employment in the "industrialized" meat sector is not likely to significantly reduce poverty levels. While there will be some individuals who will find jobs in this sector, overall however there will be less employment in the farm sector. This is because the "industrialized" meat industry is a capital and energy intensive industry rather than labour intensive.

In conclusion there is no evidence that the use of antibiotics as growth promoters in food production animals leads to improved health in the poor (or the malnourished) by increased dietary protein intake in these people or helps them economically. This practice however does increase the exposure of the human population to antibiotic resistant bacteria.

References
Beasley P., Ashley, S. and Holden, S. Livestock in Development 1999. Livestock in poverty-focused development. Outhouse Publishing Services Crewkerne.

Emborg, H., Ersboll, A., Heuer, O. and Wegener, H. 2001. The effect of discontinuing the use of antimicrobial growth promoters on the productivity in the Danish broiler production. Prev. Vet. Med. 1615, 1-18.

FAOSTAT 1998 database CD ROM. FAO Rome. 1999.

Joint Technical Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR). 1999. Australia. www.health.gov.au/-pubs/jetacar.pdf.

Mohan, B., Kadirvel, R., Natarajan, A. and Bhaskaran, M. 1996. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers. Br. Poult. Sci. May; 37, 395-401.

Proudfoot, R.G., Jackson, E.D., Hulan, H.W. and Salisbury, C.D.C. 1985). The response of male chicken broilers to the dietary addition of virginiamycin. Poultry Sci. 69, 1713–1717.

WHO. Diet, Nutrition and Prevention of Chronic Diseases. WHO/CPL/CVD/NUT/91.1. World health Organization, Geneva, 1990. Technical series report 797. http://-www.who.int/dsa/cat98/nut8.htm#Diet.

18. Discontinuing the use of antimicrobial growth promoters – the Swedish experience

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Abstract

In Sweden the use of antimicrobials for growth promoting (AMGP) was banned 1986. No negative clinical effects were reported as a consequence of the ban in production of fattening pigs, specialised beef, and turkey or in egg production (layers). In the broiler chicken production expected problems with necrotic enteritis were prevented largely by a continuous use of antibiotics during the first two years after the ban. Following the implementation of results from research during that period the general usage of antibimicrobials could be stopped and expected problems with outbreaks of necrotic enteritis was prevented.

In weaner pig production significant clinical problems emerged which created a demand for antibiotic-medicated feed at therapeutic dosages. During the subsequent four-year period the use of antibiotics increased, involving up to 75% of the pigs. That use then decreased because of improved management, and could be halved in 1993 followed by a gradual further decrease supported by the addition of zinc oxide to the feed. In 1998, compared to 1994 that use of zinc had decreased by 90% and in 1998/9 only 5% of the weaned pig producing herds used antibiotic medicated feed and 17% used zinc.

It has been shown that under good production conditions it is possible to reach good and competitive production results for the rearing of poultry, calves and pigs without the continuous use of AMGP. As a result of the ban, focus on disease prevention and correct use of antimicrobials, the total use of such drugs to animals has decreased by approximately 65% during the last 15-year period. A relatively low prevalence of antimicrobial resistance has been maintained.

Introduction

Antimicrobial growth promoters (AMGP) have been banned in Sweden since 1986. This paper summarizes the experiences gained of the ban.

Material and methods

The material and methods are previously presented (Wierup 2001). An official inquiry has presented the scientific base for the ban as required by the EU Commission (SOU 1997). These publications give all significant data and references involved. In order to limit this presentation no additional references are included apart from main source of recent data (SWARM 2001).

Results

Broiler production
The introduction of AMGP in the 1970s almost eliminated problems with necrotic enteritis. A ban of AMGP was consequently foreseen to have clinical consequences due to that disease.

Efforts including research were undertaken to find ways to control necrotic enteritis in the absence of AMGP. It was concluded that many factors such as the construction and climate of stables, hygiene, management and feed could contribute to the outbreak of necrotic enteritis.

The results and experiences gained were continuously implemented. The most important change was to reduce the protein content in the feed. Cases of necrotic enteritis can today often be related to overriding the recommended level of protein in the feed. Strong emphasis was placed on improving animal environment because many diseases including necrotic enteritis have a multifactorial background. Notably air-

flow was often inadequate, which affected animal health negatively.

The ban was implemented too quickly. Enough time to adjust to the new situation was not given. About 90% of broiler chickens were therefore continuously treated with virginamycin at a dose of 20 ppm during the first year of the ban and in 1987 the corresponding treatment frequency was 100%. Prior to the ban virginamycin was dosed at 10 ppm.

During 1987 a two-day treatment with phenoxy methyl penicillin in drinking water largely replaced the use of virginamycin. The amount of antibiotics used for treatment of necrotic enteritis subsequently decreased from about 2 tons of virginamycin in 1987 to 100 kg of phenoxy methyl penicillin in 1988. From 1995 the amount of antibiotics to prevent or cure outbreaks of necrotic enteritis is negligible, and today tylosin is used when outbreaks occur.

It should be noticed that coccidiostats of the ionophore type now used have antibacterial effects and act prophylactically against necrotic enteritis. It is also found that so far the use of the ionophores is needed to control outbreaks of necrotic enteritis. However, despite this, the sanitary situation of broiler chicken rearing gained in Sweden would not have been reached without the above-mentioned enforcement.

Contributing to the successful adaptation to the new situation in 1986, and to the continuously ongoing efforts to improve animal health was the creation of a classification system for breeding farms and production farms. A bonus is given for good animal management and care. The basic allowed population density in chicken production is 20 kg/m^2. Producers who satisfy specified requirements are permitted a limit up to 36 kg/m^2. The best growers can be rewarded, while growers with low standards are forced out of business.

The control of salmonella, started already in 1970, as well as the efforts to control also the intestinal colonisation by campylobacter, started in 1987, were certainly also of importance for the adaptation. These controls have improved the general health situation and also facilitated

further organised actions. The fact that the poultry and especially the chicken producers only to a very limited extent have been covered by subsidies and economic guarantees, in contrast to other sectors of the animal production is probably also of importance. They were used to a free market situation and had to be responsible for their own economy.

The relatively low number (200) of chicken producers at the time of the ban also facilitated organised and fast actions. They generally also had a relatively uniform production system. Their annual production was 37 million chickens. This has increased 100% to 73 million in 2001 while the producers have decreased to 175.

Turkey production
As for the broiler production the AMGP were before 1986 used as a prophylactic agent against necrotic enteritis. However, no clinical problems was observed as a result of the ban

Egg production
No AMGP were used in egg production at the time for the ban, which consequently did not influence production. Neither is the animals treated with coccidiostats during the egg production period. Problems with coccidiosis, which may occur during the growing stage, are usually concentrated to farms where the level of animal hygiene is not satisfactory. The availability of vaccine against coccidiosis has reduced the need of coccidiostats in floor raising of laying hens.

Pig production
Before 1986 practically all piglets were given the AMGP, from the start of their feeding period until delivery to the finishing units at an age of 10-12 weeks. Thereafter as finishing pigs, they were given AMGP (avoparcin or virginamycin) until slaughter at the age of about 7 months.

The ban on AMGP did not create obvious clinical problems for finishing pigs, which in 1984, consumed 7.8 tons of active ingredient of virginamacin. Since 1997 the mean daily weight gain

in herds joined to production control systems is above 850 grams and not known to be larger in countries where AMGP were used.

However, for the weaner pig production, significant problems initially emerged following the ban. The postweaning mortality during the first year after the ban was significantly higher, about 1.5 percentage units compared with 1985. Similarly, the age at 25 kg increased by 5-6 days. An extended rearing period after the ban is still also obvious from the National Pig Record by which the age of pigs tested at 30 kg increased by about two days from 1986 and onwards.

In contrast to the broiler chicken sector, the pig sector did not generally continue to use antibiotics on prescription in therapeutic doses. The use of olaquindox to weaning pigs was therefore reduced by 82% from 1985 to 1986 and by 33% from 1985 to 1987. However, more antibiotics were later prescribed and the total amount used during 1988 and 1989, was 5 to 6% higher than in 1985. Considering that the dosage prescribed from 1986 was about three times higher than the AMGP dosage employed previously, a smaller fraction of weaning pigs was treated with olaquindox after the ban or 12% in 1986, 55% in 1987, 75% in 1988 and 76% in 1989. Thereafter the use was continuously reduced and in 1993 it was 35%. From 1993-94 a further reduction occurred supported by the introduction of zinc oxide (see below). In 1995 the proportion of pigs treated with olaquindox was 12%.

In a study from 1994 it was concluded that the prescription of medicated feed to weaning pigs was clinically motivated and followed recommended guidelines. It was also found that the need for medicated feed differs markedly between herds depending on housing and management systems. The production results were related to the degree of segregated rearing systems and the level of hygiene. In herds rearing post-weaning pigs on deep litter bedding both segregation and degree of hygiene was better and the use of antibiotics three to four times lower than in herds with pigs in traditional post-weaning pens. Emerging clinical problems could not be prevented through single prophylactic actions. In many cases, more thorough

changes in production planning and in housing were needed.

Following the AMGP- ban, numerous measures are continuously being undertaken to optimise the feed and rearing and production system with the focus on receiving sectioning, age grouping and planned production. New rearing systems were created, as weaning of piglet on deep litter beds in large groups and a system, which is based on production in the same pen from birth to slaughter. The adjustment of old buildings to improved production system is expensive and until this is performed antibiotics are used to combat weaning diarrhoea in exposed herds. It is also found that the use of antibiotics prevents low production results in such herds.

Veterinary guidelines for prescription of antibiotics were developed 1990. It is emphasised that prescription should always be accompanied by recommendations on prophylactic measures. Education of veterinarians and producers was followed but intensive education of the pig producers and veterinarians on how to prevent postweaning diarrhoea started up first during 1998/99. The motivation at that time increased with the decreasing payment to the producer due to worldwide overproduction.

Since the end of 1993 zinc oxide has been used to prevent weaning diarrhoea as previously was employed in other countries in Europe. Zinc oxide was found to have a preventive effect on weaning diarrhoea equal to the effect reached when using olaquindox. The use of zinc became widespread but due to actions taken the usage from 1994 to 1998 decreased by 90%. During 1998 to 1999 17% of the piglet producers used zinc and 5% used antibiotics to weaner pigs. The launch of a programme for intensive education of producers in implementing optimal management conditions at weaning has later further improved the situation.

It is evident that the best production result can be gained without the use of AMGP. However, all herds do not have optimum conditions for production. Consequently, certain production results seen before the AMGP-ban have still not been reached. A comparison of data for 1997 with those for the first year after the ban reveals

post-weaning mortality to have decreased by 1-2 percentage units and the age at 25 kg to be reduced by 3.5- 4.5 days. The losses in these production parameters seen after the ban have thus not yet been fully recovered on a national base. However, the progressive producers report better production results than before the ban.

The relatively slow start in introduction of compensatory actions in the weaner pig production compared with broiler production may reflect that the pig industry did not anticipated clinical problems following the ban. In addition the number of the weaner pig producers at the time of the ban was large, approximately 10,000 compared to 200 producers of broiler.

Specialised beef production
The use of AMGP in specialised beef production had voluntarily more or less come to an end before the ban in 1986. Negative clinical or other effects as a consequence of the ban have not been reported. None of the AMGP used in calves is today used as therapeutics. Guidelines have been worked out when it is justified to treat all calves in a batch instead of individual treatments.

Use of antibacterial drugs
The total usage of antibacterial drugs to animals in Sweden has been regularly studied since 1980 (Figure 1; Table 1). When AMGP was banned the use of antibacterial drugs decreased by 49% (24,8 tonnes from 1984 to 1986). During the following years the total usage increased but was then, during 1988 to 1994, stable at approximately 30 tonnes per year, a level approximately 35% below the level before the new legislation was introduced. The consumption then continuously decreased to 17.3 tonnes during 2001, a level approximately 65% lower than the usage before the AMGP- ban. This decrease is at least of that magnitude when calculations is measured by dose units instead of weight of active substance. The data are analysed and e.g. presented as sales of antimicrobials divided into formulations intended for treatment of individual animals and for group treatment. Defined daily dose for cows (DDDcow) has been introduced to facilitate evaluation of data. The possible influence of the ongoing change in the animal production (e.g. decrease in pig and increase in poultry) has not been fully evaluated.

1. Antimicrobial Growth Promotor;
Source: SWARM 2
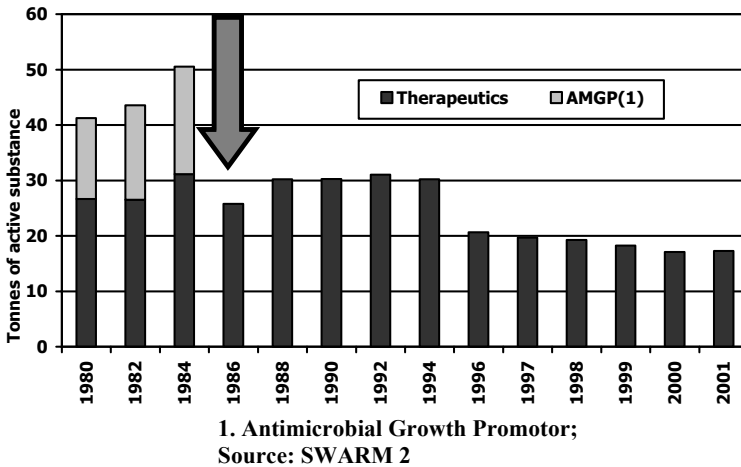
Figure 1. Total sale of antimicrobials for animal use in Sweden

Table 1. Total sale of antimicrobial drugs for animal use in Sweden (kg active substance)

ATC vet code	Substance class	Year													
		1980	1982	1984	1986	1988	1990	1992	1994	1996	1997	1998	1999	2000	2001
QJ01A, QG01AA	Tetracycline's	9.819	10.765	12.955	6.585	4.691	4.572	8.023	7.730	2.698	2.558	2.897	2.251	1.754[2]	1.453[4]
QJ01B	Chloramfenicols	47	40	49	41	35	21	-	-	-	-	-	-	-	-
QJ01CE, QJ01R, QJ 51R	G-and V penicillins[1]	3.222	4.147	4.786	5.933	7.143	7.414	7.446	10.374	8.818	8.781	8.547	8.692	8.254	8.414
QJ01CA, QJ01CR	Aminopenicillins	60	248	714	540	655	738	837	941	835	841	824	809	852	752
QJ01D	Other betalactam-antibiotics	9	6	2	-	-	-	-	-	-	53	133	245	315	474
QJ01G, QJ01R, QJ51R, QA07AA	Aminoglycosides	5.274	4.776	5.608	2.885	3.194	2.539	2.139	1.696	1.164	1.077	930	846	794[4]	770[4]
QJ01E, QA 07AB	Sulphonamides	6.600	4.931	4.325	3.093	3.072	2.510	2.362	2.323	2.198	2.151	2.345	2.403	2.338	2.485
QJ01E	Trimetoprim and derivatives	134	142	186	197	250	272	284	352	339	352	390	397	390	414
QJ01F	Macrolides and lincosamides	603	616	887	1.144	1.205	1.398	1.710	1.852	1.649	1.747	1.846	1.467	1.352	1.510
QJ01MA	Fluoroquinolones	-	-	-	--		84	147	246	173	179	175	155	156	182
QJ01XX92, QJ01XX94	Pleuromutilins	-	-	-	-	124	229	268	465	1.142	1.094	1.032	847	871	841
QJ01MB	Quinoxalines	6.250	7.700	9.900	1.300	7.164	5.778	4.917	1.904	1.098	534	-	-	-	-
QJ01XX91	Streptogramins	-	-	8.800	1.610	1.088	2.413	1.275	600	525	288	150	125	-	-
QP51AA, QJ01BA	Other substances[2]	861	823	1.637	1.575	1.567	2.304	1.634	1.764	-	-	-	-	-	-
Not classified	Feed additives[3]	8.380	9.370	700	870	-	-	-	-	-	-	-	-	-	-
Total		41.259	43.564	50.549	25.773	30.189	30.274	31.043	30.247	20.639	19.655	19.269	18.237	17.079	17.295

[1] Calculated as benzyl penicillin;
[2] Mainly nitroimidazoles, QP5 1AA;
[3] Substances included are avoparcin, bacitracin, nitrovin, oleandomycin and spiramycin;
[4] Drugs marketed with special licence are included.; (Source : SVARM 2001)

Conclusion

The results from Sweden have been confirmed following in dept evaluations both domestically and on an international basis. More recently, results from withdrawal of AMGP have been reported also from Denmark indicating that the results are of a more general application.

A change in attitude towards a more prudent use of antimicrobials, including withdrawal of AMGP and the implementation of disease preventive measures, thus can result in a considerable decrease in the over all use of antimicrobials. In Sweden that decrease is more than 60% and similar results are also reported from Norway and Finland. This decrease should also decrease the risk for the emergence of antibiotic resistant strains.

The results also demonstrate that it is possible to achieve competitive production result without a continuous use of the antibiotics as AMGP. However, even if a stopped use of AMGP generally does not need to be accompanied by increased production costs it is likely that this initially and to some extent also permanently is the case e.g. for the production of broiler and weaner pigs. The latter attributes especially to investments to optimise the management and well fare necessary in the absence of AMGP. This is also considered as a positive but challenging situation when operating in a free market.

Motivation of producers and veterinarians towards a more prudent use of antimicrobials should be based on education. It also seems to be advisable to build up a system with an economic incitement for the producer to undertake actions. Finally, an ever-ongoing surveillance of antibiotic resistance and a control of the usage of different antimicrobials, is found necessary to enable evaluation of the impact of usage of antimicrobials in livestock production.

Acknowledgements

References

SOU 1997. Antimicrobial Feed Additives. Report from the Commission on Antimicrobial Feed Additives, Stockholm 1997: 132,ISBN 91-38-20707-9, http://jordbruk.regeringen.se/antibiotika/index.htm

SVARM 2001. Swedish Veterinary Antimicrobial Resistance Monitoring. National Veterinary Institute, Uppsala, Sweden, www.sva.se

Wierup, M. The Swedish Experiences of the 1986 Year Ban of Antimicrobial Growth promoters, with Special Reference to Animal Health, Disease Prevention, Productivity, and Usage of Antimicrobials. Microbial Drug Resistance, 2001,7, 183-190.

19. Animal health as affected by management procedures in commercial pig production

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Summary

Most diseases of importance in modern pig production are of an infectious nature. Whether infection of an animal causes disease or not, is a matter of change in the balance between infection pressure (load of infection) on one side and the immunity of the animal (resistance to the infection) on the other side. Diseases can be controlled in several ways, of which elimination of the pathogens (eradication) from the herd is by far the most effective way. However, depending on the severity of the disease problem, it might not necessarily be the most cost-efficient way. Other control measures include changes in management and housing conditions to eliminate known risk factors and/or use of relevant vaccines.

Today safe and reliable methods for the eradication of several infectious diseases exist. The method of partial depopulation seems relevant for some diseases, whereas total depopulation/repopulation is the only method for other diseases.

Optimal bio-security is of crucial importance for herds to stay free form new and unwanted infections. Remote location of farms, closed herds or use of quarantine facilities, entry rooms and delivery facilities for pigs for slaughter are central parameters.

When farmers want to build new facilities, there seem to be relevant production systems that favour a healthy production. The central words are batch production (AI-AO) with a minimum of transport and mingling of pigs, e.g. the wean-to-finish system.

Introduction

Control of diseases presents a challenge for pig producers and for veterinary advisors throughout the world. Most diseases of importance in modern pig production are of an infectious nature. Whether infection of an animal causes disease or not, is a matter of change in the balance between infection pressure (load of infection) on one side and the immunity of the animal (resistance to the infection) on the other side. Thus, most if not all efforts to reduce the prevalence of disease are by changing this balance in direction of low infection load and high immunity.

Diseases can be controlled in several ways, of which elimination of the pathogens (eradication) from the herd is by far the most effective way. However, depending on the severity of the disease problem, it might not necessarily be the most cost-efficient way. Other control measures include changes in management and housing conditions to eliminate known risk factors and/or use of relevant vaccines. Medical treatment of diseased pigs is of course inevitable and crucial, whereas in-feed medication as a routine control measure no longer seems the be an option due to the potential risk of developing pathogens that are resistant to antibiotics, especially those that might be a hazard to human health.

In the following, focus will be on eradication, bio security, change in production systems, environment and potential new production systems.

Eradication

Three different principles exist for elimination/eradication of infectious diseases at herd level. These are:

1) Total depopulation followed by restocking with non-infected animals
2) Test and removal of infected animals
3) Eradication by partial depopulation.

The methods 2 and 3 seem only relevant for farrow-to-feeder or farrow-to-finish herds, whereas total depopulation seems to be the only relevant method for finishing herds.

1) Total depopulation/repopulation (SPF
production; SPF = Specific Pathogen Free) The SPF concept implies that all SPF pigs are born free of some specific infections and are kept constantly isolated from other infected animals. In Denmark the total depopulation method has been widely used since 1968 in the Danish SPF system, where herds are free from the following infections:

- *Mycoplasma hyopneumoniae* (*M. hyo*) (enzootic pneumonia)
- A*ctinobacillus pleuropneumoniae* (*A.pp*) (pleuropneumonia)
- toxigenic *Pasteurella multocida* (atrophic rhinitis)
- *Brachyspira hyodysenteriae* (dysentery)
- *Sarcoptes scabiei* (mange)
- *Hematopinus suis* (lice).

During the first 10-15 years of the Danish SPF programme it became obvious that quite many SPF herds became re-infected with *M. hyo* apparently due to airborne spread of the microorganism. As a consequence the so-called „MS" programme was launched in 1982 as a parallel to the SPF system. MS herds are identical to SPF herds except for infections with *M. hyo*. In year 2001 approx. 3700 herds were run under the SPF/MS programme, and out of these approx. 700 herds had highest health status (SPF). Approx. 70% of the Danish breeding and multiplying herds has an SPF or MS status. These herds produce 75% of all breeding and multiplying animals, which are offered for sale. The SPF/MS programme has not received equal attention among production herds. Approximately 15% of Denmark's production herds hold an SPF or MS status. Of all pigs slaughtered, 22% are produced in herds participating in this health programme.

Data from the Danish efficiency-control-programme (E-kontrol) show, that as a mean the productivity (weight gain) is higher for finishing pigs from SPF herds (842 g/day) and MS herds (831 g/day) that from herds with a conventional (unknown) health status (816 g/day) (Jultved, 2002).

2) Test and removal of infected animals
This method has been successfully applied in Denmark for some respiratory pathogens, e.g. Aujeszky's disease (Pseudorabies).

3) Partial depopulation
These procedures include temporary changes in the production flow and are usually combined with a strategic medication. They are alternatives to restocking with SPF animals: they are less expensive owing to reduced production losses, and the genetic potential of the breeding animals is preserved. These methods have been an area of strong interest through the last 10-15 years in Denmark. Today, reliable methods have been developed and tested for diseases like enzootic pneumonia (*M.hyo*) , PRRS, dysentery and mange. The estimated success rate of these methods is 80-90 %. Reliable methods remain to be developed for diseases such as pleuropneumonia (*A.pp*), porcine proliferative enteropathy (*Lawsonia intracellularis*) and atrophic rhinitis (toxigenic *Pasteurella multocida).*

In the following the *M. hyo* infection will be used as an example on the experience gained with partial depopulation. The great majority of the experiences gained have been published in case reports, where different procedures have been applied in one or a few farms. When estimating the success rate of partial depopulation programmes, one might speculate that not all the failures have been reported, by which the apparent success rate tends to seem higher that it actual is.

Partial depopulation for eradication of M. hyo
Experience shows that partial depopulation is very effective when it comes to eradication of *M. hyo* (Zimmermann et al., 1989; Bækbo et al., 1994). The procedure is based on the fact that the spread of *M.hyo* rarely takes place among adults and immune animals. Eradication of *M. hyo* is a fairly simple procedure that consists of 3 elements:

1. All young animals must be removed from the infected herd (piglets, weaners, growers and finishers). For a period of at least 14 days, a farrowing stop is imposed and only breeding animals (sows and boars) older than 10 months must be present on the farm.
2. Medication: During the 14-day-period all breeding animals are medicated with a suitable drug in drinking water or in feed.
3. All empty units and pens are cleaned and disinfected.

After this down period of at least 14 days, all antibiotic medication is ceased and normal production is resumed.

In Denmark, this general procedure has been modified in some herds to lower the costs:

- The most common change is to leave out the stop in farrowings and instead inject the piglets in the farrowing crates (on day 1, 7 and 14).
- The relevance of the age limit of 10 months has been discussed in Denmark, and a lower age (8-9 months) has been applied with success in some herds.
- Another possibility is to move younger breeding animals typically with a high genetic potential to other premises and to medicate them when the youngest pigs have reached an age of 10 months. After medication, they are moved back to the herd of origin.

It is generally believed that the success rate will decrease by decreasing age limit and if the eradication procedures are carried out in newly infected herds in which the infection is still undergoing the active phase.

Experience with partial depopulation for eradication of M. hyo
In Denmark, the method was originally tested in 4 medium-sized herds in 1991-95 (140-340 sows per herd). In these test herds, the success rate was 100% (Bækbo et al., 1994). The herds were monitored for 2½-4 years by monthly clinical inspection and by monthly blood testing of 20 blood samples using a highly sensitive (93.9 %) and a highly specific (96.2 %) mono-

clonal blocking ELISA commercialised by DAKO (Sørensen et al., 1992). Additional measures were performed in some of the herds (testing of colostrum and blood from sentinels together with inspection of lungs at slaughter).

Today partial depopulation is used as one of several routine control options in relevant farms in Denmark. Figures for the last 4 years from the Danish SPF Company show that 20-30 herds per year have carried out a partial depopulation programme – nearly all of them with success (Lassesen, 2001). The prerequisite for gaining a *M.hyo* free status is monthly clinical examination for at least 10 months and blood testing 6 times of 20 pigs with at least one month interval. Based on the experience in Denmark, the estimated success rate under field conditions seems to be approx. 80-90%.

Bio-security
At farm level high bio-security is crucial in order to ensure a low risk of reintroduction of the infection from external sources. Reintroduction can occur by airborne transmission from an infected neighbour farm, by infected replacement stock or by dirty visitors and trucks.

As for the geographic location of the herd, and thereby the risk of airborne transmission, most data is available for the *M. hyo*-infection. A Danish study shows that the distance to the nearest non-SPF herd, the distance to the nearest large non-SPF herd and the purchase of replacement stock from more than one herd per year are significant risk factors for reinfection with *M.hyo* (Thomsen et al., 1992). Based on the study, it is possible to estimate the possibility for a herd to avoid infection with *M.hyo* for one more year as a function of the three risk factors. As an example: If an SPF herd buys replacement stock from only one herd, and the nearest farm is 2 km away, the probability of maintaining its SPF status for one year more is 0.97.

Today this information is implemented in a geographical information system (GIS), a database with information about all swineherds in Denmark. This system identifies a farm by two coordinates, which can be interpreted as (x,y)-

coordinates (Mortensen & Ydesen, 1998) . The database contains information of the herd size and the health status of each herd with respect to Danish SPF diseases. The GIS can be used to print maps of herds in an area, and the herd-specific 1-year probability of survival as an *M.hyo* free herd is automatically calculated for the herd in question.

Farms should be closed and new genes should only by introduced by A.I.. Should that not be possible, quarantine facilities ought to be available for replacement stock. Data from the Danish SPF Company shows that out of 222 farms using quarantine facilities only 6% were infected by new pathogens in one year, whereas 41% out of 499 farms not using quarantine facilities were infected (SPF-Company, 2000-2001).

At farm level the only entrance to the farm should be through an entry room with change of clothes and boots. Empty, clean and disinfected trucks should be used when transporting the pigs from the farm. If this is not possible, special delivery facilities must be established at the farm. When loading the pigs onto the truck, these facilities must be closed off from the rest of the farm, and after dispatch they must be cleaned and disinfected in a way that do not jeopardize the safety of the herd.

Change in production system

Production systems with a continuous flow of finishers are regarded as a risk factor for respiratory diseases, and therefore AI-AO production (batch production) is usually highly recommended. Most new farms in Denmark apply the AI-AO production system today, either as an on-site facility or as a multi-site operation.

Meanwhile, data from a five-year Danish research project demonstrate that AI-AO as on-site production systems is not always a reliable method for reducing the prevalence of pigs with pneumonia (Anonymous, 2000). Often pigs are infected in the farrowing crates or in the weaning unit, and airborne transmissions of pathogens occur between on-site compartments of the finishing units. More segregated production systems like multi-site offer a number of advantages mainly through a break in the infection chain from dam to offspring. But partly because of a minimum weaning age of 3 weeks in the European Union not all batches of finishers will be free form pulmonary lesions due to *M.hyo* or *A.pp.*. A Danish study showed that among finishers coming from infected sow units only one out of two batches is free from *M.hyo* and one out of three batches is free from *A.pp.* (Busch et al., 2000).

Whereas to some extent traditional AI-AO systems seem to control respiratory diseases, new epidemiological studies in Denmark indicate that this production system has a higher potential for controlling infectious enteric diseases (Stege et al., 2001). Consistent batch production was shown to be associated with a reduced prevalence of *Lawsonia intracellularis* and weak betaheamolytic spirochetes (OR: 0.43 and 0.06, respectively).

Environment

Not only batch production can reduce the prevalence of enteric diseases, but the level of hygiene is also very important. A Danish epidemiological study looking for risk factors for use of antibiotics in the production of finishers, showed a high risk for oral treatment (flock treatment) in herds that never clean up between batches (RR: 4.76) (Nielsen et al., accept.). Together with a high level of hygiene, drying out of the facilities between batches is very essential. Desiccation is detrimental to nearly all pathogens and a dry environment reduces cold stress on the pigs.

Epidemiological studies and experimental studies point out poor air quality as a risk factor for the severity of respiratory disease problems (Bækbo, 1998). Clearance studies clearly implicate that dust and ammonia inflict on the normal clearance capacity of the lungs and upper airways.

Several studies find an overall correlation between the indoor carbon dioxide concentration and most air contaminants (Bækbo, 1989). Thus, high levels of carbon dioxide mean high levels of dust, ammonia, microorganisms and endotoxins. As the level of carbon dioxide is re-

lated to the ventilation rate of a building - low levels indicating a high ventilation rate - the carbon dioxide level must be regarded as a very good indicator of air quality as well as ventilation efficiency. An efficient air change is therefore crucial for maintaining a good air quality and an optimal health.

Production systems for the future?

Most segregated production systems, including multi-site, have some negative consequences for the pigs. Due to transportation and mingling of the animals, the pigs are stressed causing an impairment of their immune system. So another way of improving health, apart from interrupting the chain of infection, is to reduce the strain on the pigs (no transport and mingling) and thereby to increase their resistance to infections (Ekkel, 1996). Management systems where pigs are reared in the same pen from farrow-to-finish (FTF) or in the same pen from wean-to-finish (WTF) yield a break in the infection transmission and reduce the stress of the pigs due to minimised movement as well as mingling of the pigs. Experience from USA with the WTF concept and research from Holland and Sweden on the FTF concept seem to show a big potential for increased health and productivity adopting these production systems.

FTF and WTF in a comparative study

To validate the potential for controlling respiratory diseases in pigs produced by FTF on-site or by WTF in a two-site system, a comparative study has been performed in Denmark (Bækbo et al., 2000). The study was carried out on two commercial farms (A & B) over a three-year-period, where farm B housed the off-site WTF facility. All pigs participating in the study came from a farrow-to-finish herd with 600 sows (farm A) chronically infected with *M. hyo.* and *A.pp.*. Clinical signs of pneumonia and pleuropneumonia were distinct among growers and finishers. The study included one control group and two treatment groups.

The study showed that the prevalence of pigs with *M.hyo* associated pneumonia at slaughter was significantly lower in the FTF group as well as in the WTF group compared to the control group (Table 1). The same difference was revealed when looking on the extent of the pneumonic lesions. As for pleuropneumonia, the control group and the WTF group hardly had any, whereas 20 % of the pigs in the FTF group showed this disease at slaughter. Pleuritis was significantly low in the WTF group, whereas the control group and the FTF group did not differ.

Concurrently with the increase in health of the FTF and WTF groups a significant and remarkable improvement in productivity was revealed (Pedersen et al., 2000). The daily weight gain from wean to slaughter increased by 47 g and 98 g for the FTF and WTF groups, respectively, compared to the control group (table 2). Thus the WTF pigs reached marked weight 16 days earlier than the traditional reared pigs, while the FTF animals were slaughtered 10 days earlier. The mortality of pigs from weaning to slaughter was significantly lower in the WTF group than in the two other groups.

Table 1. Prevalence of pathological pulmonary lesions at slaughter of pigs from random sample of 3 pigs per litter (Comparative study of FTF and WTF (Bækbo et al., 2000))

	Control	FTF	WTF
Number of replica	6	6	4
Number of pigs	185	236	121
Pneumonia (MIRD)	39 %	4 %	2 %
Extent of pneumonia (volume)	8.7 %	4.4 %	2.3 %
Pleuropneumonia	2 %	20 %	0 %
Pleuritis	64 %	72 %	1 %

Table 2. Productivity (Comparative study of FTF and WTF (Pedersen et al., 2000))

	Control	FTF	WTF
Number of replica	6	6	4
Number of pigs	698	760	875
Mortality, wean-finish	4.8 %	5.4 %	1.7 %
Daily gain, growers (7-30 kg)	474 g/day	489 g/day	485 g/day
Daily gain, finishers (30-100 kg)	791 g/day	862 g/day	972 g/day
Daily gain, wean-finish (7-100 kg)	669 g/day	716 g/day	767 g/day
Age at slaughter (100 kg)	165 days	155 days	149 days

Even though a sow herd is infected, the FTF system as well as the off-site WTF system seems to be able to eliminate enzootic pneumonia. Together with elimination of the lung diseases, a positive effect is obtained on the productivity of the pigs with significantly fewer days to market.

Additional value of the FTF and WTF systems is several. Due to less transportation and mingling the systems are more animal friendly ensuring a higher level of animal welfare. The logistics of the systems are simpler and as they imply less movement of pigs and less work for cleaning of the facilities, the systems are more people-friendly too.

The FTF and the WTF systems therefore seem to be one of the central keys to a healthy pig production in the future. Today more and more farms in Denmark establish WTF systems, whereas the FTF system seems of little interest to the Danish pig producers.

Conclusion

It can be concluded that today safe and reliable methods for the eradication of several infectious diseases exist. The method of partial depopulation seems relevant for some diseases, where as total depopulation/repopulation is the only method for other diseases.

Optimal bio-security is of crucial importance for herds to stay free form new and unwanted infections. Remote farm location, close herds or use of quarantine facilities, entry rooms and delivery facilities for pigs for slaughter are central parameters.

Today, when farmers want to build new facilities, there seem to be relevant production systems that favour a healthy production. The central words are batch production (AI-AO) with a minimum of transport and mingling of pigs, e.g. the WTF system.

References

Anon. 2000. Final report of a national research program. (Respiratory diseases in pigs: the influence of production system on health, level of infection and economy). Denmark.

Bækbo. P. 1989. Air quality and pig herd health, PhD Thesis, The Royal Veterinary and Agricultural University, Copenhagen.

Bækbo, P. 1998. Effects of noxious gases, dust and microorganisms in the incidence and severity of respiratory diseases in pigs (Key-note lecture), Proceedings of the 15th IPVS congress, Birmingham, UK, 5-9 July, Vol.1, 135-142.

Bækbo, P., Hagedorn-Olsen, T., Christensen, G., Pedersen, B.K. and Wachmann, H. 2000. Health of pigs raised in the same pen from farrow to finish or from weaning to finish. P 327. 16th IPVS Congress, Melbourne, Australia.

Bækbo, P., Madsen, K.S., Aagård, M., Szancer, J. 1994. Eradication of Mycoplasma hyopneumoniae from infected herds without restocking. Proceedings of the 13th IPVS Congress in Bangkok, Thailand. 26-30 June. p 135.

Busch, M.E., Bækbo, P., Pedersen, B.K. Vigre, H. and Sørensen, V. 2000. Respiratory diseases in Danish multi-site systems. 16th IPVS Congress, Melbourne, Australia. p. 443.

Ekkel, D. 1996. Impact of farrow-to-finish production on health and welfare of pigs. Ph.D. thesis, University of Utrecht, The Netherlands.

Jultved, C.R. 2002. Rapport over P-rapporternes resultater april 2002, *INFOSVIN*, no 0222.

Lassesen, B. 2001. Personal communication. (The national SPF health control management company).

Mortensen, S., Ydesen, B. 1998. A geographic information system (GIS) for the Danish swine industry. Proceedings of the 15th IPVS congress, Birmingham, UK, 5-9 July, Vol.3, p 236.

Nielsen, E.O., Hassing, A.G., Wacthmann, H., Bækbo, P., Petersen, H.H. & Nielsen, J.P. 2002. Medicinforbrug i Dansk slagtesvineproduktion (Use of antibiotics in the Danish production of finishing pigs), Dansk Veterinær Tidsskrift, 85, 24, 6-13.

Pedersen, B.K., Jensen, T., Bækbo, P. and Ruby, V. 2000. Production in pigs raised in the same pen from farrow to finish or from weaning to finish. P 358. 16th IPVS Congress, Melbourne, Australia.

Sørensen, V., Barfod, K., Feld, N.C. 1992. Evaluation of a monoclonal blocking ELISA and IHA for antibodies to Mycoplasma hyopneumoniae in SPF-pig herds. The Vet. Rec. 130, 488-490.

SPF-Company, Annual report, 2000-2001.

Stege, H., Jensen, T.K., Møller, K., Bækbo, P. & Jorsal, S.E. 2001. Risk factors for intestinal pathogens in Danish finishing pig herds, Prev. Vet. Med. 50, 153-164.

Thomsen, B.L., Jorsal, S.E., Andersen, S., Willeberg, P. 1992. The Cox regression model applied to risk factor analysis of infections in the breeding and multiplying herds in the Danish SPF-system. Prev. Vet. Med. 12, 287-297.

Zimmermann, W., Odermatt, W., Tschudi, P., 1989. Enzootische pneumonie (EP): Die teilsanierung EP-reinfizierter sxhweinezuchtbertriebe als alternative zur totalsaniering. Schweiz. Arch. Tierheilk. 131, 179-191.

20. Animal welfare and health as affected by management procedures in commercial broiler flocks

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Summary

In the present paper, it is described how changes in management practises such as lighting programmes, feeding strategies and broiler hybrids affect walking ability and skin health of commercially grown broilers.

Introduction

After the termination of the use of antibiotic growth promoters (AGP) and meat and bone meal (M&B) in the late nineties, the broiler industry has been struggling with leg and skin problems that may compromise broiler welfare (Sanotra et al., 2001; Sørensen et al., 2002). It has never been documented, however, that the exclusion of AGP and M&B from broiler diets has played a major role in the development of leg problems and deteriorated skin health. Nevertheless, in our aim to improve food safety, we may have induced animal health and welfare problems that compromise consumer acceptance of poultry meat.

Methods

The etiology of leg and skin problems is multifactual, but may to some extend be related to several management practises (Thorp, 2000; Ekstrand et al., 1998). In this presentation, results are described from either small-scale or large scale-experiments with different management procedures. The experiments were performed on commercial broiler farms.

Walking ability was determined with the gait score method (Kestin et al., 1992) using a scale from 0 to 5. Birds without detectable gait abnormalities were given score 0. Score 5 was given to birds that were unable to walk. For the assessment of foot-pad health, one foot per bird were scored for the occurrence of *podo dermati-*

tis, using a scale from 0 to 2, as described by Ekstrand et al. (1998). Score 0 was given to feet without any discolouration or burns, while score 2 was given to feet with severe lesions. Moreover, skin health was determined through the occurrence of carcasses with skin injuries.

Results and conclusion

In summary, it was possible to reduce mean gait score by approximately 15% by introducing daily 4-hour fasting periods (Petersen, 2000) or changing from one broiler hybrid to another (Petersen, 2002b). Moreover, the mean gait score of a broiler flock tended to decrease through a change in light programme from 2 hours of darkness per day to a light programme with 8 decreasing to 3 hours of darkness per day (Petersen, 2002a).

However, light programmes with long dark periods, increased the number of birds that were rejected at slaughter because of skin injuries. Furthermore, with long dark periods, the number of birds with foot-pad burns was increased (Petersen, 2002a). The foot-pad score could be significantly decreased by changing litter material from chopped wheat straw to wood shavings (Petersen, 2002b).

Hence, changes in management practises may decrease leg problems and improve skin health in broilers.

References

Ekstrand, C., Carpenter, T.E., Andersson, I. and Algers, B. 1998. Prevalence and control foot-pad dermatitis in broilers in Sweden. Br. Poultry Sci. 39, 318-324.

Kestin, S.C., Knowles, T.G., Tinch, A.E. and Gregory, N.G. 1992. Prevalence of Legweakness in broiler chickens and its prevalence with genotype. Vet. Rec. 131, 190-194.

Petersen, C.B. 2000. Boksforsøg nr. 51. Måltidsfodring contra normal fodring udført med høner og haner adskilt V. National Department of Poultry Production, Udkaersvej 15 Skejby, DK-8200 Aarhus N, Denmark.

Petersen, J.S. 2002a. Effects of different light programmes on gait score, skin health and production traits of broilers. Archiv für Geflügelkunde. 66. Sonderheft II. p 73.

Petersen, J.S. 2002b. Boksforsøg nr. 66 og 67. Sammenligning af Ross 208 og 308 slagtekyllinger opdrættet med halm eller spåner som strøelse. National Department of Poultry Production, Udkaersvej 15 Skejby, DK-8200 Aarhus N, Denmark.

Sanotra, G.S., Lund, J.D., Ersboell, A.K., Petersen, J.S., Vestergaard, K.S. 2001. Monitoring leg problems in broilers: a suvey of commercial broiler production in Denmark. World Poultry Sci. J. 57, 55-69.

Sørensen, P., Nielsen, B.L., Petersen, J.S. & Eskildsen, B. 2002. Trædepudesvidninger hos slagtekyllinger. DJF rapport nr. 42.

Thorp, B.H. 2000. The incidence and pathology of common skeletal disorders. XXI World's Poultry Congres, Montréal, Canada August 20-24, 2000. S3.9.04.

21. Organic acids and fermented liquid feed as alternatives to antibiotic growth promoters

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Summary

Addition of organic acids to piglet/pig diets, and feeding fermented liquid feed (FLF) are two feeding strategies evaluated as alternatives to antibiotic growth promoters. The effects of these strategies on gastrointestinal ecology and animal growth performance do not always point at the same direction. The previous makes it crucial to look at the effect on each parameter separately. Organic acids have different effects on gastrointestinal ecology depending on the acid used and the dose applied. However, in general, they tend to decrease pH in the stomach, and reduce the number of micro organisms along the gastrointestinal tract (GI-tract). An exception is lactic acid, which increases the density of lactic acid bacteria and yeasts. The effect on growth performance is not always consistent and depends also on the acid used and the dose applied, but, in general, organic acids increase or tend to increase performance. Fermented liquid feed affects the gastrointestinal ecology by decreasing the gastric pH, increasing the numbers of lactic acid bacteria and yeasts (especially in the proximal GI-tract), and decreasing the number of coliform bacteria. So, feeding FLF results in a more healthy/robust GI-tract. However, the effects obtained depend on the degree of fermentation of the feed. That is, the elaboration of the FLF has great impact on the effects observed. Regarding growth performance, there are not many data available, but feeding FLF does not seem to improve performance compared to feeding dry feed. Modifications in the elaboration of FLF to improve growth performance by avoiding a microbial degradation of synthetic amino acids, like fermenting only the grain, or by avoiding a too low pH in the feed that might decrease feed intake are some of the aspects that need further investigation.

Introduction

The ban of most antibiotic growth promoters (AGP) in Europe and the total stop in the use of all AGP in Denmark has resulted in an intensive search for alternative feeding-strategies in pig production during the last five years. Among other strategies, the addition of organic acids to the feed and feeding fermented liquid feed (FLF) have been investigated and have given promising results. Optimal alternative feeding-strategies would be those that result in high health status and high growth performance of the animals. However, experience has shown that both parameters do not always follow each other. Therefore, it is necessary to describe and discuss the effects of these feeding strategies on pig health and pig growth performance separately.

Organic acids

Organic acids have been used for decades in feed preservation, protecting feed from microbial and fungal destruction or to increase the preservation effect of fermented feed, e.g. silage.

An important objective of dietary acidification is the inhibition of intestinal bacteria competing with the host for available nutrients, and a reduction of toxic bacterial metabolites, e.g. ammonia and amines, thus improving growth performance of the host animal. Furthermore, the growth inhibition of potential pathogenic bacteria and zoonotic bacteria, e.g. *E. coli* and Salmonella, in the feed and in the GI-tract benefits animal health.

Mode of action

The antibacterial activity of organic acids is related to the reduction of pH, as well as their ability to dissociate, which is determined by the pKa-value of the respective acid, and the pH of the surrounding milieu. Organic acids are lipid soluble in the undissociated form, in which they are able to enter the microbial cell. Once in the cell, the acid releases the proton in the more alkaline environment, resulting in a decrease of intracellular pH. This influences microbial metabolism inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to release protons, leading to an intracellular accumulation of acid anions (Russell, 1992). The acid anion seems to be very important regarding the antibacterial effect of organic acids and their salts. Several investigations have shown a strong bactericidal effect of organic acids without significantly decreasing the pH-value in the GI-tract.

Effect on gastrointestinal ecology

In the following, we will mainly describe and discuss results on lactic acid, formic acid and benzoic acid. The reason for it is that those are the acids we have worked most with.

pH and organic acids along the GI-tract

Data on the effect of addition of organic acids to the diet on pH and organic acid concentration in the GI-tract are not consistent. The results vary with the acid used and the doses applied.

Addition of 2.8% lactic acid to a weaner diet resulted in lower pH values in the stomach, caecum and colon of piglets, whereas addition of 0.7% and 1.4%, although decreased the pH values along the GI-tract, did not result in significant differences compared to the control (Maribo et al., 2000a). Similarly, addition of 1.4% formic acid to a weaner diet decreased the pH in stomach, whereas a dose of 0.7% decreased the pH only numerically (Maribo et al., 2000a). Canibe et al. (2001) could not detect a significant effect of 1.8% Formi (dipotassium formate) on pH along the GI-tract of piglets. Benzoic acid (2%) or a combination of 0.7% lactic acid and 0.7% formic acid did not affect

the pH along the GI-tract of piglets (Maribo et al., 2000b).

Doses of lactic acid between 0.7% and 1.4% resulted in higher concentrations of lactic acid in the stomach and proximal small intestine, but the differences were not significant in all cases. When 0.7% or 1.4% formic acid was added to weaner diets the acid was detected at higher concentrations than the control only in the stomach, which suggests that formic acid was absorbed or degraded in the proximal small intestine (Maribo et al., 2000a). Similarly, formic acid, benzoic acid and sorbic acid were only detectable in the stomach of piglets fed diets added 1% of Formi, benzoic acid or sorbic acid, respectively (Canibe et al., unpublished). However, when a dose of 1.8% of Formi was added to a weaner diet or 1.8% formic acid was added to a grower diet, formic acid was detected at significantly higher concentrations in the stomach and the whole small intestine of the animals fed the acid-added diets (Canibe and Jensen, 2001; Canibe et al., 2001). The previous indicates that the doses used influence the concentration of the acid along the proximal GI-tract. The data further show that the added acids are only detected in the proximal GI-tract, and therefore, their direct effect on microbial ecology is expected in these segments.

The effect of organic acids on the concentration of short chain fatty acids is variable, resulting in higher levels in the caecum and colon in some cases, lower in others or without effect in others (Maribo et al., 2000a; 2000b; Canibe and Jensen, 2001; Canibe et al., 2001).

Microbial populations

Lactic acid (from 0.7% to 2.8%) tended to increase the number of lactic acid bacteria and yeasts, and to decrease the number of coliform bacteria along the GI-tract (Maribo et al., 2000a). The higher the dose the larger the effect. Formic acid (1.4%, 1.8%) decreased the density of lactic acid bacteria, coliform bacteria and, as opposed to lactic acid, yeasts along the GI-tract of piglets and growers (Maribo et al., 2000a; Canibe and Jensen, 2001). Benzoic acid at a concentration of 2%, decreased the number of lactic acid bacteria, coliform bacteria and

yeasts along the GI-tract (Maribo et al., 2000b). Formi, benzoic acid and sorbic acid (1%) did not significantly affect the number of lactic acid bacteria, enterobacteria or yeasts along the GI-tract of piglets. However, numerically lower enterobacteria and yeasts densities were measured in the piglets fed the acids compared to the control group (Canibe et al., unpublished).

In order to screen various organic acids for their anti-bacterial effects in gastrointestinal contents and thereby find candidates to replace antibiotic growth promoters in feed for pigs/piglets, a batch culture system was established to simulate the conditions in the stomach (Knarreborg et al., 2002). The effect of various organic acids on growth/survival of lactic acid bacteria and coliform bacteria was tested. The anti-microbial effect of six different organic acids (formic, propionic, butyric, lactic, benzoic, and fumaric acid) were compared in stomach content at pH 4.5 (Figure 1). In contrast to lactic acid bacteria, coliform bacteria were unable to grow in stomach content at the investigated pH. Benzoic acid and fumaric acid showed the strongest antibacterial capacity against coliform bacteria, and they were able, as the only ones, to kill lactic acid bacteria. A pH-dependent inhibition of bacterial growth has been demonstrated; at the same organic acid concentration, the lower the pH the strongest the antibacterial effect (data not shown).

Growth performance

The effect of organic acids on performance shows, in general, increase growth performance or tendencies for it. Addition of 0.7% lactic acid + 0.7% formic acid or 1-2% benzoic acid improved growth performance of piglets compared to a control diet (Maribo et al., 2000b), the same was the case when doses of 0.8-1.6% lactic acid (Maribo 1999), or 2% sorbic acid (Maribo and Olsen, 1999) were added. The positive results cannot always be found, as was the case for doses between 0.7% and 2.8% lactic acid, which did not show any significant effect on growth performance of piglets (Maribo et al., 2000a). Doses of 0.7% or 1.4% formic acid did not show an improved growth performance in piglets (Maribo et al., 2000a), whereas 1.8% formic acid tended to improve growth performance of growing pigs (Canibe and Jensen, submitted).

Fermented liquid feed

Feeding liquid feed (non-fermented or partly fermented) to pigs is a practice that has been applied for many years. Some of the characteristics of liquid feed related to its effects on gastrointestinal ecology and the potential use of co-products from the food industry in its elaboration have made liquid feed a subject of interest in the scientific world. Fermented liquid feed has and is being evaluated in the search for alternatives to antibiotic growth promoters and for alternative use of co-products from the food industry.
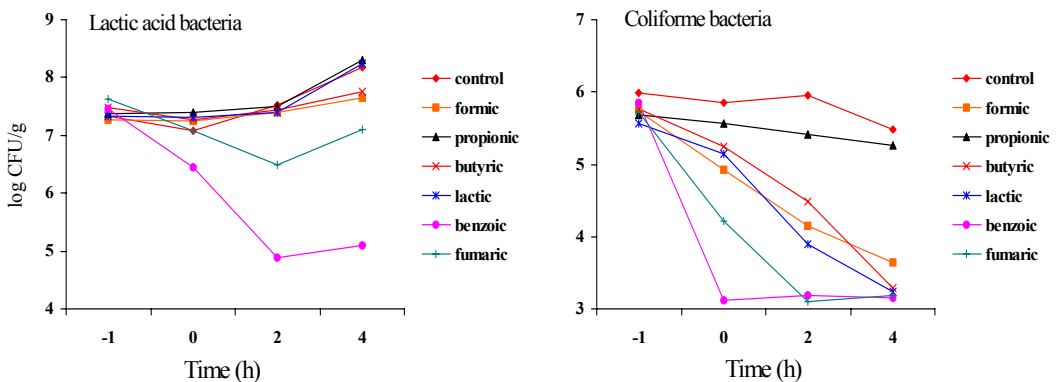

Figure 1. Survival of lactic acid bacteria and coliform bacteria (log CFU/g sample) in gastric content (pH 4.5) added various acids

From the moment dry feed and liquid are mixed, there is a risk/chance that fermentation occurs. It has been shown that lactic acid bacteria and yeasts naturally occurring in cereal grains will proliferate and produce lactic acid, acetic acid and ethanol and reduce pH if the feed is soaked in liquid for a certain length of time. The presence of lactic acid bacteria in the GI-tract is considered beneficial to the animal. High lactic acid concentrations and low pH in the stomach of pigs are also desired because they are believed to improve protein hydrolysis, and prevent the proliferation of pathogens (e.g. enterobacteria such as coliforms and *Salmonella*). From the previous it can be concluded that the characteristics of FLF, if also found in the GI-tract, makes it an attractive feed strategy for pigs. However, it is crucial to understand the course of the fermentation if the best results are to be obtained.

Elaboration and characteristics of fermented liquid feed

Studies carried out in our laboratory to determine the optimal temperature, soaking time and amount of residue to be left in the fermentation tank, showed that a temperature in the fermentation tank of 20^0C, a soaking time of 8 hours and a residue of 50% result in FLF with maximum pH values of 4.5 (considered as the maximum pH value if the growth of enterobacteria is to be inhibited), low levels of coliform bacteria, high levels of lactic acid bacteria and yeasts, and high levels of lactic acid (Jensen and Mikkelsen, 1998). Results from these studies showing the pH course with fermentation time are presented in Figure 2. It can be seen that when fermentation is initiated, a period of 3-5 days is needed to reach a steady-state. Figure 3 shows that to reach pH values below 4.5, a temperature of 20^0C is required if the soaking time is 8 hours and the residue 50%. Figure 4 shows the importance of reaching a steady state when the fermentation is initiated. During the first hours of fermentation, the number of lactic acid bacteria increases, but concomitantly there is a bloom of enterobacteria. After 3 days the further increase of lactic acid bacteria and lactic acid concentration coincides with a drop in pH (below 4.5) and the counts of enterobacteria.
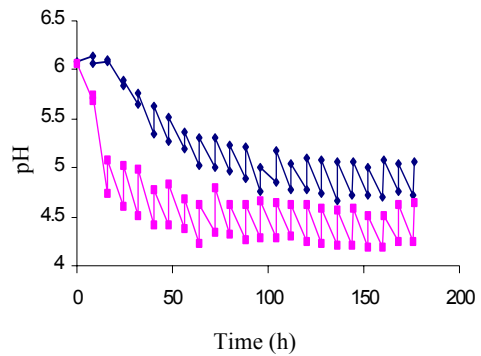
Figure 2. The effect of temperature on pH development during fermentation of liquid feed prepared with eight hours soaking time and 50% residue. -◆- 15^0C; -■- 25^0C. Each point represents the mean value from three fermentations
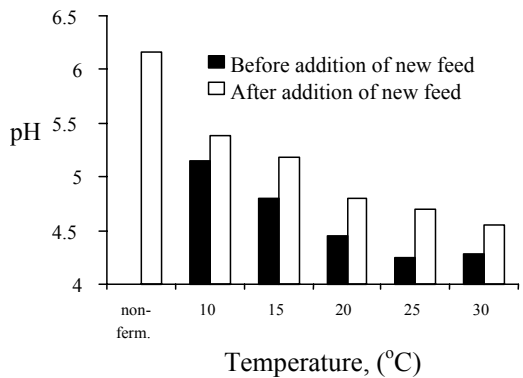
Figure 3. Effect of fermentation temperature on pH of liquid feed in steady-state prepared with 50% residue and 8 hours cycle time (^0C). Each bar represents the mean value from three fermentations
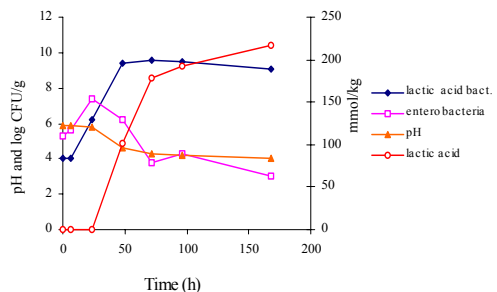
Figure 4. Density of lactic acid bacteria and enterobacteria (log CFU/g sample), pH and lactic acid concentration (mmol/kg sample) in liquid feed during fermentation

Results from our laboratory regarding microbiological characteristics of dry feed, non-FLF (feed and water mixed in a bucket, a sample taken and analysed within 2 hours, that is, maximum fermentation time of 2 hours), and FLF (20^0C, 8 or 16 hours, 50% residual left in the tank, and steady state has been reached) are shown in Table 1. The values show a ~560- fold increase in the number of lactic acid bacteria in the non-FLF (which in fact was partially fermented), but more importantly, a ~30-fold increase of coliform bacteria compared to dry feed. The relatively high microbial activity in the non-FLF is probably due to the presence of feed residues in the bucket, which acted as a „starter culture". Further fermentation results in inhibition of coliform bacteria, and the appearance of other desirable characteristics mentioned above (high lactic acid, low pH, and high counts of lactic acid bacteria). The chemical composition of the three diets elucidates some differences between them, especially regarding a decrease of carbohydrate contents (mainly low molecular weight sugars) as fermentation progresses. Lysine content seems to decrease with fermentation, too (data derive from only 4 samples and the variation is high). Pedersen (2001) has also reported a disappearance of free lysine in FLF.

Effect on gastrointestinal ecology

There is agreement in data from the literature on the effect of FLF on gastrointestinal ecology of pigs.

pH and organic acids along the GI-tract

Feeding FLF lowers the gastric pH to values of 4 or less (Jensen and Mikkelsen, 1998; Canibe and Jensen, 2000; van Winsen et al., 2001), which is probably due to the high concentration of lactic acid measured at this site in pigs fed FLF. Low gastric pH and high levels of lactic acid are desirable since these two factors inhibit the growth of enteropathogenic bacteria such as coliform bacteria and *Salmonella*. No apparent effects of FLF on large intestinal pH have been detected.

Table 1. Characteristics of dry feed (DF), non-fermented liquid feed (N-FLF) and fermented liquid feed (FLF)

| | Diet | | | | | |
	DF	n	NFLF	n	FLF	n
pH	NM[c]	-	5.98±0.18	21	4.36±0.17	24
Lactic acid bacteria (20^0C)	<4.3±0.86	11	7.2±0.59	19	9.4±0.32	23
Lactic acid bacteria (37^0C)	4.3±0.48	12	<6.9±0.71	21	9.4±0.23	24
Enterobacteria	<4.7±1.10	12	6.2±0.59	21	<3.2±0.56	24
Yeasts (20^0C)	<3.6±0.59	11	5.0±0.65	20	6.9±0.69	22
Yeasts (37^0C)	<3.3±049	12	<3.4±0.60	21	<4.3±0.92	22
Lysine	6.00±0.51	2	5.82±1.14	3	4.80±0.87	3
LMW-sugars[a]	3.60±0.13	2	2.85±0.58	3	0.08±0.06	3
Starch	46.0±0.90	2	46.8±1.68	3	46.5±2.42	3
T-NSP[b]	12.72	1	13.26±0.73	2	11.74±0.00	2

[a] Low molecular weight-sugars.
[b] Total non-starch polysaccharides.
[c] Not-measured. Values are means with their standard deviation.

The concentration of organic acids along the GI-tract, especially that of lactic acid, is affected by feeding FLF. Piglets fed FLF had higher concentration of lactic acid in the stomach and proximal small intestine and higher concentration of acetic acid in the caecum and colon than those fed non-FLF (Jensen and Mikkelsen, 1998; Scholten et al., 2002). Similarly, a higher concentration of lactic acid was measured in the proximal GI-tract of growing pigs fed FLF compared to those fed dry feed or non-FLF (Canibe and Jensen, 2003). As shown by Jensen and Mikkelsen (1998) and Canibe and Jensen (2003), higher concentration of lactic acid in the stomach coincides with a lower production of lactic acid *in vitro*. This suggests that the higher concentrations of lactic acid in the stomach can be attributed exclusively to a microbial fermentation in the feed and not to a microbial production in the stomach.

Microbial populations

Higher counts of lactic acid bacteria in the proximal segments of the GI-tract of growing pigs fed FLF (Jensen and Mikkelsen, 1998; Canibe and Jensen, 2000) can be due to the higher density of these micro organisms in the FLF compared to dry feed. Whether they are active in the GI-tract or only transient has not been elucidated. Feeding FLF decreases the density of lactic acid bacteria in the distal GI-tract, which could be partially explained by a decrease of substrate available to these micro organisms. As shown in Table 1, FLF contained less carbohydrates than dry feed. An important characteristic of feeding FLF is a decrease in the population of coliform bacteria in the GI-tract of piglets, which is beneficial because it can help diminishing weaning scours. Another enteropathogenic bacteria shown to be affected by feeding FLF *is Brachyospira hyodysenteriae,* which causes swine dysentery by colonising the large intestine of growing pigs. A study carried out by Lindecrona et al. (2000) showed lower incidence of swine dysentery and severity of clinical symptoms in pigs fed FLF as compared to those fed dry feed. Changes in the gut microbiota and/or biochemical environment were proposed as a possible explanation. In agreement with the latter, the results of Højberg et al. (2001) showed lower fermentation capacity in the large intestine of pigs fed FLF compared to those fed dry feed, and Leser et al. (2000) detected changes in bacterial community structure in the colon of pigs as a result of feeding FLF.

Higher density of yeasts has been observed in the GI-tract of piglets fed FLF compared to those fed non-FLF (Jensen and Mikkelsen, 1998; Scholten et al., 2002). Due to the presence in the FLF of high numbers of yeasts that grow at 20^0C but not at 37^0C, a large part of the yeasts are probably not active in the GI-tract. Yeasts can be considered as beneficial to the animal because they can bind pathogens and impede their attachment to the intestinal wall, or as detrimental since yeasts can cause disease in pigs. Further studies are needed to elucidate this point.

Growth performance

Piglets fed FLF had a higher feed intake and body weight gain than those fed dry feed (Russell et al., 1996). On the other hand, Pedersen (2001) observed higher growth performance in piglets fed non-FLF compared to those fed FLF or the same diet supplied as dry feed. Degradation of synthetic lysine in the FLF was suggested to be the main cause of the lower growth performance of the animals fed FLF. A too low pH of the FLF resulting in unpleasant taste for the animals could also be a reason for the lower feed intake. The piglets in the study of Pedersen (2001) were fed a dry diet the first two weeks post-weaning and after that they were offered the non-FLF and FLF. In order to obtain the best results of feeding FLF, it would have been better to offer the piglets the experimental diets from the first day postweaning. Furthermore, studies on the effect of feeding FLF in farms with health problems lack. Such studies would supply crucial information on the advantages of feeding FLF. Growing-finishing pigs fed non-FLF had also higher feed intake and daily body weight gain than those fed FLF (Canibe and Jensen, 2003). In order to avoid the degradation of the synthetic lysine, a strategy was tested which consisted of fermenting only the grain, which was mixed with the remaining ingredients (including the synthetic amino acids) immediately before feeding. This strategy resulted in higher growth performance of heavy pigs

compared to feeding non-FLF (Pedersen et al., 2002a). Further studies where all the dietary ingredients were fermented except the mineral mixture including the synthetic amino acids had negative effects on growth performance of growing-finishing pigs, probably due to low pH of the feed (4.2-4.3) (Pedersen et al., 2002b; 2002c). Investigations on the effect of extent of fermentation on lysine concentration and on feed intake will be carried out in the near future. Also, offering the animals liquid feed already during the weaning period is a strategy that needs investigation.

Conclusion

Addition of organic acids to the diet results in variable effects on gastrointestinal ecology of piglets and pigs depending on the acid used and the doses applied. The pH along the GI-tract tends to be lower after addition of organic acids but often the effect is not significant. In general, organic acids decrease the density of bacteria along the GI-tract. However, the number of yeasts are reduced by addition of formic acid, sorbic acid and benzoic acid, but increased by addition of lactic acid to the diet. The acids added in the diet are only detected in the proximal segments of the GI-tract (stomach and small intestine) which suggests that they are absorbed or degraded in these segments. Growth performance is, in general, positively affected by addition of organic acids, although with variable results, again depending on the doses and acid used.

Fermented liquid feed is a clear appropriate alternative to antibiotic growth promoters regarding gastrointestinal health (i.e., low pH in the stomach, high concentration of lactic acid, low number of coliform bacteria). However, growth performance is not improved when FLF is fed compared to dry feed, and it can be worsened compared to feeding non-FLF. Feed taste, low pH and a possible degradation of synthetic lysine as a result of fermentation could be the reason for the negative results observed when feeding FLF. Fermenting only the grain has shown improved growth performance. These aspects require further investigations. Moreover, feeding piglets with non- FLF/FLF from the weaning period is a strategy that is worth studying.

References

Canibe, N. & Jensen, B.B. 2000. Effect of non fermented- and fermented-liquid feeding on the intestinal microbiota of growing pigs. Reprod. Nutr. Dev. 40, 203.

Canibe, N. & Jensen, B.B. 2001. Effect of feed structure and processing and addition of formic acid to the feed on the microbial ecology of the gastrointestinal tract of pigs. Proceedings of the Workshop: Alternatives to feed antibiotics and coccidiostats in pigs and poultry (AFAC), Norfa network, 13 -16. October, Oslo, Norway. (Available: www-afac.slu.se/Workshop%20-Norge/Proceedings%20Oslo.htm).

Canibe, N., Steien, S.H., Øverland, M. & Jensen, B.B. 2001. Effect of K-diformate in starter diets on acidity, microbiota, and the amount of organic acids in the digestive tract of piglets, and on gastric alterations, J. Anim. Sci. 79, 2123-2133.

Canibe, N. & Jensen, B.B. 2003. Fermented and nonfermented liquid feed to growing pigs. Effect of aspects of gastrointestinal ecology and growth performance. J. Anim. Sci. 81, 2019-2031.

Højberg, O., Knudsen, B., Canibe, N. and Jensen, B.B. 2001. Characterisation of the gastrointestinal bacterial community in pigs fed fermented liquid feed and dry feed: composition, fermentation capacity and phenotypic fingerprint. 15th Forum for Applied Biotechnology, Gent, Belgium, 24-25 September, Med. Fac. Landbouww. Univ. Gent, 66/3b: 455-458.

Jensen, B.B. & Mikkelsen, L.L. 1998. Feeding liquid diets to pigs. In: Recent advances in Animal Nutrition - 1998, pp 107-126. Edited by P.C. Garnsworthy and J. Wiseman, Nottingham University Press, Nottingham.

Knarreborg, A., Miquel, N., Granli, T. & Jensen, B.B. 2002. Establishment and application of an in vitro methodology to study the effects of organic acids on coliform and lactic acid bacteria in the proximal part of the gastrointestinal tract of piglets. Anim. Feed Sci. Tech. 99, 131-140.

Leser, T.D., Lindecrona, R.H., Jensen, T.K., Jensen, B.B. & Møller, K. 2000. Changes in Bacterial Community Structure in the Colon of Pigs Fed Different Experimental Diets and after Infection with Brachyspira hyodysenteriae. Appl. Environ. Microbiol. 66 (8), 3290-3296.

Lindecrona, R.H., Jensen, B.B., Jensen, T.K., Leser, T.D. & Møller, K. 2000. The influence of diet on the development of swine dysentery. The 16th International Pig Veterinary Society Congress, 17-20 Sept., Melbourne, Australia, 7.

Maribo, H. 1999. Firm products to piglets- Lactic acid, LAFEED 80. Danish Bacon and Meat Council, no. 428 (In Danish).

Maribo, H. & Olsen, L.E. 1999. Calcium formate and sorbic acid to piglets. Danish Bacon and Meat Council, no. 445 (In Danish).

Maribo, H., Jensen, B.B. & Hedemann, M.S. 2000a. Various doses of organic acids to piglets Danish Bacon and Meat Council, no. 469 (In Danish).

Maribo, H., Olsen, L.E., Jensen, B.B., & Miquel, N. 2000b. Products to piglets: combination of lactic acid and formic acid and benzoic acid. Danish Bacon and Meat Council, no. 490 (In Danish).

Pedersen, A. Ø. 2001. Fermented liquid feed to piglets. Danish Bacon and Meat Council, no. 510 (In Danish).

Pedersen, A.Ø., Maribo, H., Jensen, B.B., Hansen, I.D., & Aaslyng, M.D. 2002a. Fermented grain in liquid feed to heavy pigs. Danish Bacon and Meat Council, no. 547. (In Danish).

Pedersen, A.Ø., Maribo, H., Kranker, S., Canibe, N., Hansen, I. D., & Aaslyng, M.D. 2002b. Fermented liquid feed to growing pigs-pelleted feed. Danish Bacon and Meat Council, no. 567 (In Danish).

Pedersen, A.Ø., Maribo, H., Canibe, N., Hansen, I.D., & Aaslyng, M.D. 2002c. Fermented liquid feed to growing pigs- home mixed with whey without formic acid. Danish Bacon and Meat Council, no.566 (In Danish).

Russell, J.B. 1992. Another explanation for the toxicity of fermentation acids at low pH: anion accumulation versus uncoupling. J. Appl. Bacteriol., 73, 363-370.

Russell, P.J., Geary, T.M., Brooks, P.H. & Campbell, A. 1996. Performance, water use and effluent output of weaner pigs fed ad libitum with either dry pellets or liquid feed and the role of microbial activity in the liquid feed. J. Sci. Food Agric. 72, 8-16.

Scholten, R., van der Peet-Schwering, C.M.C., den Hartog, L.A., Schrama, J.W. and Verstegen, M.W.A. 2002. Fermented wheat in liquid diets: effects on gastrointestinal characteristics in weanling piglets. J. Anim. Sci. 80, 1179-1186.

van Winsen, R.L., Urlings, B.A.P., Lipman, L.J.A., Snijders, J.M.A., Keuzenkamp, D., Verhejden, J.H.M. & van Knapen, F. 2001. Effect of fermented feed on the microbial population of the gastrointestinal tracts of pigs. Appl. Environ. Microbiol. 67 (7), 3071-3076.

22. Improved feed composition and feed processing as alternatives to AGP in pig production

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Abstract

With the rise in antibiotic resistance and the subsequent removal of antibiotics from pig feed there is a need to identify alternatives, which can reduce the incidence of diarrhoea caused by pathogenic bacteria such as *Escherichia coli* and that can reduce the incidence of zoonotic bacteria such as *Salmonella enterica*. Optimal alternative feeding-strategies would be those that result in as well high health status as high growth performance at the same time, however that is not always easy to achieve. Several studies have shown that the composition and processing of the feed are of great significance to maintain a healthy gastrointestinal ecosystem in slaughter pigs and decrease the numbers of pathogenic Enterobactericea (i.e. *E coli* and *Salmonella*). Especially the use of coarsely ground meal feed as well as the use of barley has been shown to reduce the occurrence of pathogenic enterobacteria significantly. However the use of these two feeding strategies causes a reduction in the production results, especially feed conversion.

Coarsely ground meal favours the growth of anaerobic bacteria, especially lactic acid bacteria, in the stomach of the pigs. The reason for this is at present unknown, but an increased microbial activity leads to an increased production of lactic acid and other organic acids and a lover pH in the stomach. Consequently, enteric bacteria like *Salmonella* and *E coli* are killed in the stomach and do not enter the parts of the gastrointestinal tract in which they will normally proliferate. In this way, the stomach acts as a barrier breaking the vicious circle where animals in problematic herds infect each other and are reinfected through consumption of faeces. These results are supported by *in vitro* studies that show a high negative correlation between undissociated lactic acid and the survival rate of *Enterobactericea* in stomach content.

Introduction

The total stop in the use of antibiotic growth promoters in Denmark has resulted in an intensive search for alternative feeding-strategies in pig production during the last five years. Among other strategies feed composition, feed processing and the use of probiotics and prebiotics have been investigated. Optimal alternative feeding-strategies would be those that result in as well high health status as high growth performance at the same time, however that is not always easy to achieve. As a consequence , both parameters will be discussed in the following, although with focus on how to improve the health status of the animal.

How does growth promoting antibiotics work?

Inclusion of growth promoting antibiotics into pig feed improves live weight gain by 5-6% and feed conversion efficiency by 3-4%. The mechanism by which growth promoting antibiotics improve growth performance is not known with certainty, but several hypotheses have been proposed, most of which involve the gastrointestinal microbiota. Although statistically significant differences in growth rate have never been well correlated with differences in bacterial counts, either in specific intestinal segments or in the gastrointestinal as a whole, several investigations have shown that growth-promoting antibiotics do produce significant changes in the gastrointestinal microbiota and the activity of the microbiota (Jensen, 1998). The results strongly indicate that the growth promoting antibiotics accomplish their effect in the small in-

testine while they have little or no effect in the large intestine. It is generally accepted that the microbiota in the small intestine competes with the host animal for easily digestible nutrients and at the same time produces toxic compounds. Experiments with slaughter pigs have shown that as much as 6% of the net energy in the pig diet could be lost due to microbial fermentation in the small intestine. On the other hand, the microbiota in the hindgut is believed to have a beneficial effect on the host animal since it produces energy by fermentation of feed material that has escaped digestion in the small intestine. Jensen et al. (1998) have calculated that on a normal Danish pig diet, 16.4% of the total energy supply for slaughter pigs is achieved by microbial fermentation in the hindgut.

Alternatives to antibiotic growth promoters

Feed composition

Probably the single most important control for microbial ecosystem in the gastrointestinal tract (and as such on animal health) of pigs is the amount and type of substrate available to the microbiota. This enables a direct control over the processes of fermentation in the gastrointestinal tract through feed composition. As shown by Jensen and Jørgensen (1994) especially the fibre content of the diet affects the microbial fermentation in the gastrointestinal tract of pigs but also the content of resistant starch and non-digestible oligosaccharides play a role. Reid and Hillman (1999) and Jensen (unpublished) have found that addition of fibre to a pig diet reduces the population of coliform bacteria in the gastrointestinal tract, while Mcdonald et al. (1998) reported an increased population of coliform bacteria in pigs fed a fibre-enriched diet. The effect of dietary fibre on the development of swine dysentery is also a little controversial. Durmic et al., (1998) and Pluske et al., (1996) reported that diets low in dietary fibre and resistant starch prevented pigs from infection with *Brachyospira hyodysenteriae* while Lindecrona et al., (2000) and Kirkvood et al., (2000) were unable to confirm these findings.

Several investigations have shown that also the fermentation of protein in the gastrointestinal tract depends on diet composition (Jensen et al., 1998). Experience from practical pig farming under Danish conditions has also indicated that high protein content in diets for growing pigs increases the incidence of diarrhoea in slaughter pigs, although that was not followed by an increase in the population of coliform bacteria (Petersen et al., 2000).

The effect of lipids on the composition and activity of the microbiota in the digestive tract of pigs and on pig health is not very well investigated. Digestion of dietary lipids in pigs occurs from the proximal part of the small intestine onwards, only negligible changes occur anterior to the small intestine. Unsaturated fatty acids are subject to bio-hydrogenation by gut bacteria. It has been known for some time that unsaturated fatty acids are toxic to many rumen bacteria (Henderson, 1973). Apart from a possible effect on the microbiota, lipids also seem to increase the occurrence of gastric ulcers (Regiena et al., 1996), which again may affect the microbiota in the stomach. Since the amount of fat in pig diets has increased during the last decade it will be of interest to elucidate how fat content affects the gastrointestinal ecosystem and pig health.

The buffer capacity of pig feed has also been postulated to affect the microbial ecosystem in the gastrointestinal tract and to affect feed digestion (Decuypere et al., 1997). Further, diets with high buffering capacity have been shown to increase salmonella shedding of pigs (Prohaszka et al., 1989). Again, very little information exists about the effect of the buffering capacity of the feed on the microbial ecosystem in the gastrointestinal tract and on the health of the animals.

Feed processing

Effect of feed processing on survival of pathogenic bacteria

Several studies have shown that the processing of the feed is of great significance for the gastrointestinal ecosystem of slaughter pigs and to reduce the counts of pathogenic *Enterobactericea* (i.e. *E coli* and *Salmonella*).

The effect of various types of feed processing on the population density of coliform bacteria in the stomach, the incidence of salmonella posi-
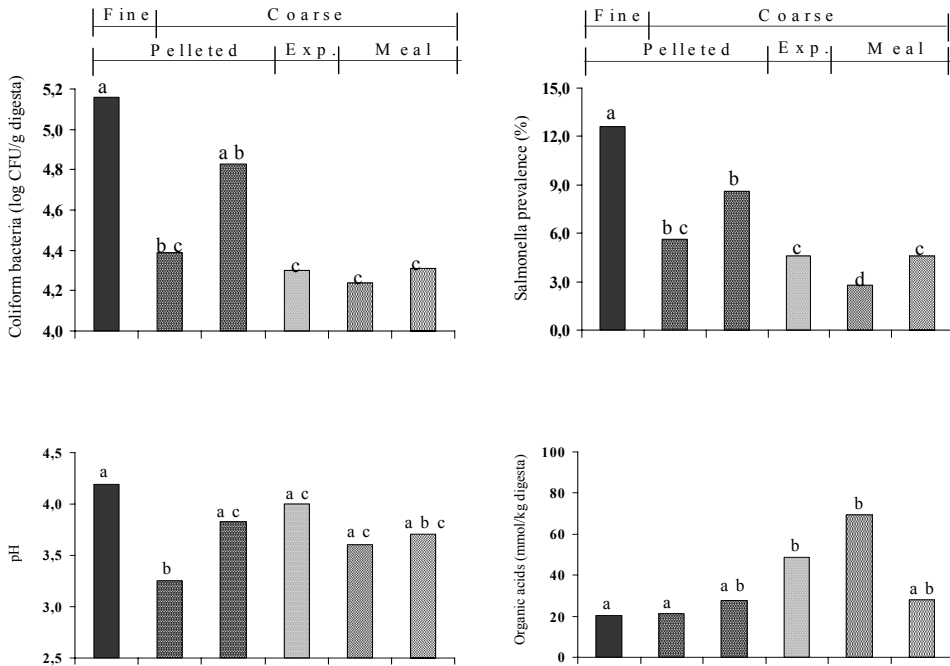
Figure 1. Effect of various types of feed processing on the density of coliform bacteria in stomach content, the prevalence of salmonella positive pigs, pH of stomach content and the concentration of organic acid in stomach content (data adapted form Jørgensen et al., 1999)

tive pigs, the concentration of organic acids in stomach content and the pH of stomach content are shown in Figure 1 (data from Jørgensen et al., 1999).

These results clearly illustrate that the effect of feed processing on the incidence of salmonella-positive pigs and the density of coliform bacteria in the stomach is similar. Indicating that the density of coliform bacteria is a good indicator for the surveillance of Salmonella in pigs. The data further indicate that feeding slaughter pigs course grinded feed compared to fine pelleted feed (diet 5 versus diet 1) reduces the pH of stomach content and significantly increases the concentration of organic acids (lactic, acetic, propionic and butyric acids) in the stomach. The increase in organic acid concentration was accomplished by an increase in the population of anaerobic bacteria, especially lactic acid bacteria (results not shown). These data are in accor-

dance with the results obtained by Canibe et al., (2001). Using the molecular fingerprinting method T-RFLP as described by Leser et al., (2000) Canibe et al., (2001) further showed that the microbial diversity was higher in pigs fed course meal compared to pigs fed fine pelleted feed (Figure 2).

The mechanism behind the stimulating effect of coarse meal on the microbial activity in the stomach has not been elucidated. A slower passage rate (Regina et al., 1999), a higher dry matter percentage, and/or other physiochemical characteristics of the gastric digesta (Hansen et al., 2001) of pigs fed coarse meal could favour the growth of lactic bacteria compared to other microorganisms. The higher density of lactic acid bacteria in the stomach of pigs fed the coarse diet explain the higher concentration of lactic acid in the gastric digesta of these animals.
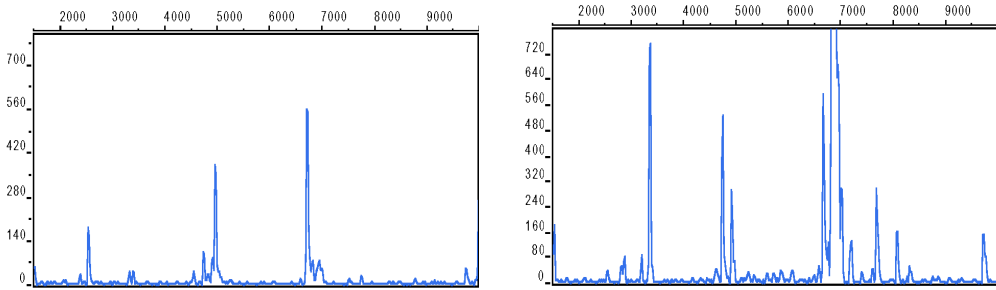
Figure 2. T-RFLP profile from stomach content of pigs fed either fine pelleted feed or course meal

The growth of enterobacteria is inhibited at high levels of lactic acid at low pH (Knarreborg et al., 2002). Further unpublished results from our lab (Mikkelsen and Jensen, unpublished) have shown a clear negative correlation between the concentration of undissociated lactic acid and the survival rate of *Salmonella* in stomach content from slaughter pigs *in vitro* (Figure 3).

In contrast to the effect on enterobacteria, lactic acid seems to stimulate the growth of yeasts (Maribo et al., 2000) this may explain the higher population of yeasts found in course meal fed-pigs compared to fine pelleted fed-pigs pigs by Canibe et al., (2001).

Effect of feed processing on gut morphology
A positive effect of feeding course diets to pigs on stomach keratinisation and lesions has been reported by various authors (Jørgensen et al., 1999; Regina et al., 1999; Wonda et al., 1995), but the mechanism behind is still unclear. It seems reasonable to suggest that the main factor that leads to the protective effect of course meal against gastric ulcers is that the gastric content became more coherent with little separation between the liquid and solid phase (Hansen et al., 2001 a,b; Jørgensen et al., 2002a).

Apart from a changed microbial ecosystem in the gastrointestinal tract of slaughter pigs, it has also been shown that pigs fed coarse diets had higher levels of epithelial cell proliferation with consequent higher levels of mucins and fewer mannose receptors than those fed a fine diet (Brunsgaard, 1998). This implies that pigs fed a coarse diet may be better protected against certain kinds of intestinal infections than pigs fed a fine diet.
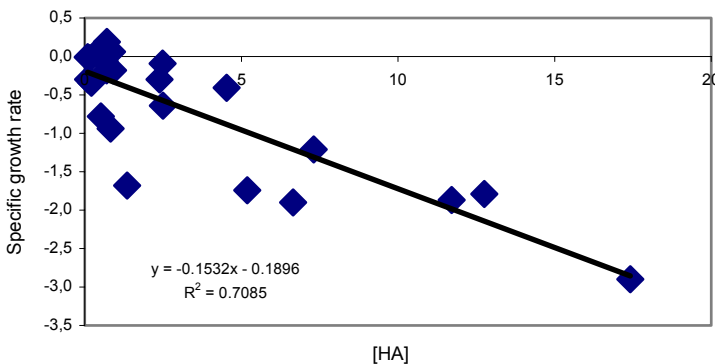
Figure 3. Correlation between the concentration of undissociated lactic acid (HA) and the survival rate of Salmonella DT12 in stomach content

The stomach as barrier

The results discussed above clearly show that coarsely- ground meal feed favours the growth of anaerobic bacteria, especially lactic acid bacteria in the stomach. The exact reason for this is at present unknown, but the increased microbial activity leads to an increased production of lactic acid and other organic acids and a lover pH in the stomach. Consequently, enteric bacteria like *Salmonella* and *E. coli* are killed in the stomach and do not enter the parts of the gastro-intestinal tract in which they will normally proliferate. In this way, the stomach acts as a barrier breaking the vicious circle where animals in problematic herds infect each other and are re-infected through consumption of faeces.

Effect of feed processing on growth performance

A big drawback with the use of coarsely-grounded meal, is the extreme reduction in production results (Jørgensen et al., 1999; Jørgensen et al., 2002a). Especially the feed conversion rate is reduced by feeding pigs course meal compared to fine pelleted feed. One of our goals at the moment, therefore, is to develop a feed compound or a feeding strategy, which is able to prevent the proliferation of pathogenic enterobacteria without reducing feed conversion.

Oligosaccharides (prebiotics)

Use of specific carbohydrates (prebiotics) especially sugar alcohols and oligo- and polysaccharides that are not degraded by host-produced enzymes have been suggested as candidates to improve animal health. Prebiotics are defined as "non digestible food ingredients that beneficially affect the host by selectively stimulating the growth/or activity of one or a limited number of bacteria, that can improve host health".

Oligosaccharides have been included in animal feed in Japan since the mid-80s with the aim of improving weight gain and health status of farm animals. However, documented effects of oligosaccharides on pig performance, animal health and effects on the composition of the indigenous microbiota are sparse and conflicting (Mikkelsen, 2001).

In a recent study carried out at our department (Mikkelsen, 2001) newly weaned four-week-old piglets were fed a semi-synthetic diet supplemented with 4% fructo-oligosaccharides (FOS) or 4% galactooligosaccharides (GOS) replacing 2% cornstarch and 2% cellulose in the control diet. In conclusion, generally little or no effect of dietary FOS and GOS on pig performance, gastrointestinal physico-chemical characteristics and gastrointestinal bacterial populations were observed in the study. The most striking result from the study was the strong stimulating effect of especially GOS and to a lesser extent FOS on the population of yeasts (Figure 4).

Figure 4. Density of yeasts in fecal or intestinal samples of pigs fed either a control diet, a diet added 4% FOS or a diet added 4% GOS

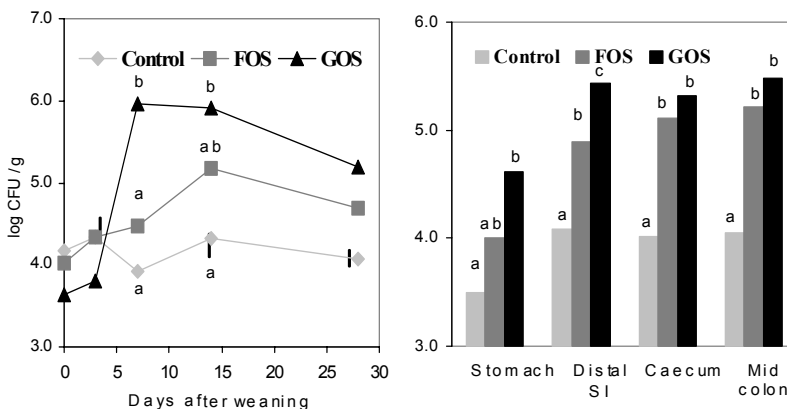Yeasts and yeast cell walls are often suggested as probiotic additives that can be applied in the pig industry as alternatives to antibiotic growth promoters in order to improve health and growth performance of pigs. Since the population of yeasts within the gastrointestinal tract of pigs seems easy to influence, any negative or positive impact of this microorganism on the growth performance of pigs would be of great interest in future studies.

Conclusion

Use of coarsely grounded meal feed is a clear alternative to antibiotic growth promoters regarding the reduction of pathogenic enterobacteria in the gastrointestinal tract. However a big drawback with this type of diets is the reduction found in the production results. A goal for the moment, therefore, is to develop a feed compound or a feeding strategy, which like grounded meal feed, is able to prevent the proliferation of pathogenic enterobacteria in the gastrointestinal tract but without affecting the production results.

References

Canibe, N. and Jensen, B.B. 2001. Effect of feed structure and processing and addition of formic acid to the feed on microbial ecology of the gastrointestinal tract of pigs. AFAC Workshop. 13-16 October 2001, Oslo Norway.

Brunsgaard G. 1998. Effects of cereal type feed particle size on morphological characteristics, epithelial cell proliferation and lectin binding patterns in the large intestine of pigs. J. Anim. Sci .76, 2787-2798.

Durmic, Z., Pethick, D.W, Pluske, J.R., Hampson, D.J. 1998. Change in bacterial populations in the colon of pigs fed different sources of dietary fibers and the development of swine dysentery after experimental infection. J. Appl. Microbiol. 85, 574-582

Hansen, C.F., Back Knudsen, K.E. Jensen, B.B. and Kjærsgaard, H.D. 2001a. Effect of meal feed coarser grinding of pelleted feed on productivity, microbiology, and physico-chemical properties in the gastrointestinal tract of finish-

ers (in Danish). Publication no 534. The national committee for Pig Production, Copenhagen, Denmark

Hansen, C.F., Bach Knudsen, K.E., Jensen, B.B. and Kjærsgaard, H.D. 2001. Effect of meal feed, potato protein concentrate, barley, beet pellets and zinc gluconate on Salmonella prevalence, gastrointestinal health and productivity in finisher (in Danish). Publication no 528. The national committee for Pig Production, Copenhagen, Denmark

Jensen B.B. 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J. Anim. Feed Sci. 7, 45-64.

Jensen, B.B and Jørgensen, H. 1994. Effect of dietary fibre on microbial activity and microbial gas production in various regions of the gastrointestinal tract of pigs. Appl. Environ. Microbiol. 60, 1897-1904.

Jørgensen, L., Dahl, J., Jensen B.B. and Poulsen, H.D. 1999. Effects of expanding, pelleting and grinding on Salmonella typhimurium infection, growth performance and gastrointestinal ecosystem in slaughter pigs (in Danish). Publication no.426. The national committee for Pig Production, Copenhagen, Denmark.

Jørgensen, L., Hansen, C.F., Bach Knudsen, K.E., Jensen, B.B., and Kjærsgaard, H. 2002a. Particle size in meal feed to slaughter pigs. Effect on productivity, Salmonella prevalence and the gastrointestinal ecosystem (in Danish). Publication no. 580. The national committee for Pig Production, Copenhagen, Denmark.

Jørgensen, L., Kjærsgaard, H.D., Wachmann, H., Jensen, B.B. and Bach Knudsen, K.E. 2002b. Effect of pelleting and use of lactic acid in feed on Salmonella prevalence, the gastrointestinal ecosystem and productivity in weaners (in Danish). Publication no. 543. The national committee for Pig Production, Copenhagen, Denmark.

Knarreborg, A., Miquel, N., Granli, T. and Jensen, B.B. 2002. Establishment and application of an in vitro methodology to study the effects of organic acids on coliform and lactic acid bac-

teria in the proximal part of the gastrointestinal tract of piglets. Anim. Feed Sci. Technol. 99, 131-140.

Kirkwood, R.N., Huang, S.X., McFall, M., Aheren, F.X. 2000. Dietary factors do not influence the clinical expression of swine dysentery. Swine Health Prod. 8, 73-76.

Lindecrona R.H., Jensen B.B., Jensen T.J., Leser T., and Møller, K. 2000. The influence of diet on the development of swine dysentery. 16th IPVS congress, Melbourne, Australia 17-20 September.

Maribo, H., Jensen, B.B. and Hedemann, M.S. 2000. Different doses of organic acids to Piglets (in Danish). Publication no. 460. The national committee for Pig Production, Copenhagen, Denmark.

Mikkelsen. L.L. 2001. Fructooligosaccharides and galactooligosaccharides as prebiotics for piglets at weaning. PhD thesis. The Royal Veterinary and Agricultural University, Copenhagen, Denmark.

Pedersen, A.Ø. Dahl, J. and Jensen, B.B. 2000. Effect of tylosin and feed protein content on occurrence of diarrhoea in slaughter pigs (in Danish). Publication no. 488. The national committee for Pig Production, Copenhagen, Denmark.

Pluske, J.R., Siba, P.M., Pethick, D.W., Durmic, Z., Mullan, B.P., Hampson, D.J. 1996. The incidence of swine dysentery in pigs can be reduced by feeding diet that limit the amount of fermentable substrates entering the large intestine. J. Nutr. 126, 2920-2033.

Prohaszka, L., Jayarao, B.M., Fabian, A., Kovacs, S. 1989. The role of intestinal volatile fatty acids in salmonella shedding of pigs. J. Vet. Med. B. 37, 560-574.

Reid, C.A. and Hilman, K. 1999. The effect of retogradation and amylose/amylopectin ratio of starches on carbohydrate fermentation and microbial populations in the porcine colon. Anim. Sci. 68, 503-510.

Regina, D.C., Eisemann, J.H., Lanf, J.A. and Argenzio, R.A. 1999. Changes in gastric contents in pigs fed a finely ground and pelleted or coarsely ground meal diet. J. Anim. Sci. 77, 2721-2729.

23. Effects of the termination of AGP use on outbreaks of *Lawsonia intracellularis* in pigs

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Porcine proliferative enteropathy (PE) is one of the most important infections causing diarrhoea, retarded growth and/or sudden death among growing/ finishing pigs in intensive pig production world-wide. The disease affects the aboral small intestines, cecum and colon but involves especially ileum. Pathologically, PE is characterised by thickening of the intestinal mucosa caused by adenomatous proliferation of immature epithelial cells infected by *Lawsonia intracellularis*, an obligate intracellular Gram-negative bacterium culturable only in cell cultures. As coculturing of the bacterium in cells is not adaptable for routine diagnostic procedures PCR, immunohistochemistry and *in situ* hybridisation have been developed and applied for ante- and post-mortem detection.

In a case control study to examine risk factors for the development of diarrhoea in growing pigs *L. intracellularis* was the most prevalent pathogen. The bacterium was detected in 75% of the herds with diarrhoeal problems compared to 39% of the control herds. *Brachyspira hyodysenteriae*, the cause of swine dysentery, and *Salmonella* were detected in 14% and 13% of the case herds, respectively. Weakly hemolytic *Brachyspira* spp including *B. pilosicoli*, the cause of spirochaetal colitis, were detected in 39% of the case herds.

In endemically infected herds *L. intracellularis* is present in all groups of animals including sows and suckling piglets. However, the prevalence peaked in 6 to 8 weeks old weaned pigs.

To determine the herd-level and within-herd prevalence of *L. intracellularis* in Danish finishing pig herds we visited 79 randomly selected herds. *L. intracellularis* was found in 94% of the herds whereas the herd prevalence of *B. hyodysenteriae* and weakly hemolytic Brachyspira spp were 2.5% and 50%, respectively. The within prevalence of both *L. intracellularis* and *B. hyodysenteriae* were 25 – 30%.

The infection dynamics of L. intracellularis from weaning to slaughter in 5 herds were studied in a longitudinal survey. Most *L. intracellularis* infected pigs were shedding at 8 -12 weeks of age. Infected pigs were shedding for 2-6 successive weeks – after 18 weeks of age all shedding was ceased. The growth rate of infected pigs seemed to decrease in the period of shedding.

Besides therapeutic use of antibiotics eradication of *L. intracellularis* by medication has been shown to be possible in sow herds.

24. Alternatives to AGPs in broiler production

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Summary

In the present paper different dietary approaches to manipulate the gastro-intestinal microflora in order to improve growth and health in broiler chickens are described. The importance of the feed structure in broiler nutrition as well as the use of feed additives like non-starch polysaccharide (NSP)-degrading enzymes, organic acids, probiotics, prebiotics and essential oils are discussed.

Among the presented feeding strategies the most promising are the dietary addition of NSP-degrading enzymes, e.g. xylanase, as well as the use of whole wheat, which is the currently common feeding practice in Danish broiler production. Xylanase decreases digesta viscosity resulting in an improved nutrient digestibility and consequently enhance broiler growth. The beneficial effect of whole wheat feeding is attributed to a reduction of the gizzard pH and an increased retention time of the feed in the gizzard. This results in a reduction of the population of lactose-negative bacteria, e.g. Salmonella, and of *Clostridium perfringens*. The gizzard can be regarded as a barrier organ in preventing the entrance of potentially pathogenic bacteria to the small intestine.

Introduction

The search for alternatives to AGPs in broiler production has occupied our minds since these substances were banned in Denmark in January 1998. In order to find appropriate substitutes, it is important to briefly reflect on the benefits and working mechanisms of AGPs .

All AGPs previously used in broiler production preferentially target gram-positive bacteria. With respect to bird health, this property makes them very suitable in the prevention of necrotic enteritis caused by *Clostridium perfringens* type A. With respect to their beneficial effect on broiler performance AGPs have shown to inhibit intestinal bacteria competing with the host for available nutrients. It is reasonable to assume that the microflora in the small intestine is of major importance in this respect, since absorption of nutrients mainly takes place at this location. The dominant bacteria in the small intestine are gram-positive bacteria belonging to the group of lactobacilli and enterococci. Furthermore, *Clostridium perfringens* is very abundant, especially in the lower small intestine. Lactobacilli are generally believed to offer health benefits for the host. The production of lactic acid resulting in a reduction of intestinal pH, as well as the production of bacteriocins, and the stimulation of the local intestinal immunity are all factors exerting a hostile environment for undesirable bacteria of the gram-negative flora, e. g. *E. coli*, Campylobacter, and Salmonella. However, the majority of lactic acid bacteria as well as *Clostridium perfringens* are capable of bile acid-deconjugation, which hampers intestinal fat emulgation, formation of micelles and consequently the absorption of lipids (Knarreborg et al., 2002). Bearing the role of the intestinal microflora in broiler growth depression in mind, it is obvious that alternatives to antibiotic growth promoters should aim at the reduction of the intestinal bacterial number and fermentation activity. This can be achieved in two ways: One way is to increase the digestibility of the broiler feed. This leads to a reduction of undigested material in the small intestine and minimises the substrate available for microbial growth and fermentation. The other way of achieving a reduced microbial growth in the small intestine and thereby growth promotion, is the addition of compounds with bacteriostatic properties. From the aspect of health it would be desirable to aim this bacteriostatic effect at undesirable potentially pathogenic bacteria including *E. coli*, Salmonella, Campylobacter and *C. perfringens*.

In the following different approaches to manipulate the broilers intestinal flora in order to improve growth and health are presented.

Non-starch polysaccharide (NSP)-degrading enzymes

The dietary addition of enzymes degrading non-starch polysaccharides (NSP), e.g. xylanases and β-glucanases, has shown to reduce intestinal viscosity and to increase nutrient digestibility, thus resulting in an improved broiler performance (Table 1), in particular when high amounts of wheat or barley are fed. Another positive effect of exogenous enzymes is a reduction of the consumption of drinking water (Table 1), which improves the quality of the litter.

Since the dietary inclusion of water-soluble viscous polysaccharides does not lead to growth depression in germ free chickens, it seems that the intestinal microflora also plays a central role in the mediation of growth response to enzymes. NSP-degrading enzymes are hypothesised to work in two steps, which may be described by an ileal phase and a caecal phase. During the ileal phase enzymes remove fermentable substrates and during the caecal phase, degradation products of sugars like xylose and xylo-oligomers are fermented by caecal bacteria, thus stimulating the production of volatile fatty acids and the growth of "beneficial bacteria" (Bedford, 2000). This is illustrated by a decrease in caecal pH following addition of xylanase to wheat based diets (Table 2). In Denmark, the feeding of whole wheat as a supplement to a pelleted compound broiler feed is common practice. Experiments carried out in our laboratory show that the addition of enzymes is particularly important when feeding whole wheat, since whole wheat increases chyme viscosity as compared to a pelleted feed. (Figure 1).

Table 1. Body weight, feed conversion ratio (FCR), and water intake of 42 d old broiler chickens fed with anaerobically stored whole wheat (ASWW), conventionally stored whole wheat (CSWW) and ground wheat (pellets) with or without addition of xylanase

	Treatment						Effect of treatment	
Form of wheat	ASWW		CSWW		Pellet		Wheat form	Xylanase
Xylanase	-	+	-	+	-	+	P-value	
Body weight (g, 42 d)	2092	2128	2075	2116	2107	2143	0.147	0.003
FCR (kg feed/kg gain)	1.68	1.72	1.64	1.58	1.74	1.67	0.001	0.229
Water (ml/bird)	6479	6259	6578	6237	6758	6508	0.001	0.001
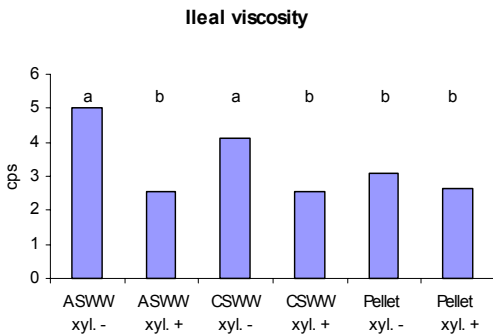
Figure 1. Ileal viscosity in broiler chickens fed with anaerobically stored whole wheat (ASWW), conventionally stored whole wheat (CSWW) and ground wheat (pellets) with or without addition of xylanase

Over the last approximately 5 years feeding enzymes have become standard in the formulation of Danish broiler diets. The use of enzymes is probably one of the main reasons why the elimination of AGPs in Denmark, where broiler diets are wheat based, did not have dramatic consequences for broiler performance.

Feed structure

The structure of the feed e.g. the feeding of a coarsely ground mash or the feeding of whole grains has a strong effect on the physiological function of the broiler gastrointestinal tract (Svihus et al., 1997, Engberg et al., 2002).

We conducted an experiment with broiler chickens, where wheat (variety Kris) harvested from the same field was either fed as whole wheat stored anaerobically (ASWW) or conventionally (CSWW) or fed as ground wheat included in pellets. All diets were fed with or without addition of xylanase. Differences between birds receiving ASWW and CSWW were only marginal, whereas distinct differences were observed between animals supplemented with whole wheat and pellet-fed birds. The feeding of whole wheat significantly improved FCR and reduced the water intake as compared to pellet fed birds (Table1). The pH value in the contents of the gizzard and proximal part of the small intestine was significantly lower in birds supplemented with whole wheat as compared to pellet fed birds, which indicates a stimulation of gastric functions e.g. hydrochloric acid secretion (Engberg et al., 2002). Further, birds fed with whole wheat had significantly higher gizzard weights and a higher amount of gizzard contents with a higher DM content (results not shown). Whole wheat feeding slightly stimulated the population of lactic acid bacteria in the upper small intestine and reduced the intestinal number of *Clostridium perfringens* and lactose-negative enterobacteria (Table 2).

It is hypothesised that the stimulation of gastric functions (low gizzard pH and higher retention time of the feed) contributes to a growth inhibition of potentially pathogenic bacteria of the gram-negative flora, e.g. *Salmonella,* which are not very acid tolerant (Naughton and Jensen, 2001). The gizzard can therefore be regarded as barrier organ in preventing the entrance of these bacteria to the distal intestinal tract. Furthermore, the low counts of *Clostridium perfringens* observed in the groups fed whole wheat indicate a beneficial influence on animal health considering the role of these bacteria in the development of necrotic enteritis.

**Table 2. Intestinal pH, lactic acid bacteria, coliform bacteria, lactose-negative enterobacteria, and *Clostridium perfringens* in selected intestinal segments of broiler chickens fed with anaerobically stored whole wheat (ASWW), conventionally stored whole wheat (CSWW) and ground wheat (pellets) with or without addition of xylanase**

Form of wheat	Treatment						Effect of treatment	
	ASWW		CSWW		Pellet		Wheat form	Xylanase
Xylanase	-	+	-	+	-	+	P-value	
pH								
Gizzard	3.05	2.96	2.88	3.05	3.34	3.56	0.003	0.386
Jejunum	5.64	5.64	5.61	5.50	5.79	5.63	0.014	0.048
Caeca	5.66	5.49	5.62	5.50	5.61	5.56	0.868	0.017
	(log CFU/g digesta)							
Lactic acid bacteria								
Jejunum	8.24	8.42	8.37	8.60	8.06	8.21	0.070	0.095
Ileum	9.08	9.17	8.80	9.12	8.90	9.12	0.279	0.019
Coliform bacteria								
Jejunum	5.10	5.60	5.53	5.78	5.74	5.91	0.345	0.252
Ileum	6.93	7.14	7.50	7.14	7.40	7.52	0.136	0.970
Lactose-negative enterobacteria								
Jejunum	4.06	4.41	4.30	4.60	5.11	5.29	0.008	0.271
Ileum	5.36	5.36	5.97	5.84	6.72	6.87	0.001	0.976
Caeca	5.72	5.19	5.97	5.59	5.98	6.06	0.224	0.304
Rectum	5.59	5.72	6.58	5.84	7.00	6.94	0.001	0.379
Clostridium perfringens								
Ileum	5.25	5.45	5.72	5.39	6.44	6.28	0.081	0.791
Caeca	5.78	5.45	5.67	5.71	6.52	6.42	0.053	0.675

The protective effect of whole wheat against Salmonella was confirmed in a study with broilers infected with a rifampicin resistant *Salmonella typhimurium* DT110 at 14d. Although caecal colonisation could not be prevented in the young bird (4 days *post infectionem*) receiving only 10% of supplemental whole wheat, in the later growth phase significantly decreased numbers of *Salmonella typhimurium* were found in the gizzard and ileum of birds receiving 30% whole wheat. This means that a possible re-infection by uptake of small concentrations of Salmonella can be naturally prevented by whole wheat, through a decrease in gizzard pH. Keeping that in mind whole wheat is suggested to be also very suitable in the nutrition of egg-layers, since the digestive tract of adult birds is more capable of whole grain digestion.

Organic acids

The antibacterial activity of organic acids is related to the reduction of pH, as well as their ability to dissociate, which is determined by the pK_a-value of the respective acid, and the pH of the surrounding milieu. The antibacterial activity increases with decreasing pH value. Organic acids are lipid soluble in the undissociated form, in which they are able to enter the microbial cell. Once in the cell, the acid releases the proton in the more alkaline environment, resulting in a decrease of intracellular pH. This influences microbial metabolism inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to release protons, leading to an intracellular accumulation of acid anions. The bacteriostatic effect of organic acids is preferentially aimed at gram-negative bacteria, since these bacteria are less acid-tolerant as compared to e. g. lactic acid bacteria. Therefore, different combinations of organic acid are extensively used as feed additives in pig production in the prevention of especially weaning diarrhoea.

In poultry, organic acids are assumed to inhibit growth of bacteria in the feed and in the upper part of the digestive tract (crop and stomach) rather than in the intestine, which results in a lower number of potentially pathogenic bacteria entering the intestine. Indeed, some organic acids, e. g. formic acid and propionic acid (Bio-Add™, 68% formic acid and 20% propionic acid) at dietary concentrations of 0.6% seem to be able to prevent Salmonella infections (Berchieri and Barrow, 1996).

Benzoic acid is not approved as a feed additive for poultry feed, but is extensively used as food preservative in human nutrition. In an experiment with broiler chickens we added benzoic acid to broiler diets at concentrations of 0%, 0.1%, 0.2%, 0.4% and 0.8%. One group received a diet supplemented with Avilamycin (10 mg/kg feed). Benzoic acid in concentrations of 0.1% benzoic acid stimulated broiler growth up to approximately 4 weeks of age in the same range as avilamycin. The increase in weight gain could be related to a significantly higher feed intake in that group (Table 3). Benzoic acid at concentrations of 0.8% resulted in a significant growth depression associated with a significantly decreased feed intake.

Table 3. Production results of broiler chickens receiving increasing concentrations of benzoic acid and avilamycin

	Benzoic acid (%)				Avilamycin	
	-	0.1	0.2	0.4	0.8	10 mg/kg	P-value
Body weight			*(g)*				
15 d	347 [ab]	363 [a]	350 [ab]	329 [bc]	315 [c]	361 [a]	0.0004
29 d	1007 [b]	1064 [a]	1020 [ab]	979 [bc]	941 [c]	1065 [a]	0.0001
43 d	1900 [b]	1947 [b]	1900 [b]	1887 [b]	1770 [c]	2037 [a]	0.0001
Feed intake			*(g/bird)*				
1-43 d	3574 [b]	3740 [a]	3593 [b]	3468 [bc]	3411 [c]	3768 [a]	0.0001
FCR			*(kg feed/kg gain)*				
1-43 d	1.92 [b]	1.96 [ab]	1.93 [ab]	1.87 [c]	1.97 [a]	1.88 [bc]	0.0009

Means in the same row followed by different superscript letters (a,b,c) differ significantly

Probiotics, prebiotic and essential oils

Probiotics are defined as live microbial feed additives, which beneficially affect the animals by improving their microbial balance (Fuller, 1989). At present 19 micro organism preparations, among them 7 for the use in poultry, are provisionally or finally authorised as feed additives within the EU. The approved organisms for the use in poultry belong to the bacterial genera of *Enterococcus, Bacillus* and in one case *Pediococcus* as well as the yeast *Saccharomyces cerevisiae*. The different modes of action of various probiotic strains are far from understood (Review by Ghadban, 2002). However, the production of bacterial metabolites in terms of organic acids in particular lactic acid (reduction of pH), as well as hydrogen peroxides and bacteriocins have been suggested to be responsible for an antagonistic effect towards possible pathogenic bacteria of the gram-negative flora, e. g. Salmonella. With respect to growth performance, some probiotics are known to produce digestive enzymes such as amylase, protease and lipase, which may enrich the concentrations of the intestinal digestive enzymes of the host and thereby support nutrient digestion. Furthermore, a reduction of bacterial ammonia production causing damages on the intestinal cell surfaces, as well as a stimulation of the local immune system were reported as beneficial effects of probiotics. Looking at the literature, it seems that lactobacilli are the most studied organisms and considering health, most beneficial effects are assumed related to these bacteria.

However, unlike human nutrition where lactobacilli can be provided via consumption of different fermented milk products, lactobacilli are not applied to broiler feed. This is manly due to their instability to high temperatures during feed processing. It is peculiar that organisms like *Enterococcus faecium* showing a strong bile acid deconjugation activity and possibly hampering fat absorption (Knarreborg, 2002), are used as probiotics where these organisms are expected to enhance growth. The published studies conclude, that the efficacy of the above mentioned probiotics seems to vary a lot. However, most of these studies report on slight improvement of bird performance considering feed conversion ratio and/or weight gain. The results of our own study with three probiotic strains (two *Enerococcus faecium* strains and one Bacillus coagulans strain) point in the same direction (Table 4). Slight improvements of weight gain and feed conversion ratio were observed at 21 d following dietary addition of probiotics.

Prebiotics are non-digestible feed ingredients, which are fermented in the digestive tract, where they selectively stimulate growth or metabolic activity of "beneficial bacteria". To this category of feed additives belong special carbohydrates e.g. fructooligosaccharides inulin, and pectin. It has been shown that inulin, which selectively stimulates bifidobacteria in humans, is fermented in the chicken intestine by Enterobacteriaceae and Streptococcaceae (Simon and Jadamus, 2002).

Table 4. Production results of broiler chickens following dietary addition of three different probiotic bacterial strains over 42 days

	Negative Control	*B. coagulans* Product A	*B. coagulans* Product A	*E. faecium* Product B	*E. faecium* Product B	*E. faecium* Product C	
Dose	-	1×10^8	*(per kg feed)* 1×10^9	1×10^8	1×10^9	5×10^8	P-value
Body weight			*(g)*				
21 d	629 [a]	629 [a]	650 [b]	643 [ab]	656 [b]	649 [b]	0.036
42 d	2145	2154	2167	2178	2177	2168	0.755
FCR			*(kg feed/kg gain)*				
1-21 d	1.46 [a]	1.44 [ab]	1.42 [bc]	1.43 [b]	1.40 [c]	1.43 [b]	0.001
1-42 d	1.68	1.66	1.66	1.68	1.68	1.68	0.807

Means in the same row followed by different superscript letters (a,b,c) differ significantly

Essential oils are extracts of different plant, which work as appetisers and stimulate digestive enzyme activity, thus improving nutrient digestibility. As mentioned before regarding the working mechanisms of NSP-degrading enzymes, an improved digestion will result in a reduction of substrate available for bacterial growth in the small intestine. At the moment research is in progress that aims at a combination of essential oils with acidifiers and probiotics (Kamel, 2002).

Conclusion and perspectives

Looking back, it seems that the broiler production in Denmark has not suffered a great deal from the removal of AGPs. This may be partly due to an improvement in the producers´ management practices as well as the extensive use of whole wheat and feeding enzymes. The composition and the structure of poultry feed are the most important factors modulating digestive physiology and the intestinal microflora. In particular problems associated with necrotic enteritis (*Clostridium perfringens* type A), which have previously been controlled by AGPs did not increase in the absence of these substances. It is very likely that the extensive continuous use of ionophore anticoccidials, which also target gram-positive bacteria, is the main reason why these problems did not occur in Danish broiler production. Although attenuated live vaccines against coccidiose as substitutes for ionophores and chemical anticoccidials are available now, their use is not very common mainly because of higher costs. Furthermore, coccidial sporozoites in the vaccine are suspected to cause small mucosal lesions, which may increase the risk of intestinal colonisation with *Clostridium perfringens* resulting in higher frequency of necrotic enteritis. If steps are taken to withdraw ionophore antibiotics from the feed, necrotic enteritis will undoubtedly be the most urgent problem to be solved. Basic research considering the factors that lead to intestinal colonisation and trigger the toxin production of *Clostridium perfringens* is necessary in order to develop appropriate feeding strategies to reduce necrotic enteritis.

References

Bedford, M. 2000. Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimise subsequent problems. World Poultry Sci. J. 56, 347-365.

Berchieri, A. Jr., Barrow, P A. 1996. Reduction in incidence of experimental fowl thyphoid by incorporation of a commercial formic acid preparation (Bio-Add™) into poultry feed. Poultry Sci. 75, 339-341.

Engberg, R.M., Hedemann, M.S., Jensen, B.B. 2002. The influence of grinding and pelleting of feed on the microbial composition and activity in the digestive tract of broiler chickens. Br. Poultry Sci. 43, 569-579.

Fuller, R. 1989. Probiotics in man and animals: A review. J. Appl. Bacteriol. 66, 365-378.

Ghadban, G. S. 2002. Probiotics in broiler production - a review, Archiv für Geflügelkunde, 66, 49-58.

Kamel, C. 2001. Plant extracts in an integrated approach. Feed Mix, 9: No.6 23-25.

Knarreborg, A. 2002. The impact of microbial deconjugation of bile salts on fat digestion in broiler chickens. ph.d.thesis, The Royal Veterinary and Agricultural University.

Naughton, P. J., Jensen, B.B. 2001. A bioreactor system to study survival of Salmonella typhimurium in pig gut content. Berliner und Münchener Tierärztliche Wochenschrift 114, 1-4.

Simon, O., Jadamus, A. 2002. Probiotics and prebiotics, Eur. Poultry Sci. 66, 45, Abstract.

Svihus, B., Herstad, O., Newman, C. W., Newman, R. K. 1997. Comparison of performance and intestinal characteristics of broiler chickens fed on diets containing whole, rolled or ground barley. Br. Poultry Sci. 38, 524-529.

25. Alternatives to AGP in pig production – practical experience

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Focus on alternatives

The National Committee for Pig Production tests various commercial products in co-operation with the companies selling the products. This is necessary as documentation for the effect is requested by pig producers, advisors and producers of feed.

A large number of Danish trials with antibiotics has been carried out. Table 1 shows the results achieved in trials with growth promoters that are legal in the EU in 2002 (Avilamycine, Flavomycine and Salinomycin) compared with a negative control group.

Antibiotic growth promoters are no longer used in Denmark. A voluntary agreement between pig producers and slaughterhouses in 2000 banned the use of antibiotic growth promoters.

The following tables show the effect of alternatives. In all the tables, the group to which the product was added is compared with a negative control group (without antibiotics).

Acid products - preservatives

Preservatives are used to extend the shelf-life of perishables particularly in the foodstuff industry. Preservatives are also used in farming, particularly products such as formic acid in whey and chemical treatment of cereals with propionic acid. Acid is often added to weaner feed to preserve and to reduce the acid binding capacity of the feed.

The group of preservatives comprises a wide range of acids and salts. The effect of the preservatives is caused by a restraint of the growth of unwanted micro organisms in feed and stomach.

Danish results with acid products – preservatives

A number of trials has been carried out in Denmark with organic acids and mixes of this to weaners and finishers, and there was great variation in the trials (table 2). One of the reasons for the great variation is the dose and the actual concentration of acids in the products, which vary greatly between the trials.

Table 1. Effect of antibiotic growth promoters on productivity, change in %

	Weaners	Growers	Finishers
No. of trials	5	6	5
Daily gain	+10.8	+2.5	+2.8
Feed conversion	-3.4	-2.5	-2.4
No. of trials with a significant effect on productivity	2	0	0

Table 2. Effect of acid products on productivity, change in %

	Weaners	Finishers
No. of trials	49	10
Daily gain	+6.3	+0.4
Feed conversion	-2.2	+0.2
No. of trials with a significant effect on productivity	14	0

The results of the individual acids and salts are shown in table 3 below.

Table 3. Effect of individual acid products on productivity, change of production result, %

Acid/acid salt	Dose, %[1]	Daily gain	FUp/kg gain	Produc-tivity	Source
Weaners					
Calprona	1.4/1.2	+10.7	-5.8	*	LU Rep. no. 364, 1997
Calprona	1.4/1.2	-4.7	-0.6	-	LU Rep. no. 364, 1997
Fra-acid	0.75/0.5	+6.8	-1.1	*	LU Rep. no. 365, 1997
Formic acid in water	0.2	+8.0	-3.1	*	LU Rep. no. 342, 1996
Formic acid in water	0.2	-0.8	+2.7	-	LU Rep. no. 342, 1996
Fumaric acid	1.5	-2.7	+2.7	-	LU Rep. 73, 1985
Fumaric acid	1.5	+5.0	-2.1	-	LU Rep. 73, 1985
Acid Lac	0.5	+3.6	-3.3	-	LU Rep. no. 322, 1995
Acid Lac dry	0.5	+2.9	0.0	-	LU Rep. no. 384, 1998
Bio Add	0.5	-0.6	-1.1	-	LU Rep. no. 322, 1995
Aciprol Micropearls	0.2	+7.8	-3.9	*	LU Rep. no. 371, 1997
Nutricid	0.4	+5.0	-2.8	tendency	LU Rep. no. 371, 1997
Probicid	0.8	+16.9	-3.5	*	LU Rep. n. 396, 1998
Bio-pro	0.2	+15.3	-2.3	*	LU Rep. no. 396, 1998
Calcium formate	1.25	+16.4	-6.9	*	LU Rep. no. 396, 1998
Bolifor FA 2000	0.65	+18.5	-5.2	*	LU Rep. no. 396, 1998
Selacid	0.5	+4.8	-2.3	-	LU Rep. no. 384, 1998
Luprocid	0.6	+8.8	-1.2	*	LU Rep. no. 409, 1999
Lafeed 80	2.0/1.0	+5.5	-2.9	*	LU Rep. no. 428, 1998
Zoolac - (g/pig)	6.4/12.8	-1.1	-1.2	-	LU Rep. no. 407, 1998
Eroacid LFPA	0.4	+6.8	-0.6	-	LU Rep. no. 441, 1999
Greenacid LBF	0.4	+7.8	-2.3	-	LU Rep. no. 441, 1999
Formi® LHS	0.6	+17.8	-8.8	-	SH Rep. July 1999
Formi® LHS	1.2	+23.1	-12.7	-	SH Rep. July 1999
Formi® LHS	1.8	+32.7	-12.7	-	SH Rep. July 1999
Calcium formiat	1.25	+3.6	-6.0	tendency	LU Rep. no. 445, 1999
Sorbic acid	2.0	+8.7	-5.5	*	LU Rep. no. 445, 1999
Bolifor FA 2000	0.3	+5.2	-1.7	-	LU Rep. no. 461, 1999
Bolifor FA 2000	0.7	+2.5	-3.3	-	LU Rep. no. 461, 1999
Lactic acid	0.7	0	+2.3	-	LU Rep. no. 469, 2000
Lactic acid	1.4	+4.8	-0.6	-	LU Rep. no. 469, 2000
Lactic acid	2.8	+3.8	+0.6	-	LU Rep. no. 469, 2000
Formic acid	0.7	+5.8	-1.8	-	LU Rep. no. 469, 2000
Formic acid	1.4	+3.6	-0.6	-	LU Rep. no. 469, 2000
Luctacid HC	0.2/0.2	+3.1	+0.5	-	LU Rep. no. 474, 2000
Luctacid Piglets	0.3/0.3	+5.7	-1.6	-	LU Rep. no. 474, 2000
Mastercid 90	0.5/0.5	-2.6	-0.5	-	LU Rep. no. 474, 2000
AciForm	0.6/0.5	+1.5	-5.0	tendency	LU Rep. no. 477, 2000
AciForm	1.2/1.0	+3.5	-5.0	*	LU Rep. no. 477, 2000
Lactic acid+formic acid	0.7/0.7	+7.4	-2.6	*	LU Rep. no. 490, 2000
Benzoic acid (not al-lowed in the EU)	2.0/1.0	+13.1	-3.1	*	LU Rep. no. 490, 2000
HSK 2000	1.2/0.6	+2.3	+0.5	-	LU Rep. no. 492, 2000
HSK 2000	1.2/1.2	+3.7	+2.7	-	LU Rep. no. 492, 2000
HSK 2000	1.8/1.2	+0.2	+8.7	-	LU Rep. no. 492, 2000

Continued ….

	Dose[1]	Daily gain	Feed conversion	*	Reference
Formic acid	0.7	+4.8	-2.6	tendency	LU Rep. no. 519, 2001
SoftAcid I	1	+4.8	-1.6	-	LU Rep. no. 537, 2001
SoftAcid II	1.5	+5.9	2.7	-	LU Rep. no. 537, 2001
SoftAcid II	2	+2.1	-0.5	-	LU Rep. no. 537, 2001
Gustor	0.3	+0.7	-1.0	-	LU Rep. no. 544, 2002
Finishers					
Acid Lac	0.3	+1.8	-2.9	-	LU Rep. no. 341, 1996
Bioacid	0.3	-1.8	0	-	LU Rep. no. 318, 1995
Bactacid	0.2	+1.0	+0.4	-	LU Rep. no. 318, 1995
Bactacid	0.3/0.25	-0.1	-0.7	-	LU Rep. no. 402, 1998
Modified Bactacid	0.3/0.25	+0.7	+1.5	-	LU Rep. no. 402, 1998
LA-Mix	0.5	-0.6	+1.2	-	LU Rep. no. 415, 1999
Genes	0.2	+1.1	+0.4	-	LU Rep. no. 450, 1999
Formi®LHS	0.6	-0.2	+1.2	-	LU Rep. no. 489, 2000
Formi®LHS	1.0	-0.5	+0.8	-	LU Rep. no. 489, 2000
Acid one	2.0	+2.5	+0.4	-	LU Rep. no. 489, 2000

*	Significant difference in productivity compared with control (P <0.05).
Tendency	Tendency towards difference in productivity compared with control (P <0.10).
-	Neither tendency nor significant difference in productivity.
[1]	If two doses are stated, the first figure represents the dose of diet 1 (4-6 weeks of age) and the second figure represents the dose in diet 2 (6-10 weeks of age). For finishers the dose is stated for the periods 30-60 kg and 60-100 kg.
LU:	The Danish Applied Pig Research Scheme, the National Committee for Pig Production, DANISH BACON AND MEAT COUNCIL.
SH:	The Danish Institute of Agricultural Sciences.

Fourteen weaner trials showed an effect on productivity. None of the finisher trials showed any effect on productivity.

Enzymes

Enzymes are found in plant and animal tissue, and they are part of the construction and decomposition of chemical compounds. Enzymes work by accelerating chemical processes without being used themselves.

Enzymes disintegrate the components of the feed of protein, carbohydrates and fat to units small enough to pass through the mucosa in the intestines and to the lymph or the blood. Enzymes are very specific, i.e. they can only disintegrate one particular kind of bond. Therefore, the enzymes used must match the ingredient composition of the individual diet. Enzymes that can be added to pig feed are e.g. phytase, xylase, β-glucanase, amylase, cellulase, and α-galactosidase.

Danish results - enzymes

A number of trials have been carried out in Denmark with enzymes in feed for weaners and finishers (table 4).

Table 4. Effect of enzymes on productivity (includes only trials comparing diets with an identical composition of ingredients), change in %

	Weaners	Finishers
No. trials	9	2
Daily gain	+2.1	+1.7
Feed conversion	-0.2	-2.9
No. of trials with a significant effect on productivity	0	1

No trials with enzymes added to weaner feed showed a positive effect on productivity. One trial with the enzyme product Porzyme 9300 for finishers showed a positive effect on the productivity, while another showed a tendency.

Micro-organisms

Microbial cultures are added to feed to stabilise the microbial activity in the intestinal tract. The effect is expected to be:

- Excretion of substances restricting pathogenic bacteria and enhancing growth of "good" bacteria
- Excretion of digestive enzymes
- Increased competition for nutrients in the intestines so that the original intestinal flora and the amount of pathogenic bacteria is reduced.

Some micro-organisms are spores, while others are added as living cells (freeze dried). The form is important to the stability of the micro-organisms particularly during pelleting. It is essential to ensure that that product used tolerates heating and pelleting and that the efficiency is not reduced by other substances in the feed (such as acids, minerals, etc.).

Danish results – micro-organisms

Trials have been carried out in Denmark with microbial cultures for weaners and finishers, cf. table 5 below.

Addition of Paciflor reduced the number of treatments for diarrhoea significantly (from 19.9% in the control group to 4.4% in the test group). When Lactiferm was added to the feed, there was a tendency towards fewer treatments for diarrhoea compared with control. The remaining trials with micro-organisms did not show any positive results. The microbial cultures are expected to have the greatest effect in periods of problems such as at weaning, when changing diets, poor climate etc.

Oligosaccharides

Oligosaccharides are not defined as additives. They are characterized as a feedstuff and can be added to the feed without registration.

Expected effect of oligosaccharides

Many pathogenic bacteria attach themselves to the surface of the intestines as do many toxins and viruses.

There are indications that polysaccharides are capable of binding the pathogenic bacteria. Thereby the pathogenic bacteria are prevented from attaching to the surface of the intestine, which makes the pathogenic bacteria unable to establish in the gastro-intestinal tract, but are "washed out" with the remaining intestinal content.

Addition of oligosaccharides to the feed may result in a changed/increased fermentation in the stomach, terminal ileum and also in the caecum and the large intestine. Hereby the natural bacteria flora is stimulated, which is likely to restrain growth of pathogenic bacteria.

Danish results – oligosaccharides

No trials with oligosaccharides showed an effect on productivity, but there was a tendency towards increased productivity in a trial with fructomix for weaners.

Table 5. Effect of micro-organisms on productivity, change in %

	Weaners	Finishers
No. trials	15	2
Daily gain	+1.01	-0.4
Feed conversion	-0.4	-0.9
No. of trials with a significant effect on productivity	0	0

Table 6. Effect of oligosaccharides on productivity, change in %

	Weaners	Finishers
No. trials	5	2
Daily gain	+1.3	+0.8
Feed conversion	-1.8	0
No. of trials with a significant effect on productivity	0	0

Aromatic and appetizing compounds

This group of additives is particularly used in feed for weaners to make the feed as attractive and tasty as possible.

Plant extracts

Plant extracts are defined as an independent group of additives and is often placed under the group of aromatic and appetising compounds. Plant extracts and herb products are not additives according to legislation, but feedstuffs.

Danish results - aromatic and appetizing compounds

One weaner trial with the product AB-naturmix showed an effect on productivity. Two finisher trials with Sangrovit and New-Add II, respectively, showed an effect on productivity.

The above trial results originate from trials carried out at the experimental stations of the National Committee for Pig Production without great disease problems and where only the individual additive distinguishes the test groups from each other. In order to assess the possibilities for reducing the problems that have occurred in many herds as a consequence of the removal of growth promoters, a number of multifactorial trials have also been carried out in herds with problems; the so-called optimised management.

Optimised management

Optimised management in the weaner unit was studied in eight problem herds and was compared with the normal practice of the herd of tending to and feeding the pigs. The aim was to improve health and production results. All the herds were characterized by a significant prevalence of diarrhoea post-weaning and use of systematic medication to control the outbreaks of diarrhoea.

Optimised management with no growth promoters was compared with use of antibiotic growth promoters.

Overall, optimised management had very varying effects. Health was improved in four herds, unchanged in two and only in one case poorer than under the normal practice of the herd where an antibiotic growth promoter was added to the feed. The productivity was substantially improved in three out of seven herds and reduced in four cases (cf. table 8).

Table 7. Effect of plant extracts on productivity, change in %

	Weaners	Finishers
No. trials	22	13
Daily gain	+2.1	+0.6
Feed conversion	-0.5	-0.6
No. of trials with a significant effect on productivity	1	2

Table 8. Effect of optimised management in problem herds compared with control (normal practice with a growth promoter in the feed)

Report no. (Herd)	504 (1)	507 (2)*	539 (3)	540 (4)	560 (5)	560 (6)*	571 (7)	572 (8)
Productivity	Reduced	Reduced	Reduced	Reduced	Improved	-	Improved	Improved
Health (diarrhoea)	Reduced	The same	Reduced	Improved	Improved	Improved	Improved	The same

* The result must be interpreted with caution as the trial only comprised few blocks.

The extra costs of optimising the management conditions were in all cases higher than the gain in the form of improved production results, fewer treatments for diarrhoea and reduced mortality. Thus, there was no immediate cover for the improvements.

It may be difficult to see how these results can be used in practice. However, they clearly demonstrate that many herds have the potential to significantly improve their health status and productivity within the existing frames. This improvement must be achieved with the lowest possible costs, and the measures in these trials can thus be used as inspiration. The aim in every herd must be to keep trying until they find the best solutions and thereby keep the costs down. In practice this means that the profit must cover the costs better then in these trials. These experiences are important to keep in mind when the existing housing system needs renovation.

Conclusion

In controlled trials with different additives added to the feed for weaners and finishers, the greatest positive effect on productivity was found in the group of organic acids, mainly when added to weaner feed. The effect depends on the dose and on the composition of the acid.

In herds with problems, it is not sufficient to simply replace an antibiotic growth promoter with organic acids. However, a combination of a number of management efforts including changes in feed has in a number of problem herds improved the health status, while the effect on productivity varied. In most of these herds, the costs connected to optimised management exceeded the improvements in health and productivity.

26. Reducing the use of antimicrobials in pig herds by improving data on disease treatment and disease

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Abstract

In Denmark data are gathered on the use of antimicrobials in animals and in humans and on antibiotic resistance in Danmap. It is important to have such a monitoring program at the national level. This paper seeks to draw attention to the herd level perspective also when dealing with use of antimicrobials; to the decision-makers at the herd level, that is the practising veterinarian and the pig manager. To assess the clinical efficacy and to make the correct decisions on treatment, they must have access to valid data on the actual disease level and on the amount of antimicrobials used. Currently, little effort has been made to use such data actively in herd-health management. Consequently, the incentive to record data accurately is low and only limited data of questionable validity are available on the actual use of antimicrobials in a herd and on the actual disease level in the herd.

At the national level it may suffice to make raw accounts of the total usage. It is a general finding in these accounts that there is a very large variation among herds when comparing the crude amounts used. However, to reflect the actual treatment level in the herd, the accounts at the herd level require additional information such as the actual disease level, the actual dosages used and the expected weight interval of the treated pigs.

Prior to making any conclusive statements about the results from an individual herd and to taking pre-emptive steps to reduce the amount of antimicrobials used, it is a prerequisite to acknowledge the necessity of having good data. Moreover, it is necessary to have a fundamental understanding of the sources of the herd to herd variation and of the complex relationship between disease and treatment. The results from our study showed that differences in disease management were very important in terms of explaining the total amount of antimicrobials. In addition to monitoring the total amount used, these issues must be addressed if the intention is to reduce the amount of antimicrobials used.

Introduction

In Denmark, there is a growing concern about the use of antimicrobials in livestock production, and a surveillance system, the Danmap system (Danmap, 2001), has been implemented to quantify the amount of antimicrobials used and to monitor the development in antibiotic resistance. More recently, the VetStat-programme has been implemented (Bager, 1999). VetStat uses the amounts of prescribed antimicrobials to the herd as its primary data source. The database can be used to monitor the usage at the national level but it also contains accounts on the usage at the herd level.

In the existing data there are two general findings. There has been an increase in the total amount of antimicrobials used for livestock production and there is a considerable variation among the herds in the amount of antimicrobials used. The relationship between treatment and disease is a contentious issue in the ongoing debate about the use of antibiotics in livestock production. This is particularly the case for pig production, which in Denmark is responsible for 74% of the total amount of antimicrobials used for food animals (Danmap, 2001). It is the purpose of this paper to focus on the relationship between disease and treatment in a herd to gain a better understanding of the observed variation among the herds in their use of antimicrobials. With this information concrete steps to reduce the amount used can be outlined.

The inextricable connection between disease and treatment

In clinical practice it is generally acknowledged that criteria for treatment decisions and disease management strategies may vary considerably among herds, and that this fact often jeopardises any unequivocal relationship between treatment and disease. In Figure 1 the theoretical relationship between disease and treatment is illustrated. If an obvious treatment effect were present, one would expect to see a decline in the disease level, when the treatment level increases (A). However, with increasing disease level in a herd it is conceivable that one could observe the opposite relationship as an indication of a reaction to the change in the herd (B). If no relationship were present one would expect to see (C). If high disease levels are accompanied by high use of antimicrobials, is this finding an indication proper reaction to disease or an indication of poor treatment effect? Low disease levels and high treatment levels is not always accompanied by a decline in the treatment level. Why change a strategy that works?

Relationship between disease and use of antimicrobials in a herd
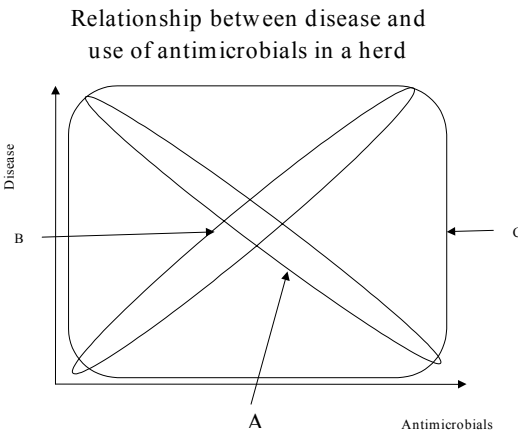
Figure 1. The theoretical relationships between disease and use of antimicrobials in a herd. With an evident clinical treatment effect one would expect to see (A). However, with increasing disease levels one might also see an increase in the amount of antimicrobials used (B). If there is no relationship between the treatment level and the disease level the picture could be like in (C)

Conclusively, when dealing with these relationships one seem to be faced with an inextricable cause-effect relationship.

In 2001 a study was conducted at the Department of Animal Health and Welfare at the Danish Institute of Agricultural Sciences, where the objective was to study the relationship between disease and treatment. In this study the use of antimicrobials and disease, measured as clinical disease prevalence among the weaners, were monitored in the 15 herds. The relationship between clinical disease and treatment was very poor in these herds, and these findings led us to investigate further into the causes of the large variation among herds in the actual amount of antimicrobials used. We encountered two major issues. The first issue was related to the data used for measuring disease and treatment, and the second issue was related to variation in treatment criteria among the study herds. Qualitative interviews were conducted with the farmers and the prescribing veterinarians, and the specific purpose was to identify important variables that could explain the herd to herd variation and some of the equivocal relationships between clinical disease and treatment.

Quantification of disease and the use of antimicrobials in a pig herd

Measuring disease

In the study we used clinical disease prevalence as the major response to treatment. Although alternative measures like daily weight gain and mortality are also relevant the clinical status of the population was considered a prerequisite to any decision made on treatment. Currently in clinical practice systematic recordings of the clinical disease status of the herd are generally not made.

Measuring the use of antimicrobials in crude amounts

The manager and the practising veterinarian have access to some data on the use of antimicrobials in the herd. The practising veterinarian visits most pig herds on a regular monthly basis. At each visit an account of the amount of antimicrobials prescribed and used must be rendered.

The amount of antimicrobials is calculated on the basis of mandatory farmer recorded disease treatments in the preceding time period. The account thus provides data on the amount used measured in raw weight of each drug for each age group in the herd.

From 2002 the practising veterinarian had access to herd accounts on the amount of prescribed antimicrobials measured in kg active compound specified in age groups and diagnoses from VetStat. There are, however, some major disadvantages to these accounts for the persons involved in making treatment decisions. First, the account does not provide the user with the denominator for interpreting the data correctly. Differences in population sizes (i.e. herd sizes) may account for a large part of the observed variation. Moreover, summarising kg active compound is a poor measure of the actual treatment level in a herd, because this number is not a measure of the strength of the compounds. Other important missing variables are the actual weight of the treated animals. Because the data consists of the prescribed amount and not the actual amount used in a specified time interval, the data cannot be used to make inferences on changes in the disease management and the dynamics of the treatment level in the herd. Finally, there may be considerable discrepancies between the diagnoses and age groups indicated by the prescribing veterinarian and the farmer's actual treatment. Conclusively, these accounts only provide information on the crude variation in the total amount used among the herds.

Measuring the use of antimicrobials using animal dosages
A preferable and recommended approach (Danmap, 2001) would be to estimate the number of dosages used. Hence the number will provide a measure of the estimated kg pig meat treated in the herd. Figure 2 provides an example of how such an account could look like for 15 sow herds. In these herds the number of dosages was calculated from the actual amount used per month using the manufacturers recommended dosages. When these were given as a range the maximum value was chosen (VetStat uses the median value). To account for different herd sizes, the number of dosages used has been cor-

rected for the number of sows in the herds. As seen in the previous figure the variation among the herds is immense. Another common finding is that the majority of the consumption is among the weaners, where oral compounds constitute the majority of the dosages used. However, this account is also only meant for making crude herd comparisons for, say, one year, and it still lacks the necessary information to make inferences on the actual treatment strategy in the herd.
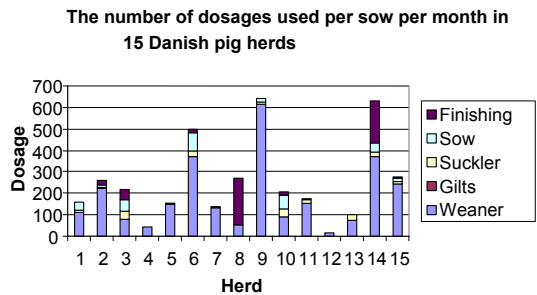

Figure 2. A comparison of the total number of antimicrobial dosages used per sow per month in 15 Danish sow herds in 2001. The dosages have been calculated using the maximum dosage recommended by the manufacturer

Measuring the use of antimicrobials as the estimated treatment level
A third option would be to define the treatment level in a herd according to the general formula (Baadsgaard, 2001):

$$T = \frac{A}{D * W * L * P * R}$$

In the formula T denotes the treatment incidence. The treatment incidence, T, is obtained from the nominator, A, which is the amount of active compound. Dividing A with the dosage, D ~measured in mg or ml per kg body weight, yields the estimated number of dosages. Dividing again with W, which denotes the weight of the pigs, yields a number that is comparable to what is denoted Animal Defined Dosages in Danmap, with the important exception that W

denotes a normal probability distribution rather than a standard weight. In this distribution information about the mean and the standard deviation of the treated pigs can be included. Furthermore, by including the time interval, L, and the population size, P, an incidence can be calculated as the number of treatments per pig-days at risk. Finally, one may wish to adjust the treatment incidence according to the number of days the pigs have been treated; symbolised with R. If L, P and R are omitted, T will simply denote the treatment frequency. Besides the expected weight distribution (W), it would make sense also to let the dosage (D) and the population size (P) be included as distributions instead of point estimates. Consequently, the treatment incidence will also become a probability distribution, with a mean and a standard deviation that will reflect our uncertainty regarding our estimate of the expected treatment incidence in the herd.

Figure 3 shows an example of the development in the estimated treatment frequency in a herd based on the actual amount of dosages used. The two curves show the estimated treatment frequency for two different weight groups (i.e. age groups). The upper line shows the expected treatment frequency for a mean of 10 kg body-weight and a standard deviation of 2 kg, and the lower line shows the expected treatment frequency for a mean of 20 kg body weight and a standard deviation of 2 kg. The vertical lines show the 95% confidence intervals. As is evident from the figure the expected weight distribution has a large impact on the estimated treatment level in the herd. These calculations only require some very simple calculations on already existing data, and they can easily be done in a simple spread sheet program.

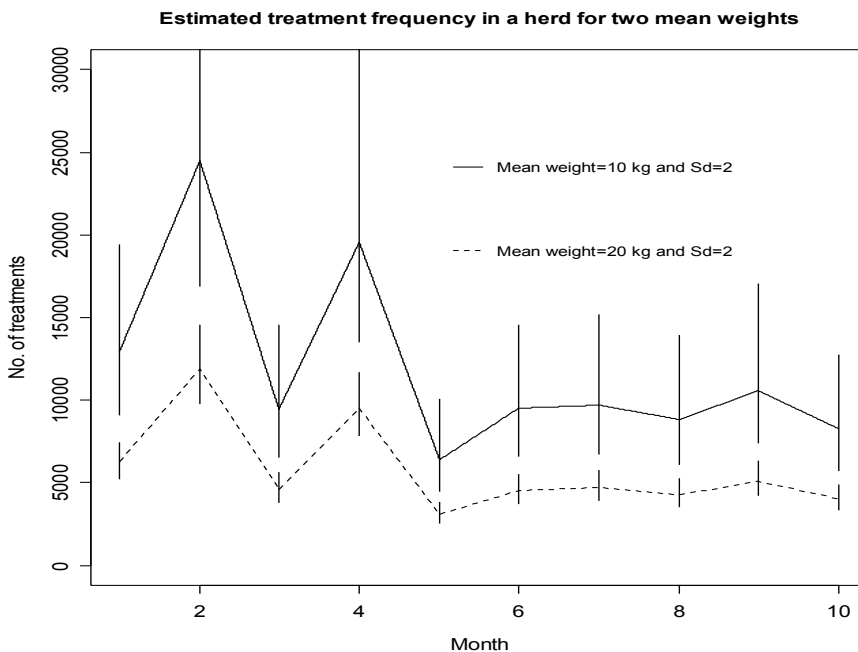**Estimated treatment frequency in a herd for two mean weights**

Figure 3. Estimated treatment frequency in a herd calculated from the number of dosages used each month to the weaners. The treatment frequency has been calculated assuming two different mean body weights in this particular age group

The treatment criteria

From clinical practice it is well known that treatment strategies vary considerably among herds. Based on interviews with the farmers and their veterinarians we identified the following key issues:

- Within the 15 project herds disease management strategies varied from injection and isolation of individual diseased pigs and subsequent clinical assessment of the treatment effect for these pigs on day 2 and 3, to water-medication in 8 days of whole sections (800 pigs or more), if disease was observed in one pen.

- The mere presence of a medicator for mixing therapeutic drugs into the drinking water or into the feed affects the treatment strategy.

- In our study we used the veterinarian's clinical assessment of the treatment effect as the response. Although the veterinarian is the person responsible of the amount of prescribed antimicrobials to the herd, it is the farmer who actually performs the treatments and assesses the treatment effect. The interaction between the decision-makers is therefore vital to the understanding of the eventual treatment level in the herd.

- The population dynamics of a pig herd and the intervals between the veterinarian's visits to the herd implies that the veterinarian very seldom sees the same pigs twice. For example, with monthly intervals of the visits, 3-weeks batch operation systems imply that the age composition of the weaners will vary considerably from visit to visit. The dynamics of the population therefore has a major impact on the veterinarian's clinical observations.

- In our study the treatment level was calculated as dosages per pig-day at risk and not treatments per pig day at risk. Hence we did not correct for the differences in treatment levels that occurred due to treatment of, say, 10 kg's weight as opposed to 20 kg's weight. In addition we did not correct for the number of days the pigs were treated. General recommendations on how to calculate the actual treatment level in the herds are therefore needed.

- Feeding strategies and treatment strategies were changed continuously during the observation period and added to the large variation that was observed within the herds.

- Recommendations on dosage and number of days necessary for treating the pigs varied considerably among the participating veterinarians.

- Generally treatment costs were perceived as being low. The average treatment costs per sow per month in the 15 project herds was approximately 1 euro per sow per month. Most farmers stated that treatment costs were not a factor, when decisions on treatment were made.

- Labour costs. Individual clinical assessment and treatment of pigs require much labour. The lack of qualified labour is a key issue in today's pig production in general.

- Decisions to treat are not always based on the presence of clinical disease. "Poor growth" is also a commonly used diagnosis.

- Using pig's daily water consumption to calculate the amount of antimicrobials needed may yield quite different results compared to a strategy, where the weight distribution of the pigs is used to estimate the necessary number of dosages.

- The eventual amount of antimicrobials used was affected by actual procedure for adding antimicrobials to the food or to the drinking water.

Discussion

In addition to issues related to lack of data, poor quality of data and specific accounting issues, there are a number of factors that have a large impact on the eventual use of antimicrobials in a herd. These factors may in some cases be related to the disease level in the herd, but in other cases they may be independent of the actual disease level. It is important to realise that the mere presence of a medicator reflects the attitude and the values of the particular farmer (Vaarst et al., 2002). In some herds, individual treatment and care is the rule, while in others such a strategy is out of question. The overall costs, when decid-

ing on a treatment strategy, therefore, seem to become more and more important. Results from this study indicate that treatment costs in pig production are low. From dairy production it is well known that the withdrawal period for delivering milk after treatment for mastitis can have a significant impact on the observed treatment level in the herd (measured as veterinary recorded disease treatments). Bennedsgaard et al. (2002) thus showed that converting to organic milk production decreased days in antibiotic treatment for mastitis. It is conceivable that this result can be attributed to the costs of treatment, because the withdrawal period for milk is 3 times the lawfully established period, and because the farmer does not have access to treatment with antimicrobials in organic production.

While arguing for strategies that involve more intensive disease surveillance and individual treatment (as a tool to reduce the use of antimicrobials) as opposed to treatments of, say, sows after farrowing to prevent MMA, sucklers or weaners to prevent infectious diarrhoea, the absence of qualified labour also becomes a critical factor. The focus on systems like automated monitoring of water consumption (Nejsum, 2001) is a consequence of the growing labour issue in pig production.

It may be seen as a disadvantage to allow more or less subjective assessments to be included in the general formula for calculating the treatment level. However, these assessments are prerequisites to making inferences on any changes in the actual treatment level in the herd, and the graphs can serve as a starting point for a discussion on the eventual effect of the treatment and treatment strategy. In arguing for better data, it is an underlying hypothesis that better data on treatment and disease in the herds will improve the basis for making decisions on treatment in the herd. Meaningful accounts on the dynamics of the treatment level in the herds as well as accounts on herd comparisons, where reasonably comparable herds are included, may become an incentive to the farmer to make more precise recordings.

The relationship between disease and treatment is not simple. At the same time, there is a lack of common definitions, and consequently good data, on treatment and disease. The results and the conclusions from central surveillance systems, such as the VetStat system, rely heavily on the quality of the data that enter the database. Therefore, the quantification of the use of antimicrobial agents and the improvement of the precision of the veterinarian's prescription is not limited to serve merely as a decision-aiding tool for the clinician and the farmer. For 10-15 years ago, when herd sizes were considerably smaller than today the lack of good data on treatment and disease might not have been critical. However, dealing with population sizes in today's pig production implies that the veterinarian must assess the health status, the treatment effect and the expected prognosis until next visit to the herd (the future treatment level) in populations that readily can be counted in thousands. Access to valid data therefore becomes decisive.

References

Baadsgaard, N.P. 2001.Development of Clinical Monitoring Methods in Pig Health Management. Ph.D. thesis.

Bager, F., Jacobsen, E. 1999. Registrering af brug af receptpligtig medicin til husdyr. Dansk Veterinær Tidssskrift, 21, 926-927.

Bennedsgaard, T., 2003. Reduced use of veterinary drugs in organic dairy herds- Potentials and consequences. Ph.D. thesis. The Royal Veterinary and Agricultural University.

DANMAP 2001. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals and humans in Denmark. ISSN 1600-2032.

Nejsum, T.M. 2001. Tools for Monitoring Growing Pigs. Ph.D. thesis.

Vaarst, M., Paarup-Laursen, Houe, H., Fossing, C. and Andersen, H.J. 2002. Farmers' choice of medical treatment of mastitis in Danish dairy herds based on qualitative research interviews. J. Dairy Sci. 85, 992-1001.

27. Automatic data as early disease indicators in pig production

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Abstract

A uniform water or feed disappearance pattern is required for assessment of changes in pig health and well-being. Water disappearance rate was measured and monitored continuously in pigs from 4 to 11 weeks of age. The study comprised three herds and 18 batches of pigs, which were managed all in all out. In one herd feed disappearance was measured continuously as well. Water and feed disappearance was measured electronically and data were transferred to a computer for time series analysis. Management interference such as change of diet and treatment of pigs were recorded daily in a logbook. The results indicated that water disappearance was associated with a distinct circadian rhythm. Water disappearance rate peaked between 4 and 6 pm and was lowest between 3 and 5 am regardless of herd and housing system. The circadian pattern persisted throughout the growing period while total water disappearance rate increased.

The pigs showed a very stable diurnal drinking pattern as long as they were healthy whereas the pattern often changed when the pigs were affected by a disease.

A method using a state-space model in conjunction with a Consume control chart is presented as a tool for online monitoring of young pigs, based on their water consumption. By an example it is shown that an outbreak of a disease (diarrhoea) can be detected by the monitoring method approx. one day before physical signs are seen on the pigs.

The monitoring method has been implemented as a commercial software package (Farm-*Watch*), which is available for pig producers.

Introduction

Management decisions in modern pig production are frequently based on subjective judgments. Normally, such decisions derive from a combination of information sources including visual observations of pigs and (e.g., aggression) pens (e.g., signs of diarrhoea), or other senses (e.g., temperature) as well as results from monthly or quarterly performance records.

Outbreak of a disease such as diarrhoea often spread fast within a group of pigs and if it is not detected and treated immediately the outcome might be losses in terms of reduced growth rate and increased mortality. Similarly, poor feed quality might result in reduced growth rate and poor feed conversion.

Because of the general trend toward larger herds and more animals managed per person there is little time available for observing individual animals in weaning and finishing units. Formerly, when daily care taking was associated with manual feeding and mucking out more time was spent among the animals, which increased the chance of detecting outbreak of disease or other problems.

Modern pig housing includes fully slatted flooring, automatic climate control, automatic feeding and watering, which allow the caretaker to manage a large number of animals. Theoretically, labour saving equipment should increase time available for inspection and supervision of animals. However, in commercial practice improved technology has meant that time spend on manual labour has been converted to more animals managed per person, the average labour input being only 10-12 min per finishing pig produced. Therefore, it is important that time

available for inspection is devoted to those animals that are at risk of being infected with disease or exposed to stressors. However, the problem is to determine the risk level or health status of various groups of pigs. Of course, the manager might have some prior knowledge that certain disease outbreaks appear at a given stage of the production period. But it must be assumed that some kind of guidance might be helpful as to where to concentrate management efforts. Thus, it has been suggested that automatic monitoring systems and use of time series analysis might be promising management tools (Frost et al., 1997). Yet, surprisingly few attempts have been made in terms of using such methods in modern pig production.

There is general agreement that animal well-being might be measured indirectly using indicators such as animal behaviour (Smidt, 1983). Changes in eating and drinking patterns are usually the first visual signs that pigs are experiencing environmental stress. Eating pattern in pigs has been reported in several studies (Nienaber et al., 1991; Xin and DeShazer, 1992; Young and Lawrence, 1994; Hyun et al., 1997). A common finding was that the eating pattern is characterized by a distinct diurnal rhythm. Bigelow and Houpt (1988) have shown that pigs' drinking behaviour is closely correlated to their feed consumption which leads to the hypothesis that changes in pig health that affect feed intake also impinge on water intake and thus drinking behaviour.

Statistical quality control methods are quite commonly used in the manufacturing industry, but rarely in animal husbandry. Monitoring daily milk yields in dairy cows, detection of oestrus and disease in dairy cattle based on time series analysis; detection of changes in feed consumption in broilers and detection of changes in daily milk production in cows (Thysen, 1992) are some examples. Common for all these monitoring systems are, that they: 1) incorporate an automatic method for collection of production

traits and 2) include an adequate model for analysis of the collected data.

Modern computer technology has extended the possibilities of real-time monitoring at farm level. Process computers known from factory automation and electronic water flow meters can easily be installed in pig barns to provide real time data of water consumption in a computer. The real challenge lies in processing the recorded data in order to achieve as much information as possible. Monitoring production traits is often difficult because of random as well as more systematic variation in responses, which may complicate the interpretation of data. However, advanced mathematical modelling might help overcoming some of these problems.

The purpose of this paper is to present new methods of real-time monitoring of pig performance while using modern information technology.

Water monitoring - methods

In a study under the Danish Applied Pig Research scheme (DAPR) (Pedersen et al., 1995) it was shown that monitoring water disappearance rate in pigs might be a powerful method of detecting health or systems problems at an early stage (Pedersen and Madsen, 2001). The trial was carried out in 3 commercial pig farms and water disappearance was measured in 18 batches of 4-11 wk old pigs. In one farm feed disappearance was measured as well. Each batch of pigs originated from the same weaning. Pigs were housed in rooms comprising 400-900 animals, which were managed all in all out. Pigs were fed a standard cereal soy diet according to Danish feeding standards. Production conditions are shown in Table 1.

Electronic water flow meters and weighing scales were installed in four and three weaning units, respectively, for measurements of water and feed disappearance (Figure 1).

with similar peaks and troughs and a curvilinear shape as that found for water disappearance in the present study (e.g., Walker, 1991). It is well known that rates of many bodily functions vary systematically over time and therefore, it is likely that the water intake pattern in pigs is an indicator of the feed intake pattern.

Variation in water disappearance rate was large and systematic over a 24 h period as shown in figure 2. Water disappearance was at its lowest point between 3 and 5 am. Pigs increased water intake gradually during the day except for a 2 h period between 11 am and 1 pm when water disappearance rate was reduced. Water consumption peaked between 4 and 6 pm followed by a reduction in intake until the lowest point was reached again between 3 and 5 am. Then a new cycle was initiated.
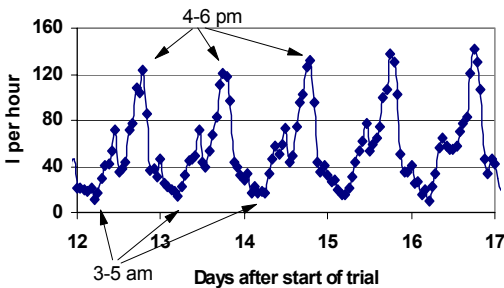

Figure 2. Water disappearance rate, peaks and troughs of water intake in a batch of weaners monitored over a 5 d period (data processed on an hourly basis)

With each new diurnal cycle the level of water disappearance increased a fraction corresponding to the pigs' growth rate.

Comparison of diurnal water disappearance patterns for the three farms indicated that the shape of the curve as well as peaks and troughs of drinking activity were similar. Apparently, housing and pen design did not affect drinking pattern even although there was large variation in housing between farms.

A systematic water and feed disappearance pattern is required for prediction of any disturbance

of pig well-being. In the present study data were recorded every two minutes. Summation of numbers with this interval led to too high random variation while the diurnal water disappearance pattern became more discernible the longer the summation interval. However, a long summation interval also meant that any deviation in water disappearance rate might not be recognized. An evaluation of 20 min, 1 h and 4 h summation intervals revealed that a 1 h interval provided the best estimation.

The next step included computing water disappearance rate using a dynamic linear model (DLM) while employing Bayesian statistics. A DLM is well suited for modelling the dynamic/-cyclic pattern for water disappearance (West and Harrison, 1997).

These methods of treating water and feed disappearance data were integrated in a new computer based monitoring system (Farm*Watch*®) for automatic monitoring of pig well-being in weaning and finisher units, which includes an electronic logbook for notification of management interference or any relevant daily observations in the pig barn.

Tests of the Farm*Watch*® prototype programme employing data from the three farms indicated that several changes in water and feed disappearance were detected, which could be attributed to diarrhoea or tail biting. In fact, diarrhoea after weaning was associated with an increased water disappearance rate 24 h before symptoms were visible in the barn.

The caretaker must be able to distinguish between relevant and irrelevant changes in water disappearance rates and therefore the software should assist him in taking action only when relevant. Normally, a DLM is used as a tool for making forecasts of events based on prior knowledge. Within the present framework, the DLM was used to make predictions of the water disappearance rate one step ahead in time. The difference between the one step forecast and the observed water disappearance was then used as a measure of the deviation from the „normal" level of water consumption.
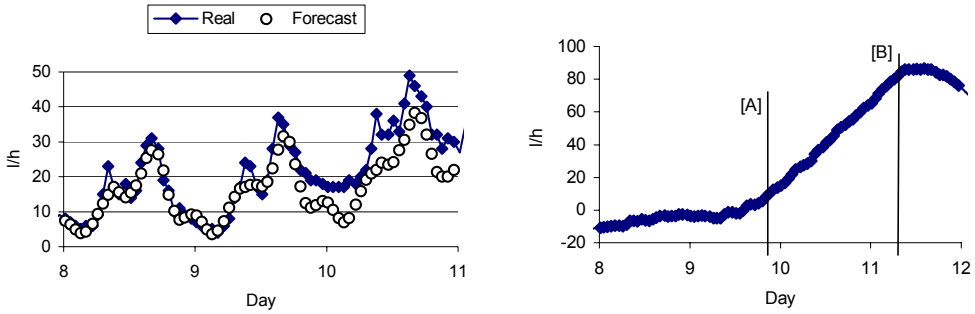
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Figure 3. (a) Observations (real) and one-hour forecasts of water disappearance from the Dynamic Linear Model with quadratic growth between time steps. (b) Sum of forecast errors. An alarm is issued because of deviating drinking pattern at point [A]. Outbreak of diarrhoea is detected and treated at point [B]

The deviation or forecast error might be considered an independent random error term with zero mean as long as the process model is valid. If, on the other hand, the pigs change their drinking behaviour, data will no longer conform to the model predictions, and the numerical value of the forecast errors will increase. Traditional statistical process control or quality control (CUSUM control chart) was then used to evaluate the series of forecast errors and to present an alert when deviations from normal behaviour.

*Farm*Watch® – *real time monitoring*
With a production period of 6-8 months and 12-14 months per weaner and finisher batch, respectively, it is difficult to know whether pig performance is on track. Therefore, the main idea behind Farm*Watch*® was to create a monitoring system, which could assist the caretaker in detecting and correcting any problem that might affect pig well-being in due time. Moreover, it was assumed that the pigs themselves might provide the best cue as to how they were performing. Therefore, a direct measure such as water disappearance was preferred as an indicator of pig condition over an indirect measure such as room temperature.

The Farm*Watch*® system does not provide expert advise when a significant change in water disappearance occurs. Thus, it is up to the caretaker to find causes and solutions to any prob-lem that might have arisen. Over time he might learn that similar changes in water disappearance rate might occur at the same time between batches. Thus, he might be able to moderate or eliminate a specific problem such as *E. coli* diarrhoea, which usually occurs at a certain time period after weaning.

Farm*Watch*® is being used as a proactive management tool in several Danish farms and some examples are presented below:

- In a boar test station all weaners were vaccinated against pneumonia. One hour after vaccination Farm*Watch*® issued an alarm because of reduced water disappearance rate, which was stabilized again after one or two days. Eating and drinking activity was clearly reduced shortly after vaccination. The negative effects of vaccination might be reduced by developing better vaccines or by changing vaccination procedure such as time of vaccination.

- In an 1150 farrow-to-finish farm water disappearance rate was monitored in 6 weaning and 5 finishing rooms, respectively. With respect to weaners, Farm*Watch*® delivered an alarm because of increased water disappearance 24 hrs before visual symptoms of weaning diarrhoea were present. Thus, the caretaker could apply medicine to the drinking water of the weaner rooms in question. Moreover, the amount of drugs could be reduced due to the fact that the medicine could be applied before the infection

escalated. In the finishing units a feeding error led pigs to increase their water intake dramatically, which triggered Farm*Watch*® to deliver an alarm. In this case application of too much soy meal to the feed mix was responsible for the problem. However, it took a while before the caretaker found the cause of the problem.

- Recently, Farm*Watch*® was used in a feed trial for weaners to identify changes in water intake associated with diets. Medication was applied to the drinking water to prevent diarrhoea. However, shortly after the trial had started Farm*Watch*® issued an alarm indicating that water disappearance rate had dropped significantly. The drug applied to water system had plugged up the water nipples.

- In a finishing herd Farm*Watch*® delivered an alarm due to significant reduced water disappearance rate in three rooms of pigs, which was caused by an outbreak of swine influenza. In one room water intake rate returned to normal after a couple of days while water disappearance continued to drop in the two other ones. Thus, it was decided to intervene with medical treatment in the latter rooms. During the cold season water disappearance rate dropped by 10% over a few days in one finishing room while pigs started coughing. The problem was instigated by one of the fresh air inlets, which led cool air toward the thermostat of the ventilation system. Therefore, the ventilation system was alternating between low and high ventilation rate.

IT-based monitoring of pig production
The FARM*WATCH*® software package is an example of how modern information technology might benefit the pig industry. However, it might just be the first system in a range of new integrated management systems, which will enable the pig industry in meeting tight product specifications, while satisfying society's demands to reduce environmental impact of modern production methods (Wathes et al., 2001).

The fastest and easiest way of applying information technology in livestock production is probably to make use of process control techniques that have been useful in other industries. Thus, image analysis, statistical quality control

measures, Bayesian and mathematical modelling and time series analysis are some of the analytical tools, which might be applied to biological processes to provide real time evidence animal performance. We are currently working on a number of projects, which incorporate some of these features:

- Automatic growth check and weighing of weaners and finishers using image analysis technology.
- Bayesian modelling of reproduction rate to forecast use of all in all out facilities.
- Economical evaluation model regarding replacement strategy and culling of sows.
- Monitoring of water intake in individual sows.
- Monitoring of sow visits in electronic sow feeding systems.

Thus, as has been prophesised by professor Wathes (1993) at the Silsoe Research Institute, "In the third millennium, the farmer's role may be more akin to that of the process engineer than the peasant".

References
Bigelow, J.A. and Houpt, T.R. 1988. Feeding and drinking patterns in young pigs. Phys. and Behav. 43(1), 99-109.

Frost, C., Schofield, S. Beaulah, T. Mottram, J. Lines and C. Wathes. 1997. A review of livestock monitoring and the need for integrated systems. Comput. Electron. Agr. 17, 139-159.

Hyun, Y., Ellis, M., McKeith, F.K. and Wilson, E.R. 1997. Feed intake pattern of group-housed growing-finishing pigs monitored using a computerized feed intake recording system. J. Anim. Sci. 75, 1443-1451.

Iwersen, J. 1997. Statistical Process Control for autocorrelated processes: a case study. Frontiers in Stat. Qual. Contr. 5, 138-155.

Madsen, T.N., 2001. Tools for Monitoring Growing Pigs. PhD Thesis. The Royal Veterinary and Agricultural University, Department of Animal Science and Health, Copenhagen. Denmark.

Nienaber, J. ., McDonald, T.P., Hahn, G.L. and Chen, Y.R. 1991. Group feeding behaviour of swine. Transactions of the ASAE, 34(1), 289-294.

Pedersen, B.K. and Madsen, T.N. 2001. Monitoring water intake in pigs: prediction of disease and stressors. In Sixth International Livestock Symposium, Louisville, KY. American Society of Agricultural Engineers.

Pedersen, B.K., Ruby, V. and Jørgensen E. 1995. Organisation of applied pig research: the Danish approach. In: Manipulating Pig Production, Austral-Asian Pig Society. Canberra, Australia.

Smidt. D. 1983. Advances and problems of using integrated systems of indicators as compared to single traits. In: D. Smidt. (Ed.) Indicators Relevant to Farm Animal Welfare. Martinus Nijhoff Publishers, Boston, Mass.

Thysen. 1992. Detection of changes in daily milk production by a multi-state kalman filter. Dina Report, No. 3.

Walker, N. 1991.The effects on performance and behaviour of number of growing pigs per mono-place feeder. Anim. Feed Sci. Tech. 35, 3-13.

Wathes, C.M. 1993. Animals in man's environment: a question of interest. In Fourth International Livestock Symposium, Warwick. American Society of Agricultural Engineers, 1255-1266.

Wathes, C.M., Abeyesinghe, S.M. and Frost, A.R. 2001. Environmental design and management for livestock in the 21st century: resolving conflicts by integrated solutions. In Sixth International Livestock Symposium, Louisville, KY. American Society of Agricultural Engineers.

West, M. & Harrison, J. 1997. Bayesian Forecasting and Dynamic Models. Springer Verlag. 680 pp.

Xin, H. and DeShazer, J.A. 1992. Feeding patterns of growing pigs at warm constant and cyclic temperatures. Transactions of the ASAE, 35(1), 319-323.

Young, R.J. and Lawrence, A.B. 1994. Feeding behaviour of pigs in groups monitored by a computerized feeding system. Anim. Prod. 58, 145-152.

28. Breeding pigs for resistance to clinical and subclinical disease

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Summary

The objective of this paper is to demonstrate that selective breeding for resistance provides a viable method to control clinical and sub-clinical disease in pig production. Research to date indicates that genetic variation for resistance to clinical and sub-clinical disease exists in pigs. Furthermore, there are potential selection criteria, such as immunological parameters, becoming available by which to select pigs for resistance. Although current pig breeding programs do not select for resistance to disease, these findings give pig breeders reason to be optimistic. They demonstrate that selective breeding for resistance does provide a viable method to control clinical and sub-clinical disease in pig production. A successful selective breeding program would have the added advantage of reducing the reliance upon traditional methods of disease control, such as vaccination and medication (includes growth promoters).

Introduction

Pig production is often hindered by clinical and subclinical disease, which cause mortality, reduced production performance, increased costs, and poor animal welfare. In Denmark, clinical and sub-clinical diseases of most economic importance to pig production are diarrhoea, respiratory problems, lameness, and mastitis-metriisagalactia (MMA).

Traditional methods used to control disease include eradication, sanitation, quarantine, culling, vaccination, and medication (includes growth promoters). Pig production currently relies solely upon these methods to control disease. A complementary, albeit longer term, approach is to selectively breed pigs for resistance.

There are two main reasons for implementing a selective breeding program for resistance. First,

any genetic improvement obtained is cumulative and permanent. That is, genetic improvement obtained in a particular generation is added to the improvement obtained in previous generations. Furthermore, the genetic improvement obtained in previous generations is not lost, should selection for resistance stop. The second reason is that a successful selective breeding program reduces the reliance upon traditional methods of disease control. Of particular interest is a potential reduction in the use of vaccines and medicines, which helps fulfil public demands for food free of such products.

The objective of this paper is to demonstrate that selective breeding for resistance provides a viable method to control clinical and sub-clinical disease in pig production. In doing so, it is assumed that the breeding goal is to increase resistance of the pigs to clinical and sub-clinical disease, where resistance is assessed as the incidence of disease during production.

Genetic variation for resistance

To selectively breed pigs for resistance, resistance to clinical and sub-clinical disease must express genetic variation. In its simplest form, this means that there must be variation for resistance amongst the pigs (i.e., some of the pigs are resistant to disease, some are susceptible, while others are within these two extremes), and at least part of this variation must be due to the genes they have inherited from their parents.

In most, if not all, animal populations, there exists individuals that are genetically resistant to specific pathogens (Nicholas, 1987; Müller and Brem, 1991; Straw and Rothschild, 1992). This is also the case for pigs in relation to the pathogens that have been investigated (Straw and Rothschild, 1992; Rohrer and Beattie, 1999), suggesting that there are genes within pig popu-

lations that convey resistance to specific diseases.

Resistance to specific diseases is not surprising. To be resistant, pigs only need genes that code the immune system to defend against a specific form(s) of pathogen. However, under commercial production conditions, pigs are reared under many different production systems, both indoors and outdoors, and are exposed to a wide range of pathogens (i.e., clinical and sub-clinical diseases). Therefore, pig production would benefit from using animals that have general resistance to clinical and sub-clinical disease. To have general resistance, pigs require genes that code the immune system to defend against a wide range of pathogens. Despite this, there still tends to be genetic variation for general resistance to clinical and sub-clinical disease in pigs.

Two studies involving the Danish Institute of Agricultural Sciences (DIAS) indicate that genetic variation for resistance to clinical and subclinical disease exists in the Danish pig population. In the first study, Henryon et al. (2001) detected genetic variation for resistance of growing pigs to five categories of clinical and subclinical disease: (i) any clinical or subclinical disease (ii) lameness (iii) respiratory diseases, (iv) diarrhoea, and (v) other diseases [i.e., any clinical or sub-clinical disease with the exception of (ii), (iii), and (iv)]. In the second study, Berg et al. (unpubl.) detected genetic variation for birth assistance in sows. However, there was very little genetic variation for resistance to MMA in sows and neonatal diarrhoea in piglets.

The existence of genetic variation in the Danish pig population tends to be supported by the few other studies carried on piglets, growing pigs, and sows. Specifically, Lingaas and Rønningen (1991) detected genetic variation for resistance to neonatal diarrhoea in the Norwegian pig population. Genetic variation was also detected for resistance of sows to MMA, mastitis, and metritis. Smith et al. (1962) and Lundeheim (1988) reported genetic variation for resistance of growing pigs to diarrhoea and respiratory diseases in the British and Swedish pig populations.

There are few estimates of genetic variation for resistance to clinical and sub-clinical disease in pigs. However, the available evidence is encouraging. It indicates that genetic variation for resistance to clinical and sub-clinical disease does exist in pigs, and that selective breeding for resistance can be successful.

Selection criteria

Selective breeding programs for resistance involve the selection of pigs which possess genes that convey resistance. By using these pigs for breeding purposes, genes that convey resistance are passed onto the next generation, while genes that do not convey resistance are removed from the population. Geneticists often refer to the level of resistance of a pig due to the genes it possesses as its breeding value for resistance.

The success of selective breeding programs is largely dependent upon the identification of pigs with the highest breeding values for resistance. However, there is a catch, as the breeding values of pigs (i.e., the genes they possess) cannot be directly observed. The challenge for pig breeders is to find indirect sources of information (i.e., selection criteria) that can be used to estimate the breeding values of the pigs.

Breeding value estimates can be obtained using the incidence of clinical and sub-clinical disease (i.e., health records) during the life of the pigs. However, using the incidence of disease as the sole selection criterion generally results in unreliable breeding value estimates (i.e., degree of certainty by which the most resistant pigs can be identified). There are two reasons. First, it is often difficult to correctly diagnose live pigs for many clinical and sub-clinical diseases. Second, much of the variation for the incidence of disease is due to environmental factors, such as unpredictable exposure to pathogens. Consequently, selective breeding would be more successful if other traits, which reflect the resistance of the pigs, could be identified and used as additional selection criteria.

There are two prerequisites required for a trait to be suitable selection criterion. First, the trait

needs to express genetic variation. Second, the trait needs to be genetically correlated (i.e., genetically related) with resistance to clinical and sub-clinical disease. With few exceptions, all of the studies to date have only addressed the first of these prerequisites, the expression of genetic variation.

In the following section, the suitability of immunological parameters as additional selection criteria is investigated. Immunological parameters are by no means the only potential criteria. In theory, there can be any number of potential candidates. Other traits that have attached attention include post-mortem lesions and quantitative trait loci, while further traits are sure to become available as our understanding of diseases and genetics grows.

Immunological parameters

The immune system is the natural capacity of pigs to resist infection (Janeway et al., 1999). It provides a remarkably effective defence mechanism that ensures that, although pigs spend their lives surrounded by potentially pathogenic microorganisms, they become ill only relatively rarely.

Measures of the immune system (i.e., immunological parameters) may provide suitable criteria by which to select pigs for resistance to clinical and subclinical disease. The principle is simple. Given that the immune system is the natural capacity of pigs to resist infection, immunological parameters may reflect the functional capacity of the immune system (i.e., immuno competence) and, in turn, the ability of pigs to resist infection.

Immunological parameters that have attracted attention in Denmark are:

- Concentration of white blood cells (i.e., leukoytes) as the immune system is dependent upon the activities of leukocytes.

- Concentration of acute phase proteins. Acute phase proteins are a series of proteins secreted into the blood by the liver after the onset of infection. These proteins mimic the action of antibodies but, unlike antibodies, they have broad specificity for pathogens.

- Amounts of the major histocompatibility complex class I and II (MHC I and II) molecules on the surface of the leukocytes. MHC I and II molecules are important in the control of specific components of the immune system.

A study involving DIAS is currently underway to assess the suitability of (a) concentration of leukocytes, (b) concentration of acute phase proteins, and (c) amounts of MHC I and II molecules on the surface of the leukocytes as selection criteria for resistance in pigs. To date, genetic variation has been detected for concentration of leukocytes (Henryon et al., 2002), concentrations of acute phase proteins, and the amounts of MHC I and II molecules on the surface of the leukocytes. Genetic variation has also been detected for other immunological parameters in other pig populations (Rothschild et al., 1984a,b; Mallard et al., 1992; Edfors-Lilja et al., 1994). These findings indicate that many immunological parameters measured in pigs will respond to selection. Certainly, a research group in Canada was successful in producing genetic lines of pigs with high and low values for specific immunological parameters (Wilkie and Mallard, 2000).

It appears that most, if not all, immunological parameters in pigs express genetic variation, thereby fulfilling the first prerequisite required for the parameters to be a suitable selection criterion. However, before these parameters can be considered suitable selection criteria, it needs to be demonstrated that they fulfil the second prerequisite, namely that they are genetically correlated with resistance. This premise is currently being tested at DIAS. Preliminary results suggest that there is at least a weak genetic correlation between some immunological parameters and resistance. The amount of evidence is not overwhelming. However, these correlations do provide optimism, as they suggest that at least some immunological parameters are genetically correlated with resistance, and will provide suitable criteria by which to select pigs for resistance.

Breeding programs

Current selective breeding programs for pigs concentrate solely on the improvement of production traits. However, evidence from other species (e.g., mice, dairy cattle) indicates that there are unfavourable genetic correlations between production traits and resistance to some diseases. Should pigs resemble these species in any way, the implications are clear. Breeding pigs solely for the improvement of production traits is likely to have adverse effects on the resistance to at least some clinical and sub-clinical diseases. This, in itself, ought to justify the use of selective breeding as a method of disease control.

Registration system

The success of a selective breeding program for resistance to clinical and sub-clinical disease is dependent upon a well-established registration system to record the incidence of disease in pigs. Pig production in Denmark does not have a well-established registration system. At best, young boars from the nucleus breeding population of the Danish Pig Breeding Program are recorded each time they are treated for a clinical or subclinical disease. A boar is assumed to be infected with a clinical or sub-clinical disease when it is treated for the disease. This registration system has two major drawbacks. First, many non-diagnosed pigs are treated for preventive measures (i.e., majority of pigs within a pen group are treated for a disease to reduce the risk of disease although only few individuals in the pen are diagnosed with the disease). Second, many of the clinical and sub-clinical diseases are poorly defined. These two drawbacks will need to be addressed, and well-established registration system will need to be implemented, before selective breeding can be used as a method of disease control in Danish pig production.

Other considerations

There are several areas that require consideration when selecting pigs for resistance to clinical and subclinical disease. First, it may be unrealistic to hope to achieve resistance to all forms of disease. Diseases differ in their aetiologies, each requiring a different mechanism of immunity on the part of the pigs to prevent infection. Furthermore, there is evidence to suggest that some of these mechanisms of immunity may be negatively intercorrelated (c.f. Biozzi et al., 1982). Second, during selection of pigs resistant to a pathogen, the pathogen is likely to evolve to survive in the pig (c.f. Nicholas, 1987). Increased resistance in the pathogen may offset at least some of the progress made in the resistance of the pigs. These two considerations are certain to make selective breeding for resistance challenging. However, it may only be necessary to make small increases in the resistance of the pigs. This is because a successful breeding program for resistance would not only reduce the number of infected pigs at any time, but simultaneously reduce the risk of susceptible pigs being infected (c.f. Knap and Bishop, 2000). In this way, breeding for resistance can have a dramatic impact on the incidence of clinical and subclinical disease at the population level, whereby the population could carry a sizeable proportion of susceptible pigs without the risk of disease outbreak.

A further consideration is genotype x environment interactions. Genotype x environment interactions exist when the relative genetic differences of genetic groups (i.e., breeds, or full- and half-sib families) varies between environments. The existence of genotype x environment interactions are important in breeding programs for resistance because pigs selected for resistance within a particular environment may not be resistance under a range of environments. In such situations, selection programs may need to be developed for different environments.

Finally, as our knowledge of the immune system grows, it is becoming clear that the immune system needs to be stimulated to keep it functionally sharp. By increasing the emphasis on clean environments, pig producers could actually be increasing the susceptibility of the pigs to clinical and sub-clinical disease by removing the stimulus required to keep the immune system fine-tuned.

Conclusion

This paper demonstrated that selective breeding for resistance does provide a viable method to

control clinical and sub-clinical disease in pig production. Genetic variation for resistance to clinical and subclinical disease exists in pigs, and there are potential criteria, such as immunological parameters, becoming available to select pigs for resistance. These findings give pig breeders reason to be optimistic, as they demonstrate that selective breeding programs for resistance will be successful. However, pig breeders are yet to respond. With research attention directed towards areas that will further develop selective breeding programs for resistance (i.e., estimation of genetic variation, identification of suitable selection criteria, establishment and development of registration systems), such programs are likely to reduce the reliance upon traditional methods of disease control, such as vaccination and medication.

References

Berg, P., Andersen, S., Henryon, M., Nielsen, J. 2003. Genetic variation for birth assistance and MMA in sows and diarrhoea in their litters (unpubl.).

Biozzi, G., Mouton, D., Heumann, A.M., Bouhillier, Y. 1982. Genetic regulation of immuno responsiveness in relation to resistance against infectious diseases. Proc. 2nd Wld. Congr. Genet. Appl. Livest. Prod. 5, 150-163.

Edfors-Lilja, I., Wattrang, E., Magnusson, U. and Fossum, C. 1994. Genetic variation in parameters reflecting immune competence of swine. Vet. Immun. Immunopath. 40, 1-16.

Henryon, M., Berg, P., Jensen, J., Andersen, S. 2001. Genetic variation for resistance to clinical and sub-clinical diseases exists in growing pigs. Anim. Sci. 73, 375-387.

Henryon, M., Juul-Madsen, H.R., Berg, P. 2002. Genetic variation for total and differential counts of leukocytes exists in growing pigs. Proc. 7th Wld. Congr. Genet. Appl. Livest. Prod. 31, 607-610 (CD-Rom Communication No. 13-02).

Janeway Jr., C.A., Travers, P., Walport, M., Capra, J.D. 1999. Immunobiology: The Immune System in Health and Disease, 4th edition. Current Biology Publications and Garland Publications.

Knap, P.W., Bishop, S.C. 2000. Relationships between genetic change and infectious disease in domestic livestock. In The Challenge of Genetic Change in Animal Production (eds. W. G. Hill, S. C. Bishop, B. McGuirk, J. C. McKay, G. Simm and A. J. Webb), Brit. Soc. Anim. Sci., Occasional Publication 27, 65-80.

Lingaas, F., Rønningen, K. 1991. Epidemiological and genetic studies in Norwegian pig herds. V. Estimates of heritability and phenotypic correlations of the most common diseases in Norwegian pig production. Acta Veterin. Scand. 32, 115-122.

Lundeheim, N. 1988. Health disorders and growth performance at a Swedish pig progeny testing station. Acta Agric. Scand. 38, 77-88.

Mallard, B.A., Wilkie, B.N., Kennedy, B.W., Quinton, M. 1992. Use of estimated breeding values in a selection index to breed Yorkshire pigs for high and low immune and innate resistance factors. Anim. Biotechnol. 3, 257-280.

Müller, M., Brem, G. 1991. Disease resistance in farm animals. Experientia 47, 923-934.

Nicholas, F.W. 1987. Veterinary Genetics. Oxford University Press, Oxford.

Rohrer, G.A., Beattie, C.W. 1999. Genetic influences on susceptibility to acquired diseases. In Diseases of Swine, 8th edition (eds. B. E. Straw, S. D. Allaire, W. L. Mengeling and D. J. Taylor), Iowa State University Press, Ames, 977-984.

Rothschild, M.F., Chen, H.L., Christian, L.L., Lie, W.R., Venier, L., Cooper, M., Briggs, C., Warner, C.M. 1984a. Breed and swine lymphocyte antigen haplotype differences in agglutination titres following vaccination with B. bronchiseptica. J. Anim. Sci. 59, 643-649.

Rothschild, M.F., Hill, H.T., Christian, L.L., Warner, C.M. 1984b. Genetic differences in serum neutralization titres of pigs after vaccination with pseudorabies modified live-virus vaccine. Am. J. Vet. Res. 45, 1216-1218.

Smith, C., King, J.W.B. and Gilbert, N. 1962. Genetic parameters of British large white bacon pigs. Anim. Prod. 4, 128-143.

Straw, B. E., Rothschild, M.F. 1992. Genetic influences on liability to acquired disease. In Diseases of Swine, 7th edition (eds. A.D. Leman, B.E. Straw, W.L. Mengeling, S.D. Allaire, D. Taylor), Iowa State University Press, Ames, pp. 709-717.

Wilkie, B., Mallard, B. 2000. Genetic aspects of health and disease resistance in pigs. In Breeding for Disease Resistance in Farm Animals, 2nd edition (eds. R.F.E. Axford, S.C. Bishop, F.W. Nicholas, J.B. Owen), CAB International, 379-396.

29. Natural resistance as an alternative to AGP in poultry

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Summary

In spite of antibiotic- and vaccination-programmes, infectious diseases are still a major problem in our animal productions due to the development of more and more virulent microorganism. It is therefore important to develop new strategies to withstand these problems. One way to contribute to this is to get more concrete knowledge about the molecular and cellular interactions between the micro-organism and the host during the pathogenesis. This gives the possibilities to make use of the hosts' own immune defence mechanism through breeding and management procedures. A molecule of interest in that connection is mannan-binding lectin (MBL) that belongs to the first-line innate unspecific part of the immune system. People deficient in MBL experience a substantial increase in infections especially during early childhood indicating the importance of MBL for host defence.

At the Danish Institute of Agricultural Sciences (DIAS), chickens have been selected for high or low concentration of MBL in serum for seven generations resulting in two unique lines of chickens with very different amounts of MBL in the serum. After an experimental virus infection, the acute MBL response rises 1.5–6 fold in chickens with a low genetic level of MBL whereas the rise was only 1–3 fold in chickens with a high genetic level of MBL. However, when the specific antibody titer against the virus was measured, the chickens with the highest genetic level of MBL had a significantly lower level of specific antibodies. This may be due to antiviral activity of MBL performed by neutralisation of the virus before the humoral antibody response was activated.

The MBL acute phase response is not only affected by genetics but also by management procedures since chickens infected with a virus in the morning had a higher MBL response compared with chickens infected in the evening. Furthermore, an inverse relationship to the humoral immune response was observed. Chickens with the highest MBL response had the lowest specific antibody response and visa versa.

In order to develop better vaccines without simultaneously contributing to the development of even more virulent virus strains, a simple blocking of the innate immune system may be a possibility.

Introduction

MBL selectively recognises a number of structural oligosaccharide components on the surface of micro organisms in the presence of calcium (Turner, 1996). When MBL has bound to a micro-organism, the complex promotes killing of the micro-organism via the MBL-associated serine proteases (MASP-1 and –2) either by acting directly as an opsonin or by activating the lectin complement pathway, thereby playing a major role in the first-line innate immune defence against bacteria, viruses, and parasites. Similar to humans, chickens produce MBL and up-regulate the production during acute stages of virus infections (Laursen and Nielsen, 2000; Juul-Madsen et al., 2002). MBL may therefore play an important role in infections as well as in vaccination responses in chickens as well.

Material and Methods

To study the host defence role of MBL to infections in chickens, the acute phase response to inoculation with Infectious Bronchitis Virus (IBV) was measured in genetically different chickens and in chickens that was inoculated with virus at different times of the day.

The serum MBL concentration and the complement activation ability of MBL were analysed by ELISA- and a complement activation assay, respectively as described in Juul-Madsen et al. (2002). The Restriction Fragment Length Polymorphism (RFLP) profiles of DNA were determined by the method described in Juul-Madsen et al. (1993).

Results and Discussion

At DIAS, chickens have been selected for high or low concentration of MBL in serum for seven generations resulting in two unique lines of chickens with very different amount of MBL in the serum (Figure 1A).

By RFLP analysis using the cDNA gene for chicken MBL that has recently been isolated (Laursen et al., 1998), specific profiles have been found in these lines indicating that the polymorphism may also carry out functional differences (Figure 1B).

Offspring from the F6 generation was used in an experiment where the chickens were inoculated with IBV. Using a sandwich ELISA, the acute phase response of MBL was measured post inoculation (Figure 2). The rise in chickens with low MBL was measured to be from 1.5-6 fold whereas the rise in chickens with high MBL only was between 1-3 fold (Figure 2A) even though the amount of MBL in mg/mL serum was still very different in the two lines (Figure 2B).

However, when the specific antibody titer against IBV was measured, the chickens with the highest amount of MBL had a significantly lower level of specific antibodies ($p < 0.05$) (Figure 3). This may be explained by antiviral activity of MBL resulting in neutralisation of the virus before the humoral antibody response was activated.

The MBL level is not only affected by genetics but also by management procedures. The acute phase response of MBL in commercial chickens with different genetic MBL basis concentrations was also investigated by experimental inoculation with IBV at different times. The MBL acute phase response was followed and a significant difference depending upon the time of inoculation of the virus isolate was found (Figure 4).
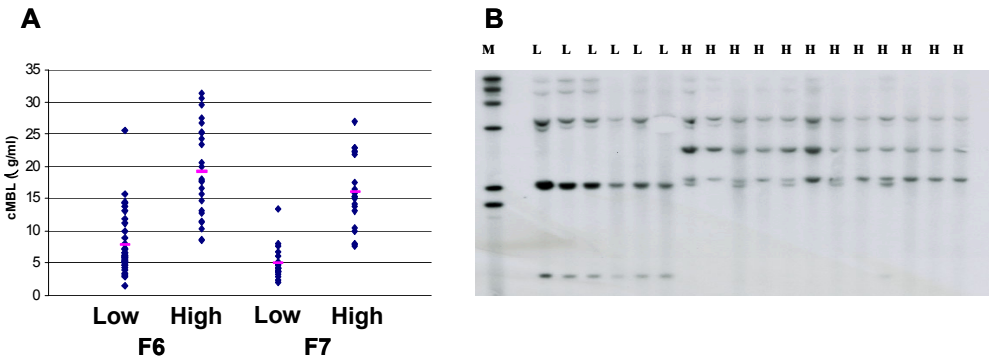

Figure 1. A: Two lines of chickens selected for high or low level of MBL in serum (μg/mL). Chickens from the F6 and the F7 generation are shown. B: RFLP profiles of DNA digested with the restriction enzyme Bgl II from chickens with low (L) or high (H) level of MBL in serum
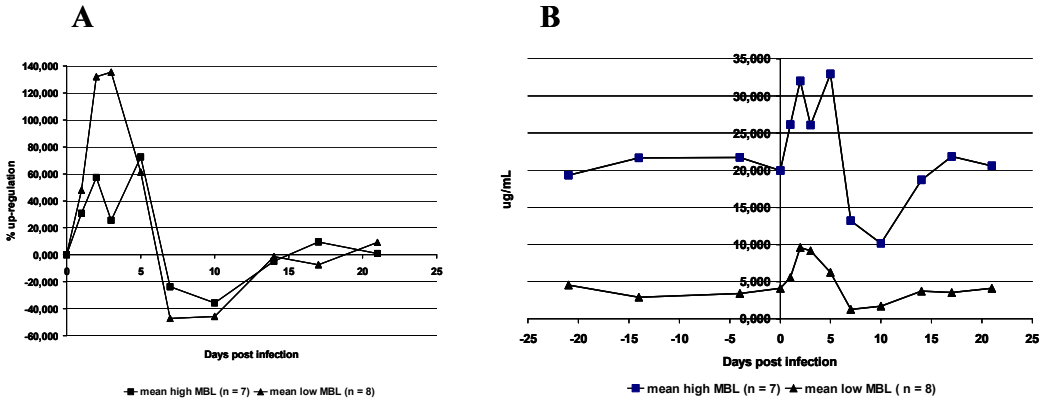
A

B

Figure 2. The mean MBL response after infection with IBV in chickens with low and high serum concentration of MBL shown in % up-regulation (A) and in μg/mL (B)
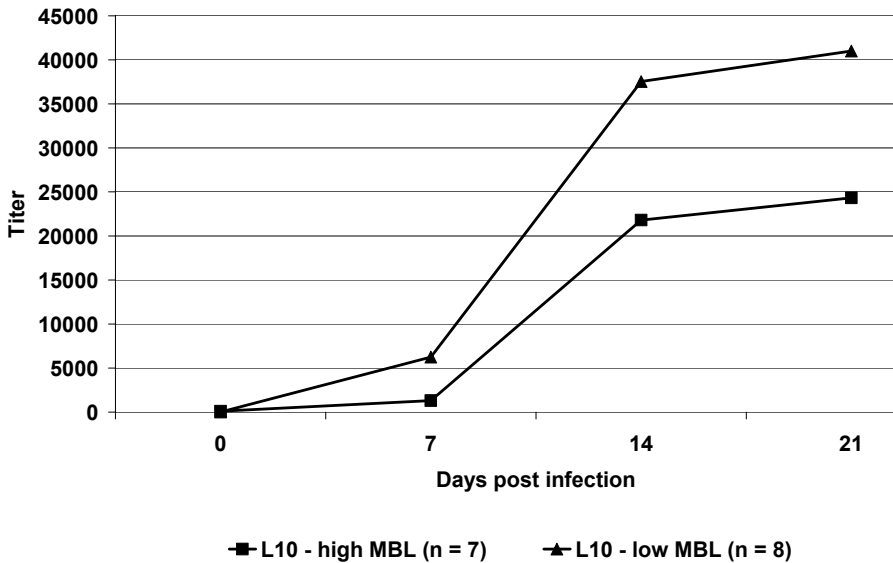

Figure 3. The mean specific antibody titer after infection with IBV

One group was mock infected at 0900 h, one group was IBV infected at 0900 h, and one group was IBV infected at 2100 h. The acute phase response is presented as the relative up-regulation in relation to a determined basic MBL level in each chicken. Each dot at the curves represent 10 chickens from each of 2 separate experiments: one where the chickens were bled on days 1, 3, 5, 9, 14, and 18 PI and one where the chickens were bled on days 2, 4, 7, 11, 16, and 21. The values from these two experiments were gathered in one curve showing the period with different responses between treatments.
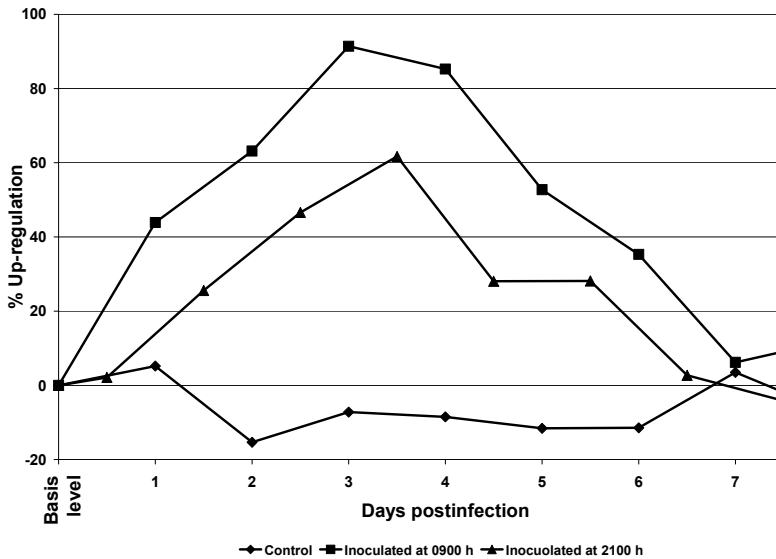
Figure 4. The relative MBL acute phase response in chickens inoculated with IBV at different times of the day

Chickens inoculated at 0900 h (after a long period of dark) had a significantly higher level of MBL in serum ($p < 0.0068$) compared with chickens inoculated at 2100 h (after a long period of light). These results may indicate that diurnal rhythm of hormones can modulate the MBL acute phase response.

The specific antibody titer against IBV in this experiment was measured as well and showed – as for the selected lines - an inversely proportional response compared with the MBL response (Figure 5). Chickens infected in the morning had a significantly ($p < 0.0091$) lower concentration of specific antibodies than chickens infected in the evening.

Finally, chicken serum from the different treatment groups of the last experiment was tested for the ability of chicken MBL to activate the complement system in a heterologous system by deposition of human C4 on the chicken MBL/MASP complex (Figure 6).

The complement activation was found to be directly associated with the concentration of MBL in serum. This result supports the idea that the level of MBL in serum affects the degree of neutralisation of the virus before the humoral antibody response takes over and thereby determines the level of the specific antibody response or pushes the immune response into a more cellular response. This has to be investigated further.

Applying the results to vaccinations strategies, blocking part of the chicken's own innate immune system may be a way to develop new and better vaccines without simultaneously contributing to the development of even more virulent virus strains. This has to be investigated in the future. The effect of the variation in the diurnal rhythm and environmental factors on the acute MBL response and the subsequent specific antibody is another interesting question to analyse further.

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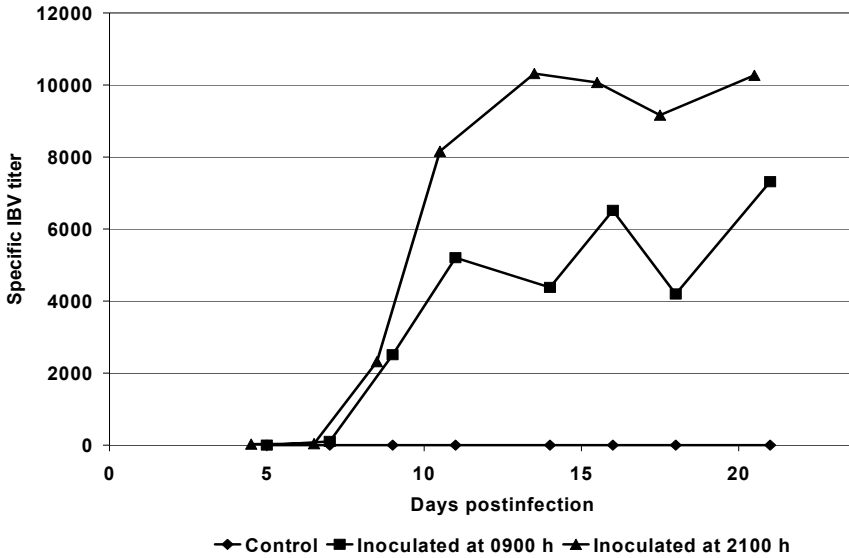
Figure 5. The mean IBV specific antibody titer in chickens inoculated with IBV at different times. Only blood samples from days 4.5 to 21 were analysed

Figure 6. The complement activation values measured as deposition of human C4 on the chicken MBL/MASP complex. The means of two animals of each indicated treatment group are shown. The MBL concentration was measured as 0-16 μg/ml whereas the C4 deposition was measured as 0–800 mUnits/ml. A: Control; B: Inoculated at 0900 h with IBV; C: Inoculated at 2100 h with IBV

Conclusions

- The MBL basis concentration was affected by genetics.
- DNA from chickens with different levels of MBL has different RFLP profiles.
- A polymorphism was found in the promotor region of the MBL gene.
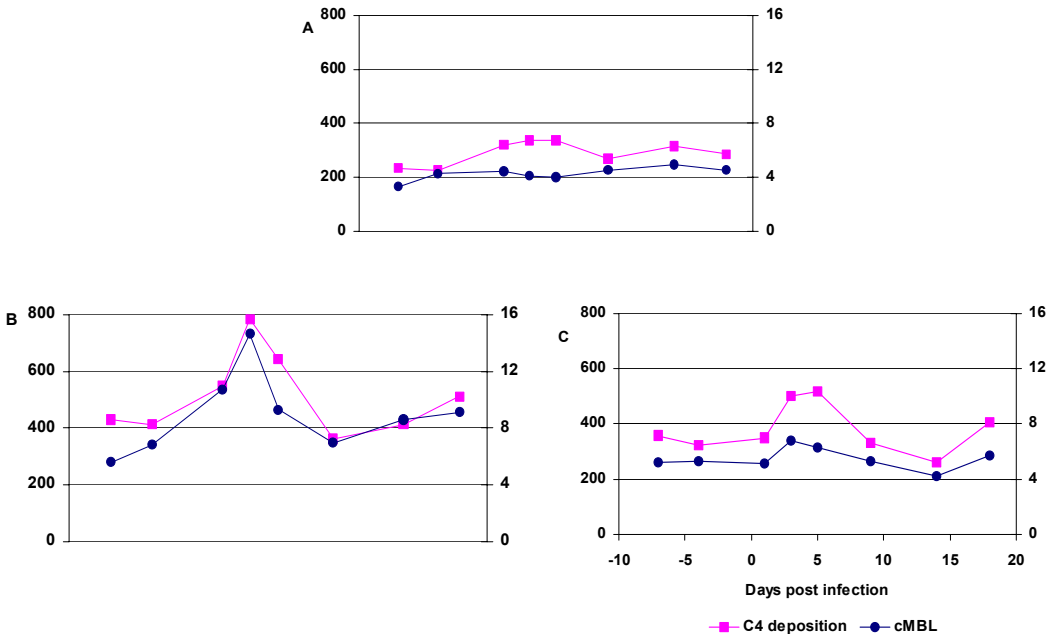- The MBL acute phase response after inoculation of IBV in chickens with a low genetic level of MBL was a 1.5–6 fold increase.
- The MBL acute phase response after inoculation with IBV in chickens with a high genetic level of MBL was only a 1–3 fold increase.
- The MBL acute phase response was affected by the time of the inoculation of virus.
- The MBL concentration was directly associated with the ability to activate the complement system.
- Chickens with a higher (genetic or stimulated) level of MBL had a lower IBV specific antibody response.

References

Juul-Madsen, H.R., Hedemand, J.E., Salomonsen, J. and Simonsen, M. 1993. RFLP analysis of the chicken B-F and B-L genes and their association with serologically defined B haplotypes. Anim. Genet. 24, 243-247.

Juul-Madsen, H.R., Munch, M., Handberg, K.J., Sørensen, P., Johnson, A.A. and Jørgensen, P.H. 2002. Serum levels of Mannan-Binding Lectin (MBL) in chickens prior to and during experimental infection with avian Infectious Bronchitis Virus (IBV). Poultry Sci. 82, 235-241.

Laursen S.B., Dalgaard T.S., Thiel S., Lim B.L., Jensen T.V., Juul-Madsen H.R., Takahashi A., Hamana T., Kawakami M., and Jensenius J.C. 1998. Cloning and sequencing of the cDNA encoding the chicken mannan-binding lectin (MBL) and comparison with mammalian analogues. Immunology 93, 421-430.

Laursen S.B. and Nielsen O.L. 2000. Mannan-binding lectin (MBL) in chickens: molecular and functional aspects. Develop. Comp. Immunol. 24, 85-101.

Turner M.W. 1996. Mannose-binding lectin: the pluripotent molecule of the innate immune system. Immunol. Today 17, 532-540.

30. Use of alternatives to AGP in animal food production in China

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Abstract

In china, there are about 3000 metric tons feed antibiotics used as growth promoter per year. Along with the vigorous development of feed industry and the increase in social concept of food and environment safety, most people are becoming deeply aware of that the feed safety is important for keeping food and environment healthy. Now, the feed antibiotics are now one of the most important factors that affect the feed safety, in spite of their distinctive effect on performance of animals. So, the first thing to solve the problem is to develop alternatives to AGP. In china, alternatives to AGP mainly include enzymes, probiotics, herbal medicines and oligosaccharides.

The history of researching and using of feed enzymes in china is just about ten years. But now more and more feed producers and consumers use the additive. These enzymes mainly include phytase, cellulase, ²-glucanase, ±-galactosidase, xylanase, amylase, protease, pectinase. So far, the number of the plants which can produce 2000 tons of feed enzyme per year is 40 in china. It may be forecasted that feed enzymes will became a key technology to improve the development of feed industry in 21st century.

In China, the research of probiotics started in 1980's. In 1999, the Ministry of Agriculture of China announced the following 12 kinds of probiotics additives: *Lactobacillus casei, Lactobacillus plantum, Lactobacillus acidophilus, Streptococcus faecalis, Streptococcus faecium, Streptococcus lactis, Pediococcus acidilacticii, Bacillus subtilis, Bacillus natto, Candida utilis, Rhodospseudomonas palustris, Saccharomyces cerevisiae.* In fact, some other kinds of probiotics such as *Bacillus cereus, Bacillus licheniformis, Bifidobacterium adolescentis, Bifidobacterium sp.* have been in use.

One of the more recently celebrated concepts is that of using herbs and the active substances contained therein. They have many possible modes of action to animals. Some herbs are well known to have antimicrobial effects, others have an astringent effect on the intestine, and can encourage efficient digestion. The latest nation-wide survey reveals that about 11200 kinds of Chinese medicinal herbs are being used in China, and among them about 500 kinds were supplied as feeding supplement.

In addition, the following oligosaccharides are in use to test their effects on performances of animals in China: α-Glucooligosaccharides, α-GOS; Mannanoligosaccharides, MOS; Fractooligosaccharides, FOS; Galactooligosaccharides, GAS; Xylooligosaccharides, XOS, and so on.

In general, most of the alternatives to AGP are still in the stage of researching and testing about their effects on animals in China. And it's very difficult to use them on a large scale in present animal production because of the following factors: (1) Cost is High; (2) Effects are unstable or indistinctive; (3) Activity is easy to be lost in pelleting process.

But along with further researching and testing, the use of alternatives to AGP in animal food production will become more and more popular in China. Key words: feed additive, antibiotics, alternative, China.

Introduction

Antibiotics are compounds produced by microorganisms. These compounds have the properties of inhibiting the growth or metabolism of other harmful microorganisms. Antibiotics have, in general, been effective as growth promoters when fed at low levels to young and

growing animals. The response of antibiotics in growth and feed efficiency are apt to be variable among species, ages and environmental conditions. For example, there is little or no response in very clean surroundings or in germ-free animals raised under aseptic conditions. Generally, the most pronounced responses in young animals occur in situations where organisms such as *E. Coli* or other organisms causing diarrhoea are in high concentrations because antibiotics, as a rule, will help to reduce the incidence or severity of several types of diarrhoea. Recent research has demonstrated that adding antibiotics to animal diets will improve growth performance or nutrient availability. In china, there are about 3000 metric tons feed antibiotics used as growth promoter per year. Up to date, some efficacy studies have been conducted with antibiotics in monogastric animals (Table 1).

However, Long term use of antibiotics in feed will cause (1) microbes to become resistant to drugs, (2) immunity of animals to decline, (3) endogenous infection and reinfection in animals, (4) residues of antibiotics in animal products and environment.

Especially, the residual problems of antibiotics in carcasses are limiting its use in diets of pigs and broilers, and the concerns in the use of antibiotics are growing, although lots of researchers have been demonstrated the effects of antibiotics in pig as a growth promoter. In short, along with the development of feed industry and the increase in social concept of food and environment safety, most people are becoming deeply aware of that the feed safety is important for keeping food and environment healthy. Because the feed antibiotics are now one of the most important factors that affect the feed safety, animal producers have to find alternatives to AGP. In this aspect, responses of China government is very positive. Research on antibiotic residue in animal products was listed in the Eighth and Ninth Five-year National Key Research Projects. In 1997, the standards of maximum quantity of antibiotic residue in animal products were enacted. In 2000, the Feed Safety Project was

started. All these showed that China government has paid much attention to the development of „Green feed additives". So far, the alternatives to AGP mainly include enzymes, probiotics, herbal medicines and oligosaccharides in china.

Alternatives to AGP in China

Enzymes

Until now, there have been numerous studies and reviews on the use of enzymes for improvement of feed utilization in monogastric animals. Monogastric animals cannot utilize cell walls to the same extent as ruminants except through products of hindgut fermentation.

Therefore the primary objective of the use of enzymes as feed additives is the extensive degradation of 'fiber' prior to the ileum to render nutrients available for digestion within the small intestine. Such an approach may improve the utilization of currently used feedstuffs and increase the range of feed ingredients suitable for inclusion in animal diets. Because of the complex nature of non-starch polysaccharides (NSP), an enzyme addition must contain a mixture of enzymes with different specifications. Thanks to the increase in knowledge of cell wall composition and structure and of the digestive physiology of the animal, the use of enzymes in livestock production has been evolved in recent years.

The history of researching and using of feed enzymes in china is just about ten years. But now more and more feed producers and consumers use the additive. These enzymes mainly include phytase, cellulase, ²-glucanase, ±-galactosidase, xylanase, amylase, protease, pectinase. So far, the number of the plants which can produce 2000 tons of feed enzyme per year is 40 in china. It may be forecasted that feed enzymes will became a key technology to improve the development of feed industry in 21st century. Table 2 shows the positive effects of various enzymes on growth performances in as reported by some researchers.

Table 1. Effect of key antibiotics on the performances of pig and broiler in China (Tong, 2000)

Antibiotics	Pig (1-60 day old)			Broilers(1-21 day old)		
	Add. (mg/kg)	Gain (+%)	FCR (+%)	Add. (mg/kg)	Gain (+%)	FCR (+%)
Chlorteracyeline	75	27	9.8	35	7.6	6.9
Bacitracin Zinc	40	15	5.6	20	12	5
Flavomycin	20	21	7.6	5	4.1	5
Verginiamycin	20	10	5	10	5.4	4.3

Table 2. Effects of feed enzyme additives on pigs and broilers in China

Enzyme	Add. (U/kg)	Test animal	ADG (+%)	F:G (-%)	References
α-Galactosidase	60	piglet	10.45	6.37	Pan, 2002
Xylanase	1500	broiler	2.27	4.44	Wu, 2002
Pectinase	60	broiler	5.97	1.75	Wang, 2002
Phytase	1200	piglet	56.94	28.48	Liu, 1997
Phytase	800	growing pig	50	10	Chao, 1995

Probiotics

The importance of maintaining an normal intestinal microflora in monogastric animals has been recognized for many years. In the past decades, the most common method of repressing the harmful microorganisms has been treated with anti-bacterial agents. Since the regulation prohibits the use of subtherapeutic levels of antibiotics, the use of probiotics as a possible alternative to antibiotics has received renewed interest.

In China, the research of probiotics started in 1980's. In 1999, the Ministry of Agriculture of China announced the following 12 kinds of probiotics additives: *Lactobacillus casei, Lactobacillus plantum, Lactobacillus acidophilus, Streptococcus faecalis, Streptococcus faecium, Streptococcus lactis, Pediococcus acidilacticii, Bacillus subtilis, Bacillus natto, Candida utilis, Rhodospseudomonas palustris, Saccharomyces cerevisiae*. In fact, some other kinds of probiotics such as *Bacillus cereus, Bacillus licheniformis, Bifidobacterium adolescentis, Bifidobacterium sp.* have been in use. Some test results of probiotics are listed in the following tables.

Herbal medicines

One of the more recently celebrated concepts is that of using herbs and the active substances contained therein. They have many possible modes of action to animals. Some herbs are well known to have antimicrobial effects, others have an astringent effect on the intestine, and can encourage efficient digestion. The latest nationwide survey reveals that about 11200 kinds of Chinese medicinal herbs are being used in China, and among them about 500 kinds were supplied as feeding supplement. Some test results of herbal medicine are showed in the following tables.

Oligosaccharides

In addition to all above alternatives, oligosaccharides are expected to become an alternative to AGP. The following oligosaccharides are in use to test their effects on performances of animals in China: α-Glucooligosaccharides, α-GOS; Mannanoligosaccharides, MOS; Fractooligosaccharides, FOS; Galactooligosaccharides, GAS; Xylooligosaccharides, XOS, and so on. Some test results in China are listed in Table 7.

Table 3. Effects of probiotics on egg hens

Probiotics or antibiotics	Supplement (mg/kg)	Number of hens	Laying rate (+%)	Feed/Egg (-%)	Mortality rate (-%)
Bacillus cereus	1000	2400	3.04	0.89	0.36
Bifidobacterium adolescentis	1000	2400	4.37	2.54	0.69
Lactobacillus acidilacticii	1000	2400	3.59	1.16	0.77
Bifidobacaterium sp.	1000	2400	5.65	3.49	1.23
Flavomycin (Pos. Control)	4	2400	5.12	2.38	0.92
Neg. Control	-	2400	-	-	-

Table 4. Effects of probiotics on weanling pigs (Wang, 2002)

Probiotics or antibiotics	Supplement (mg/kg)	Number of pigs	ADG (+%)	Feed/Gain (-%)	Diarrhoea rate (-%)
Bacillus cereus	1200	276	3.53	2.76	18.7
Lactobacillus acidilacticii	1200	276	3.35	2.51	20.5
Bifidobacaterium sp.	1200	276	7.06	4.42	31.4
Flavomycin (Pos. Control)	5	276	7.12	4.37	29.3
Neg. Control	-	276	-	-	-

Table 5. Effects of Chinese herbal supplementation on growth performance in piglets (Piao, 2002)

Add.	ADG (g/d)	ADFI (g/d)	FCR
0.08% Olaqindox	538	891	1.66[a]
0.04% Olaquindox + 0.4% Herb	541	852	1.58[ab]
0.8% Herb	592	913	1.54[b]
P value	0.35	0.68	0.03

Table 6. Effects of different herbal polysaccharides on the performance of broilers (Chen, 2002)

	Add. (mg/kg)	ADG (g)	ADFI (g)	F/G	Mortality %
Aloe polysaccharide	200	38.3	73.8	2.09	4.2
Astragalan polysaccharide	200	35.9	71.1	1.98	5.2
Achyranthan polysaccharide	200	38.5	78.5	2.04	3.8
Control (CTC)	80	35.5	73.1	2.06	4.2

Table 7. Effects of oligosaccharides on weanling pigs (Wang, 2002)

Probiotics or antibiotics	Supplement (mg/kg)	Number of pigs	ADG (+%)	Feed/Gain (-%)	Diarrhoea rate (-%)
Glucooligosacchari	2000	36	5.36	0.21	9.17
Froctooligosaccharide	2000	24	4.21	0.55	8.26
Flavomycine	4	36	7.18	3.97	12.58
Neg. Control	-	36	-	-	-

Alternatives to AGP and environment
Another effect of the alternatives to AGP is to alleviate environmental pollution through the following pathways: (1) Decrease odor gas. In this aspect, probiotics have a significant effect. For example, EM in feed can decrease the density of bad odor by 92%~97%, and improve significantly the environmental quality of chicken houses (Liu, 2002); (2) Decrease excretion of nutrients such as P and N. Because monogastric animals lack the enzyme phytase which is required to release the organically-bound phytin phosphorous in cereal grains, their feed must be supplemented with inorganic phosphorous. The end result is phosphorous pollution of land and ground water through manure application. Addition of microbially derived phytase to animal feeds is being implemented to reduce or even replace the need for inorganic phosphorous supplementation through enhanced utilization of phytin phosphorus by monogastric animals. Pigs and poultry wastes account for nearly half of the total P excretion by animals. The high amount of P returned to the environment is a great concern in many countries, and is of even more concern in those areas of the world where animal populations are dense, and land and water resources are limited.

Conclusion
In short, alternatives all above have some effects on performances of animals to some extent, but most of them are still in the stage of researching and testing in China. And it's very difficult to use them on a large scale in present animal production because of the following factors: (1) Cost is High; (2) Effects are unstable or indistinctive; (3) Activity is easy to be lost in pelleting process. But along with further researching and testing, the use of alternatives to AGP in animal food production will become more and more popular in China.

References

He, W.L. and Qi, D.S., 2002. Effects and problems of alternatives to antibiotics. Feed Research, (9), 10-12.

Liu, H., 2002. Research and application of "green" feed additives. Feed Research, (7), 18-21.

Tong, J.M., 2000. Feed antibiotics and feed safety. 2000 APEC China Seminar(Public Health Issues in Animal Production/Animal Products), China, 110-112.

Wang, H.D., Zeng, Q.L. and Wang, M.D., 2002. The research about alternatives of feeding antibiotics. Feed and Animal Husbandry, (1), 16-21.

31. Use of Alternatives to AGP in Animal Food Production in the USA

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Abstract
There are inadequate quantitative data available on the use of alternatives to AGP in the USA, so this paper is based on informal observations and conversations. The industry expects progressively tighter constraints in the future, and is very interested in technology that will smooth the transition to animal production without AGP. Antibiotics for use in animal feeds are selected more on the basis of their perceived value in protection against disease than on the basis of their efficacy in promoting growth. There are significant differences in pig production systems between the USA and western Europe, and these differences may impact the use of both AGP and alternatives. The US industry is characterized by fewer constraints on the use of AGP, widespread adoption of all in/all out pig flow, aggressive bio security measures, early weaning, and sophisticated nursery feeding programs. The US industry uses very high levels of lactose, mostly from dried whey, in early nursery diets. Spray-dried plasma in the early diets increases growth rate dramatically, and is widely used in the USA. Zinc oxide and copper sulfate are used extensively in early nursery diets in the US pig industry. Diet acidifiers are used much less in the USA than in Europe, because they appear less effective in diets containing high levels of milk products, such as those we use. Other products including mannan oligosaccharide, egg immunoglobulins, and direct-fed microbials are also used in the USA, but less widely.

Introduction
Restrictions on the use of antibiotics in animal production in the USA are tightening, but are much less constraining than those in the European Union. The industry expects progressively tighter constraints in the future, and is very interested in technology that will smooth the transition to animal production without AGP.

Antibiotics for use in animal feeds are selected more on the basis of their perceived value in protection against disease than on the basis of their efficacy in promoting growth. Where AGP are withdrawn, the loss of growth promotion has a cost, but the loss of disease control is of much greater concern to producers and veterinarians.

There appear to be no reliable quantitative data on the use of alternatives to AGP in the USA. Therefore, the following comments are based on my informal observations and conversations, as an academic and an active participant as a consultant in the pork production industry. I am not close enough to the poultry industry to make useful comments about it.

Related issues
Production systems
The US pig industry has adopted new production systems designed to improve health. All in/all out pig flow, often by site (farm), is a central component of these systems. All in/all out flow allows interruption of disease circulation within a population when the facility is emptied, cleaned, and disinfected, and is substantially more effective when applied to the farm than when applied only to the building or room within a farm. The industry also has adopted aggressive bio security policies and sanitation programs. The shift to large production systems has facilitated adoption of these strategies.

Weaning age
The US industry weans piglets earlier than the European industry does, often between two and three weeks of age. The younger weaned piglet

requires more sophisticated facilities, diets, and care than if weaned later. The move to earlier weaning has been driven partially by economic considerations, but also by health concerns. Early weaning interrupts the vertical transmission of pathogens from the sow to the piglets, which occurs at various ages depending on the pathogen. Also, the younger pig at weaning has substantially more colostrum-derived immunity than the pig weaned at three to four weeks of age.

Nursery diets

The feeding programs used for weaned pigs in the USA are complicated, consisting of three to six different diets fed in sequence. The first diet is especially sophisticated, containing high levels of several special ingredients that are not fed to older pigs. It is economically feasible to feed so expensive a diet only because we feed only a small amount of it, less than 1 kg/pig. As the pig matures, it receives progressively less sophisticated (and less expensive) diets, until it gets a simple diet based on maize and soybean meal by perhaps 15 kg body weight.

The pigs are dramatically healthier when given these sophisticated feeding programs than when fed simpler diets and simpler programs. We could not realistically have reduced the weaning age so far without these dietary improvements.

Alternatives to AGP

There is enormous interest in the USA in technologies for reducing the amount of antibiotics used in pig production. There is ubiquitous use of some of these technologies, along with AGP, and current interest in adopting others.

In general, the data on feed ingredients proposed as alternatives to AGP suggest that some may be useful, but most are less powerful in either promoting growth or protecting against disease than are antibiotics. Some of these products are likely to provide benefits even in the presence of AGP.

Milk products

In many cases the first diet the pigs receive after weaning contains more than 20% lactose, mostly from dried whey. The level of lactose is gradually reduced in successive diets, and usually reaches zero by the time the pigs are about 15 kg body weight. It is clear that lactose improves growth performance, but it is not clear how. Perhaps it alters bacterial populations and(or) pH in the digestive tract.

There may be also benefits from the milk proteins, including immunoglobulins. Products containing casein, such as dried skim milk, are rarely used because of cost.

Spray-dried plasma

A meta-analysis conducted in The Netherlands (van Dijk et al., 2001) showed that inclusion of spray-dried animal plasma in the diet of weaned pigs in several studies increased the growth rate by an astounding 27%, on average. Few technologies in animal production are so powerful! The mode of action of plasma is not known, although some evidence suggests that it affects the immune system.

Spray-dried plasma is included in many of the early nursery diets in the USA.

Zinc oxide and copper sulfate

The use of high levels of zinc oxide (usually >2,000 ppm Zn) is ubiquitous in early nursery diets in the USA. Conversations with pig producers and veterinarians indicate they have a great deal of confidence in the ability of zinc oxide to protect pigs against enteric infections, primarily with *E. coli*. Nutritionists appreciate the growth promotion it provides.

High levels of copper sulfate (>125 ppm Cu) are also widely used for growth promotion.

Acids

Diet acidifiers are used much less in the USA than in Europe, although much of the early re-

Table 1. Pen and housing design

	Farm A	Farm B	Farm C
Pens per section	12	24	4
Pigs per pen	35	30	200-250
Flooring	partly slatted	partly slatted	deep litter
Feeding system	tube feeder	tube feeder	tube feeder
Feeding regimen	ad lib	ad lib	ad lib
Pigs per drinking bowl	17	15	30
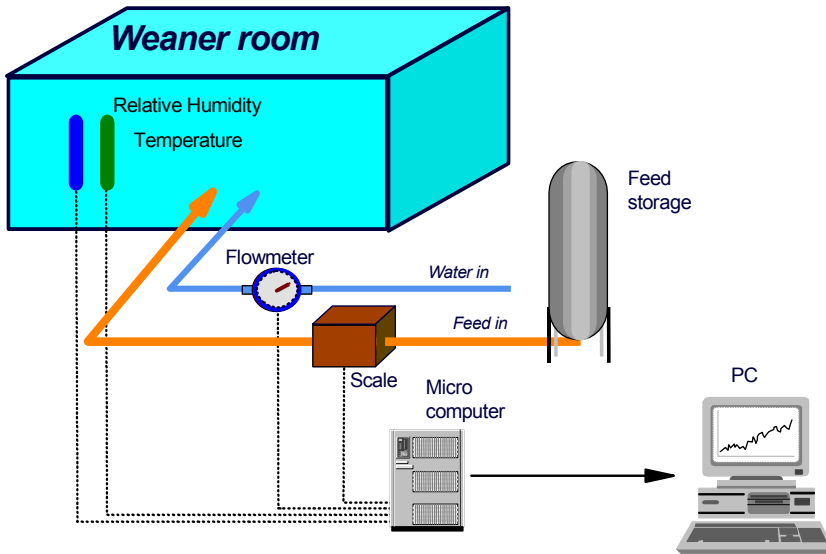
Figure 1. Schematic diagram of experimental set-up

Water disappearance was recorded in 2 min intervals while feed disappearance was measured in 10 kg intervals. Temperature (inside and outside) and relative humidity was recorded continuously via sensors connected to the ventilation system. All data were logged every two minutes and stored in a local microcomputer. Every 24 hours data were transmitted to a central database via modem link. In addition, the farm staff recorded any management intervention such as treatment of sick animals, change of feed, removal of dead animals, etc., in a logbook.

Water monitoring - results

Utilization of water and feed disappearance data in pig management implies that it is possible to distinguish between changes in water or feed consumption, which are associated with stressors or disease, and changes in intake, which are related to natural diurnal variation. The results from monitoring water disappearance rate in two-minute intervals indicated that it was characterised by a distinct circadian pattern.

A similar pattern was not detected for feed disappearance, which was monitored in 10 kg portions. This measure was too large to indicate a specific eating pattern in weaners.

Pigs' water intake pattern has not previously been reported, while water intake has been shown to be closely linked to ingestion of feed. Thus, Bigelow and Houpt (1988) found that 75% of water disappearance was associated with feed disappearance. Moreover, pigs' eating pattern has been described as a cyclical event

search on organic acids was done in the USA (e.g. Easter, 1988). Some studies (Burnell et al., 1988; Allee et al., 1999) have shown that acids are less beneficial in diets that include milk products than in simpler diets, and that observation has diminished the interest in practical use of acids in the USA. There has emerged the concept that lactose is converted to lactic acid in the stomach, and therefore mimics the effects of acids. This concept and other aspects of potential roles of diet acidifiers are now under renewed investigation in my laboratory and elsewhere in the USA.

Mannan oligosaccharide
This product derived from yeast cell walls produces a small but clear increase in growth rate in weaned pigs (Miguel et al., 2002). Its mode of action is unclear, but it may include prevention of pathogen binding to the gut wall and(or) effects on the immune system. Several feed companies and pork producers use it.

Egg immunoglobulins
Hens are immunized against specific porcine pathogens, and immunoglobulins from their eggs are prepared into a feed additive. A battery of hens immunized against different pathogens supports development of a product providing passive immunity to several diseases. The industry uses this product to some extent. It is sometimes combined with spray-dried plasma.

Direct-fed microbials
Direct-fed microbials have been available for decades. The industry chooses to use them to some extent, but they are not ubiquitous in nursery feeding programs. There continues to be a significant amount of research reported on such products.

Others
There is continuing interest in botanicals and in fructo-oligosaccharides, but in my judgment they are not used extensively by the US pig industry. There is research activity with competitive exclusion.

Conclusions
The degree of government control of antibiotic use, pig production systems, weaning age, and nursery diet formulations all impact the use of AGP and alternatives by the pig industry. These factors tend to be different in the USA from Europe, so use of AGP and alternatives are different also. There is acute and widespread interest in methods for producing pigs without AGP in the USA.

The US pig industry uses milk products, spray-dried plasma, zinc oxide, and copper sulfate very widely. It also uses acids, mannan oligosaccharide, egg immunoglobulins, and direct-fed microbials, but to a lesser extent. Acids are used much less in the US than in Europe.

References
Allee, G.L., Touchette, K.J. and Liu, H. 1999. The effect of lactose and acidification on nursery pig performance. J. Anim. Sci. 77 (Suppl. 1), 62 (Abstr.).

Burnell, T.W., Cromwell, G.L. and Stahly, T. S. 1988. Effects of dried whey and copper sulfate on the growth responses to organic acid in diets for weanling pigs. J. Anim. Sci. 66, 1100-1108.

Easter, R.A. 1988. Acidification of diets for pigs. In: Recent Advances in Animal Nutrition 1988 (W. Haresign and D. J. A. Cole, eds), Butterworths, London, UK, 61-71.

Miguel, J.C., Rodriguez-Zas, S.L. and Pettigrew, J. E. 2002. Practical effects of Bio-Mos® in nursery pig diets: a meta-analysis. In: Nutritional Biotechnology in the Feed and Food Industries, from Niche Markets to Mainstream: Proceedings of Alltech's 18th Annual Symposium (T. P. Lyons and K. A. Jacques, eds), Nottingham University Press, Nottingham, UK, 425-443.

Van Dijk, A.J., Everts, H., Nabuurs, M.J.A., Margry, R.J.C.F. and Beynen, A.C. 2001. Growth performance of weanling pigs fed spray-dried animal plasma: A review. Livest. Prod. Sci. 68, 263-274.