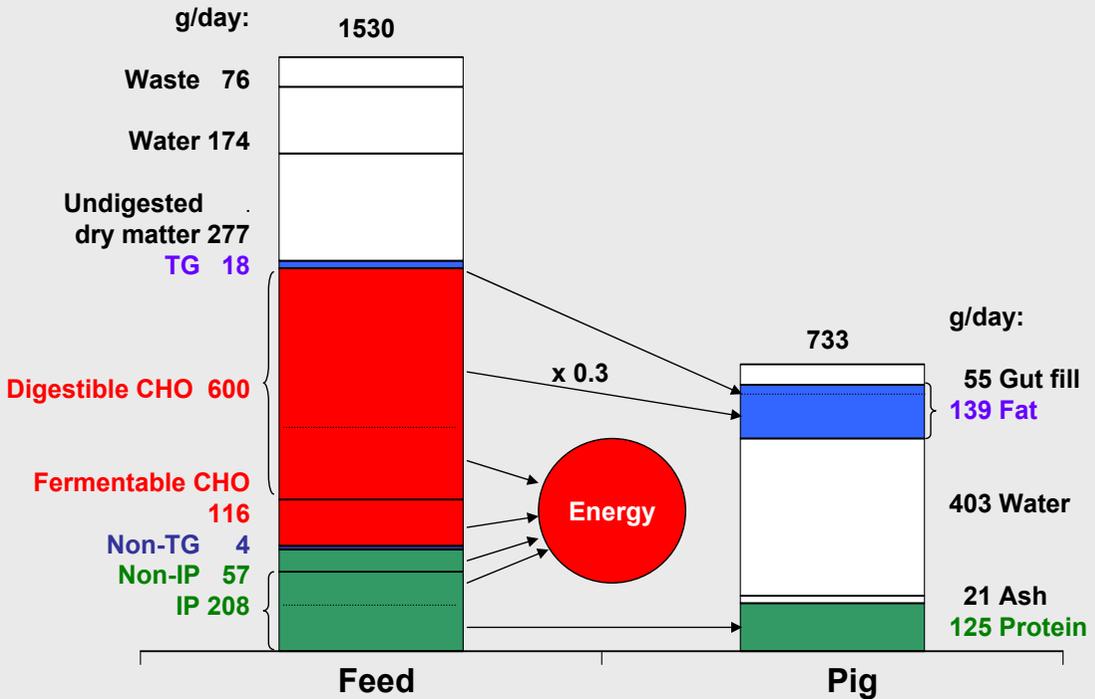


A New Concept for Practical Feed Evaluation Systems

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ISBN: 87-91949-18-1

Preface.....	4
Summary.....	5
Introduction.....	9
Properties of the feed	11
Basic principles for feed evaluation.....	13
Potential physiological energy	13
Potential digestibility of nutrients	15
Standardised digestible amino acids	18
Ideal protein	21
Protein quality of feedstuffs	23
Energy evaluation of the major components in feedstuffs and pig diets	24
Starch as energy reference for other nutrient fractions	24
<i>Ileal</i> digestible carbohydrates	24
<i>Ileal</i> digestible lipids	25
<i>Ileal</i> digestible protein	25
Fermentable carbohydrates	26
Energy costs of other components in pig diets	26
Enzyme indigestible dry matter at <i>ileal</i> level (EIDMi).....	26
Surplus protein	27
Anti-nutritional factors (ANF's).....	27
The practical performance of the new Danish feed evaluation system.....	29
Basal chemical analyses, factors and equations.....	29
Calculation of energy value (PPE).....	29
Standardised digestible amino acids (SDAA) in feedstuffs and diets	30
Amino acid recommendations	31
Feed optimisation based on SDAA and PPE	33
The new Danish feed evaluation system compared with other systems.....	35
Further developments and improvements in feed evaluation and pig production.....	39
Feed evaluation and optimisation of diets in practise	39
Feeding techniques and feeding strategy	41
General principles for a step-wise feed evaluation based on the new Danish system	42
General discussion.....	45
References.....	47

Preface

Starting from the nineties more focus was initiated on the nutrient surplus from the intensive husbandry in animal production. In particular the surplus of nitrogen (N) and phosphorus (P), which resulted in problems with evaporation of ammonia from the animal manure and a surplus of phosphorus discharged in streams, lakes and internal seas causing serious environmental problems.

Consequently, more focus on feed evaluation and basic principles for feed optimization for the different production animals and procedures, respectively, was initiated. Thus, feed evaluation for pigs in Denmark and other countries was still strongly influenced by classical analytical methods and principles for feed evaluation. However, during the last decennials a considerable development in the understanding of digestion, metabolism and utilisation of the individual nutrients has occurred. Thus, it was afterwards understood that a practical utilisation of the new knowledge would be of great impact for a future sustainable husbandry animal production. In particular for the slaughter pig production, which increased significantly, and became more and more important for the national economy.

Danish Institute of Agricultural Sciences (DIAS) and the Danish Meat Association (DMA) therefore cooperated in developing and implementing a new system which was based on new principles and methods for feed evaluation. Thus, DMA was responsible for the implementation of the system, including the performance of ring tests between official and commercial laboratories, and for the development of the final equations for calculation of nutrient fractions and feed units for pigs (FUp). Furthermore, Per Tybirk (DMA) contributed throughout the process with many inspiring discussions.

Carsten Pedersen contributed during his PhD study focussing on the protein value of pig feeds with particular attention to the standardized digestibility of amino acids in feedstuffs. Ole Hartvig Olsen has contributed with data collection, statistical analyses and drawings. Many scientists throughout the world are thanked for valuable critical comments to the manuscript. Sissel Rønning Christiansen and Mette Holme Janum are thanked for preparing the final set up of the report.

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May 2007
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Summary

Feed evaluation has been under development during the last century. The classic Weende analyses from 1888 for chemical characterisation, based on analyses for crude protein, crude fat, crude fibre and nitrogen-free extracts (NFE), have been a central basis for characterisation of the feed quality. However, different systems for feed evaluation have, generally, been based on animal experiments and have moved from systems based on digestible energy (DE) and metabolisable energy (ME) to different principles based on net energy (NE). The philosophy has been to describe more accurately the feed's production value for the animals.

However, the actual value of a feed is influenced by its specific use and, therefore, animal experiments, performed under experimental conditions, cannot be the optimal basis for defining the feed value under a variety of practical production conditions. Alternatively, the feed value can be based on the properties of the feed itself, and recommendations for the optimal feed composition can then be based on all relevant information according to the specific production.

Optimisation of pig diets from actual feedstuff batches is, generally, based on linear programming. In the new Danish feed evaluation system, the composition of standardised digestible amino acids and potential physiological energy, respectively, are optimised according to the recommendations for the specific pig category and weight range for slaughter pigs. In the practical feed evaluation, the content of digestible nutrient fractions in actual batches of feedstuffs are analysed and commercially produced pig diets are, furthermore, analysed by the official controlling authority.

Standardised digestible amino acids (SDAA) are presently based on table values for digestibility of amino acids in feedstuffs, whereas the other digestible nutrient fractions are based on *in vitro* digestibility analyses corresponding to *ileal* and faecal level, respectively. Values of *in vitro* digestibility reflect the potential digestibility and correspond to the real digestibility of protein, from which standardised digestible amino acids can be calculated in the actual feed batches. The *in vitro* analysis methods have proved to give reliable measures for the variation in digestibility in the actual feed samples and, thus, contribute to a more precise production of diets for pigs.

Potential physiological energy (PPE) is based on the potential production of ATP when the different nutrients are oxidised at cellular level.

Starch is commonly the major energy source in pig diets and can, furthermore, be considered as a pure energy source without additional properties like all other nutrient fractions. Starch is, therefore, an obvious energy reference for the other nutrients in feed optimisation.

In the new Danish feed evaluation system, the energy value of all other nutrients is related to the effect on the energy value of the diet when they supplement starch. The main effect in practical feed optimisation is that dietary lipids are credited for their sparing effect on the alternative lipid syntheses from dietary starch in growing pigs, because they deposit considerable more lipids than they eat.

Finally, the *in vitro* determined indigestible fraction of dry matter at *ileal* level contributes with a negative energy value due to the extra endogenous losses of protein and lipids, which are induced by this feed component.

The new Danish feed evaluation system for pigs is unique compared to other systems, which are all based on results obtained with animal experiments. These systems are, therefore, dependent on specific experimental conditions, which do not include the effects of a variety of influencing factors under practical production conditions.

On the other hand, in the general feed optimisation the relative energy values for the different nutrient fractions are more important than the absolute values. Interestingly, the relative energy values of the different nutrient fractions in recent proposals for NE systems are quite close to those

in the PPE system. Therefore, due to the relatively imprecise performance which is still commonly used in the practical production of feeds and pigs, the practical benefit of a scientifically correct system may still be questioned.

However, with more refined techniques in feed production, feed analyses and practical feeding strategies, the new principles for feed evaluation, based directly on the properties of the feed itself, offer the opportunity for considerable improvements in practical pig production. Furthermore, a feed evaluation system, which is not influenced by specific experimental conditions and animal responses in different countries, appears to be the only realistic choice for a common international system. The agreement on a common international feed evaluation system would be the optimal basis for future systematic scientific developments as well as general advantages in international feed trade.

Dansk sammendrag

Fodervurdering har været under udvikling igennem de sidste 100 år. De klassiske Weende-analyser, som var baseret på analyser for råprotein, råfedt, træstof og NFE (kvælstoffrie ekstrakter), udgjorde en central baggrund for karakteriseringen af foderets kvalitet. Igennem denne periode har udviklingen af fodervurderingssystemer været baseret på dyreforsøg og bevæget sig fra systemer baseret på fordøjelig energi og omsættelig energi til forskellige principper baseret på netto energi. Filosofien har været at give en mere præcis beskrivelse af foderets produktionsværdi for dyrene.

Imidlertid er den aktuelle værdi af foderet påvirket af dets aktuelle anvendelse, hvilket betyder, at dyreforsøg, der er udført under eksperimentelle betingelser, ikke er velegnede til at definere foderets værdi under de forskellige praktiske produktionsbetingelser. Dette forudsætter, at foderets værdi beskrives direkte ud fra dets basale egenskaber, medens anbefalingerne for den optimale blanding baseres på alle relevante informationer i relation til både den specifikke og aktuelle produktion.

I Danmark er optimeringen af svinefoder, ud fra de aktuelle foderstofpartier, baseret på lineær programmering. Med denne metode optimeres sammensætningen af standardiseret fordøjelige aminosyrer og den potentielle fysiologiske energi fra de aktuelle foderstofpartier i henhold til de officielle anbefalinger for den pågældende kategori og vægtklasse af svin. I den praktiske fodervurdering analyseres de aktuelle foderpartiers indhold af fordøjelige næringsstoffraktioner, og de kommercielle svinefoderblandinger kontrolleres desuden gennem stikprøver udtaget af Plantedirektoratet, der er den officielle kontrolinstans.

Foderets indhold af standardiseret fordøjelige aminosyrer baseres indtil videre på tabelværdier for standardiseret fordøjelighed i de enkelte foderstoffer, medens de øvrige fordøjelige næringsstoffraktioner er baseret på *in vitro* fordøjelighedsanalyser, der simulerer fordøjeligheden på henholdsvis tyndtarms- og fæcesniveau. Værdier for *in vitro* fordøjelighed afspejler den potentielle fordøjelighed og korresponderer desuden til den reelle fordøjelighed af protein, hvorfra indholdet af standardiseret fordøjelige aminosyrer vil kunne beregnes i de aktuelle foderstofpartier. De udviklede *in vitro* metoder har vist sig at kunne give pålidelige bestemmelser af de variationer, der kan forekomme i fordøjeligheden i forskellige foderstofpartier og vil således kunne bidrage til en mere præcis produktion af svinefoder i relation til ændringerne i grisenes behov under deres vækst og udvikling.

Foderets indhold af potentiel fysiologisk energi er baseret på den potentielle produktion af ATP, når næringsstofferne oxideres på celleniveau. Stivelse betragtes som energi reference for de andre næringsstoffraktioner, dels fordi stivelse normalt er den dominerende energikilde i svinefoder, dels fordi stivelse kan betragtes som en ren energikilde uden supplerende egenskaber, sådan som det er tilfældet for de øvrige næringsstoffraktioner.

Det betyder, at energiværdien af de øvrige næringsstoffraktioner bestemmes ud fra deres effekt på blandingens energiværdi, når de erstatter stivelse. Herved sikres bedst muligt, at blandingens reelle energiværdi kan holdes konstant i forhold til indholdet af standardiserede aminosyrer ved forskellige sammensætninger af de energiholdige næringsstoffraktioner. Den vigtigste effekt af dette er generelt, at foderfedtets energiværdi øges, fordi det tillægges den energi, der ellers skulle forbruges til fedtsyntese ud fra glucose (stivelse). Korrektionen er fysiologisk korrekt, eftersom foderfedtet normalt aflejres direkte i grisen uden fysiologisk omsætning, og fordi det generelt lave fedtindhold i svinefoder altid vil være i underskud i forhold til den mængde, der aflejres i voksende grise. Ud over de energiholdige næringsstoffraktioner indgår den *in vitro* bestemte ufordøjelige tørstoffraktion på tyndtarmsniveau, som bidrager med en negativ værdi pga. de ekstra omkostninger denne fraktion giver i forbindelse med ekstra tab af protein og fedtstoffer under foderets fordøjelse.

Det nye danske fodervurderingssystem for svin er enestående sammenlignet med andre landes eksisterende systemer, der alle er baseret direkte på resultater opnået i dyreforsøg. Den generelle begrænsning i disse systemer skyldes, at de forudsætter at foderets værdi i den praktiske produktion svarer til resultater opnået i forsøg, der er gennemført under specifikke forsøgsbetingelser. Den vigtigste forudsætning for en korrekt fodervurdering er imidlertid en korrekt angivelse af de relative energibidrag fra de enkelte næringsstoffraktioner i foderet. Det er interessant, at de relative værdier for potentiel fysiologisk energi i de vigtigste næringsstoffraktioner er sammenlignelige med de relative energiværdier for såvel de seneste versioner af NE systemer fra Frankrig og Holland, som et nyt forslag fra Tyskland mht. korrektion for energibidragene fra hhv. protein og fermenterbare kulhydrater. Motivationen for at ændre praksis vil dog formentligt være forholdsvis lav pga. den relativt upræcise styring af fodringen, der stadig hersker i de fleste praktiske svineproduktioner i de forskellige lande.

På den anden side vil en generel mere raffineret teknik i foderproduktion, foderkontrol og fodringspraksis i fremtiden i højere grad kunne udnytte den nyeste viden om foderets mange forskellige egenskaber. Et fodervurderingssystem, der er baseret direkte på foderets specifikke egenskaber, vil være den eneste realistiske mulighed for at opnå enighed om et fælles internationalt system. Et sådant system ville give mulighed for optimale betingelser for såvel forskningsmæssigt samarbejde omkring videreudvikling inden for fodring og produktion som for den generelle samhandel af foder.

Introduction

Feed evaluation has been under development during the last century. The classic Weende analyses from 1888 for chemical characterisation, based on analyses for crude protein, crude fat, crude fibre and nitrogen-free extracts (NFE), made a central basis for characterisation of the feed quality. During this period the development of feed evaluation systems, based on animal experiments, has moved from systems based on digestible energy (DE) and metabolisable energy (ME) to different principles and several methods based on net energy (NE). The philosophy has been to more accurately describe the feed's production value for the animals (Chiba, 2000).

Thus, a system based on NE was introduced from Rostock (Schiemann et al., 1972) and was used for feed evaluation in the former East Germany. Similar systems were developed in Denmark (Just, 1982), The Netherlands (CVB, 1993) and France (Noblet & Henry, 1993). Recently, a new principle for NE system based on the potential for NE retention (NER) and expressed in relation to the energy value from ATP was published from Rostock (Jentsch et al., 2003)

However, although it is generally agreed that systems based on DE and ME do not provide a sufficient basis for feed evaluation, such systems are still used in many countries, most probably, because the relevance for using a system based on NE has been discussed during the last three decades. Thus, it has been stated that the use of NE is too sensitive to be of practical use (Wiseman & Cole, 1985), and that the estimation of NE is difficult and imprecise and influenced by many factors (NRC, 1988) and, therefore, unlikely to provide any greater precision in formulating diets or predicting responses compared with the ME or DE system (Whittemore, 1993).

Based on these facts, Fuller (1997) stressed that "The more the system attempts to describe the productive processes, the more the values depend upon the animal itself, and since the animal factors increase the variability of the response, the less precise the measure becomes" and, finally, Emmans (1999) concluded that: "It is much easier to recognise that the energetic efficiencies of maintenance, lipid retention and protein retention are different and not to get involved in trying to collapse these functions into one measure called NE".

Alternatively, researchers have developed advanced computer models for predicting relevant production parameters based on digestible nutrients (Black *et al.*, 1995). Furthermore, as stated by France *et al.* (2000): "Energy retention *per se* is no longer an adequate index of the performance of the animal or of the nutritive value of the feed because it is the composition of the animal products (e.g. fat and protein in meat, milk and eggs) which is important".

In conclusion, NE is not a suitable basis for feed evaluation because this measure is only valid for a specific production, generally obtained under experimental conditions. Therefore, calculated values of NE for the actual diet may be very different from the NE value obtained under the actual production conditions in practise. Consequently, a feed evaluation system based on NE appears not to be relevant for practical feed evaluation, and neither for efficient developments within modern feed science.

Alternatively, feed evaluation should be considered as a step-wise process in which the feed value is based solely on the properties of the feed itself. From relevant information of actual feedstuff samples, i.e. digestible nutrient fractions, which contribute to the potential energy value and digestible amino acids, respectively, diets can then be optimised according to recommendations for the specific production. These principles are the basis for a new Danish system.

The purpose of this report is to introduce this new concept for practical feed evaluation and to describe the basic principles for the new official feed evaluation system for pigs in Denmark.

Properties of the feed

The basic purpose for feed evaluation is to use the feed value as a suitable tool for optimisation of diets from available batches of different feedstuffs and with different combinations of feedstuffs, for a specific production of husbandry animals.

In Danish pig production a large number of different feedstuffs are available for production of pig diets. The properties of these feedstuffs vary considerably and represent a high variation in chemical composition, *i.e.* from pure sources of proteins, lipids, carbohydrates and minerals to very complex feedstuffs, which include a variety of different nutrients and anti-nutritional compounds. Furthermore, many feedstuffs may be contaminated by a variety of myco-toxins, which can result in a variety of specific negative effects on the feed quality (de Lange *et al.*, 2000).

In the future, new analysis equipments based on physical analysis methods, e.g. NIR, NIT, NMR, chemo-metrics etc. are expected to be able to provide fast and reliable on-line analyses of the nutritional value of the samples of feedstuffs, which are used for the actual production of optimised diets.

However, at present it is not possible to include all the properties of a feedstuff in the feed evaluation process. Firstly, because the specific nutritional effects of the different dietary compounds are not yet completely understood; secondly because their contribution may vary considerably in different batches of the same feedstuff. Furthermore, the availability of nutrients, as well as of anti-nutritional compounds, may be considerably influenced by processing, e.g. milling, heat treatments and enzyme supplementations, as well as of storing.

Therefore, practical feed evaluation and diet production is, generally, based on mean table values, which are adjusted according to actual analyses of the most important properties for the involved feedstuffs. Moreover, relevant analyses are performed for control of the produced diets.

It follows, that practical feed optimisation needs to be based on a relatively simple feed evaluation system, which focus on the most important properties, *i.e.* the energy value and the protein value, respectively. Because both properties are very much influenced by a number of factors related to the actual pig production and feeding strategy, a common relationship between the feed value and the actual production value of the feed cannot be expected. Thus, the actual NE of a specific diet is always influenced by the actual production conditions and, consequently, the feed value needs to be related directly to the properties of the feed itself!

The fundamental properties of feedstuffs and diets, respectively, are based on the potential physiological energy (PPE) contributed from the different digestible nutrient fractions and standardised digestible amino acids (SDAA) contributing to the ideal protein profile for the specific pig category, respectively (Boisen, 2003a).

Optimisation of diets is generally based on recommendations for digestible amino acids relative to the energy value of the feed for the different categories and weight ranges of pigs. Thus, the practical feed optimisation in Denmark is related to specific recommendations for optimal composition of SDAA relative to PPE of the diet for the actual feeding purpose. The energy value of all relevant nutrient fractions and components are precisely defined. Thus, the two fundamental properties in feed evaluation and production are well-defined properties of the feed.

The integration of well-documented and up-to-date scientific developments in experimental and practical feed evaluation offers new challenges for the field of feed science, as well as for improvements of the practical production conditions of husbandry animals.

In conclusion, the energy and protein value of the feed is directly related to the properties of the feed itself and should not be generalised from production results obtained under specific experimental conditions.

Basic principles for feed evaluation

Potential physiological energy

The physiological energy is a measure for the cellular synthesis of adenosine tri-phosphate (ATP), which is the universal energy donor for all energy-requiring processes in living organisms. A dominating portion of ATP is produced from Acetyl-Coenzyme A (AcCoA), which is a central metabolite in the oxidative degradation of nutrients (Figure 1). The potential physiological energy (PPE) value of nutrients is the energy value of produced ATP during their complete oxidation in living cells.

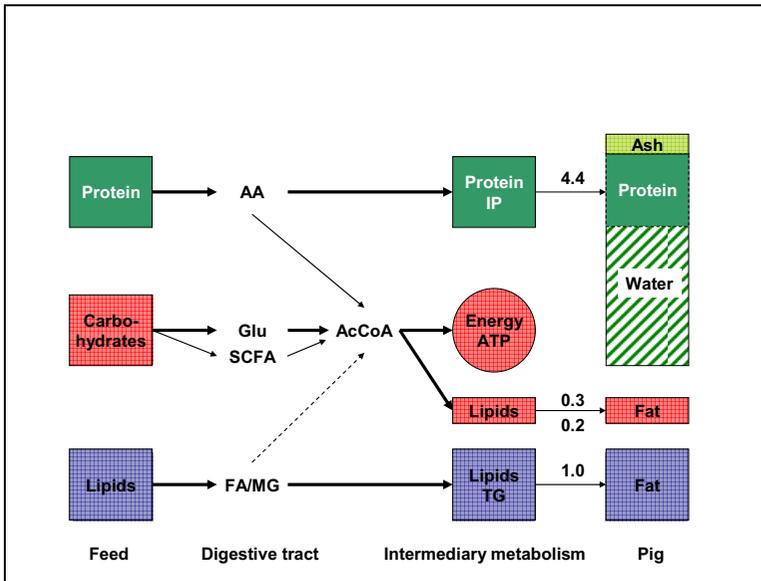


Figure 1. Metabolism of digestible nutrient fractions to energy or deposited nutrients in the pig.

Abbreviations: AA: amino acids; IP: ideal protein; Glu: glucose; SCFA: short-chained fatty acids; AcCoA: Acetyl Coenzyme A; FA: fatty acids; MG: monoacyl-glycerols; TG: triacyl-glycerols. (Boisen & Verstegen, 2000). See text for further details.

PPE of the different nutrient fractions is not influenced by their actual utilisation (oxidation or deposition) and, consequently, the contributions of PPE from ingredients are additive in diets.

The actual metabolism of the nutrients, and their contribution into processes of oxidation and deposition, respectively, is integrated in the recommendations. Furthermore, these processes are integrated in requirement models, for the different pig categories and live weight ranges in slaughter pig production. Generally, PPE is a well-documented property of the different nutrient fractions in feedstuffs and diets (Table 1).

Table 1. Potential physiological energy (PPE) of nutrients and their constituents

Compound	Gross energy (kJ per g)	Potential physiological energy ¹ (kJ per g)	Potential physiological energy utilisation (%) ¹
<i>Protein and other nitrogenous compounds:</i>			
Crude protein (av. source)	23.7	10.4	44
Phenylalanine	28.2	12.4	44
Isoleucine	27.6	16.6	60
Leucine	27.6	16.0	58
Tryptophan	27.5	12.1	44
Valine	25.0	14.8	59
Tyrosine	24.9	12.2	49
Proline	23.7	13.0	55
Lysine	23.5	11.5	49
Histidine	21.7	6.9	32
Arginine	21.4	8.6	40
Methionine	18.6	6.3	34
Cystine	18.4	6.8	37
Alanine	18.2	9.3	51
Glutamine	17.6	8.6	49
Threonine	17.2	9.1	53
Glutamic acid	15.3	9.2	60
Asparagine	14.6	5.8	40
Serine	13.8	6.5	47
Glycine	12.9	4.9	38
Aspartic acid	12.1	6.7	55
<i>Carbohydrates and related compounds:</i>			
Starch	17.5	11.7	67
Sucrose	16.5	11.1	67
Glucose	15.6	10.5	67
Cellulose	17.5	0	0
Lactic acid	15.2	9.9	65
Acetic acid	14.6	8.6	59
Propionic acid	20.8	12.7	61
Butyric acid	24.9	15.9	64
<i>Lipid compounds:</i>			
Crude fat (average source)	38.9	26.1	67
Caprylic acid, C8	32.4	21.4	66
Laurylic acid, C12	36.4	24.3	67
Palmitic acid, C16	39.1	26.2	67
Stearic acid, C18:0	39.9	26.7	67
Oleic acid, C18:1	39.7	26.6	67
Glycerol	18.0	11.3	63

¹For production of ATP. From Church & Pond (1982); Boisen & Verstegen (2000).

PPE is a scientifically correct measure for the physiologically relevant energy in feeds and the logic choice for energy evaluation in modern research and feed evaluation.

Finally, PPE of digestible nutrients is a universal property for all farm animals. Therefore, when taking into account the differences in the digestive physiology of the different species, PPE is also an obvious common basis for feed evaluation across animal species.

Potential digestibility of nutrients

Digestibility is not a specific property of the feed as is the case for the chemical composition. The actual digestibility of a feed can be influenced by many different factors in the production. These factors include not only effects related to the feed itself, *e.g.* processing and storing, but also factors related to the animals (breed, sex, age, live weight, health status), feeding conditions (*ad libitum* feeding, number of feedings, meal size, dry or liquid feeding), and the environment (temperature, air humidity) may directly or indirectly influence the actual digestibility of the feed.

Furthermore, it is well known that experimentally determined digestibility values in pigs are influenced by several factors related to the specific techniques, *e.g.* cannulation technique, feeding and collection strategy etc. as refereed by Boisen & Moughan (1996a,b). Obviously, such analyses are very resource consuming and unsuitable for use in the practical feed evaluation of the actually produced feed batches.

The digestibility of the actual feed batches can, alternatively, be analysed with simple laboratory methods, which simulate the digestion in the animals. For pig feeds, different incubation steps corresponding to the nutrient degradation in the stomach, small intestine and hindgut, respectively, has been demonstrated to be a suitable basis for this purpose.

Thus, two different *in vitro* methods for simulating the digestibility of nutrients at *ileal* and faecal level, respectively, have been developed (Boisen & Fernandez, 1995, 1997). These methods (Figure 2) are now implemented in the Danish feed industry for routine analyses of feedstuffs, pre-mixtures and pig diets and, furthermore, integrated in the official control of commercial pig diets.

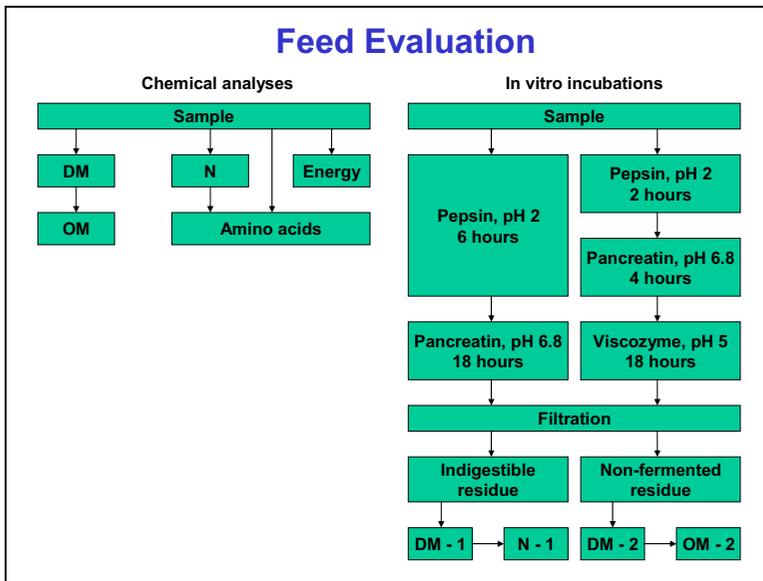


Figure 2. Flow-diagram of *in vitro* incubations of feeds for simulating *ileal* and total tract digestion, respectively. The chemical analyses for calculating the *in vitro* digestibilities of the sample are shown.

Abbreviations: Dry matter (DM); organic matter (OM); nitrogen (N); and their *in vitro* digestibility corresponding to *ileal* (1) and faecal (2) level, respectively (Boisen, 2000a).

The degradation profiles for the two common feedstuffs, barley and soya bean meal are quite different as illustrated in Figure 3. The profiles illustrate the effects of the three different incubation steps according to the contributions of protein, starch and fermentable fibre in the two feedstuffs. Generally, each feedstuff has its own individual degradation profile and, furthermore, the degradation profile ends up in a plateau for each incubation step. This assures that the obtained values correspond to the potential digestibility, which is essential for a well-defined property and reproducibility of results obtained from different laboratories.

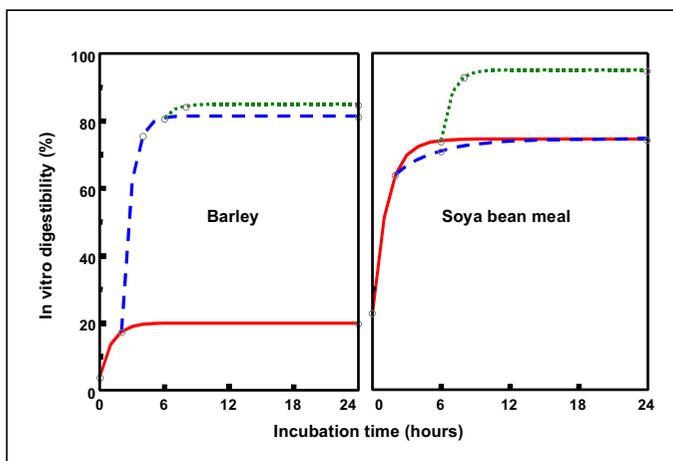


Figure 3. Degradation profiles of dry matter in barley and soya bean meal after enzyme incubations. The samples were incubated with pepsin (—), pancreatin after a preliminary incubation with pepsin for two hours (---), and with Viscozyme after preliminary consecutive incubations with pepsin and pancreatin for two and four hours, respectively (...). From Boisen & Fernandez (1997).

A close relationship between *in vitro* enzyme digestibility of organic matter (EDOM) and *in vivo* digestibility of energy (DE) has been documented in a study with 90 samples from 31 different feedstuffs covering almost all feedstuffs used in the Danish pig production (Figure 4).

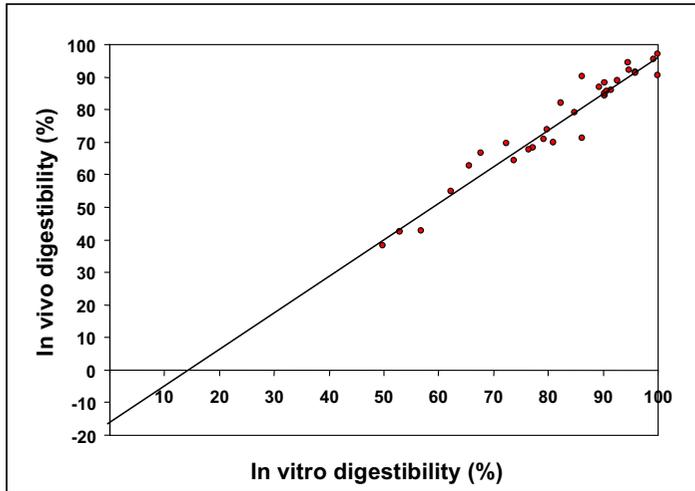


Figure 4. Relationship between the *in vivo* enzyme digestibility of organic matter and the *in vivo* total tract digestibility of energy in growing pigs determined in 90 samples for 31 different feedstuffs. Mean values for each feedstuff is given in the figure (Boisen & Fernandez, 1997).

From this study the relationship was described by the general equation:

$$\text{DE, \%} = -14.0 + 1.106 \times \text{EDOM, \%} \quad (R^2 = 0.94; \text{RSD} = 3.4; \text{CV} = 4.4)$$

The generally lower faecal digestibility *in vivo* compared to the *in vitro* digestibility corresponds to the endogenous losses of protein and lipids which are included in the measurements of apparent digestibility.

Similarly, the difference between values of apparent *ileal* digestibility of protein and *in vitro* values of the real digestibility of protein can be directly related to endogenous protein losses (EPL) and can be described by the linear equation:

$$\text{EPL, g kg}^{-1} \text{DM}_{\text{intake}} = 13.2 (+/- 3.1) + 0.066 (+/- 0.01) * \text{UDMi, g kg}^{-1} \text{DM},$$

where UDMi is undigested dry matter at *ileal* level (Figure 5). According to the figure the intercept of 13.2g per kg DM intake corresponds to a basal endogenous loss for digestion, whereas the linear slope corresponds to an extra endogenous loss, which is specific for the actual diet and related to the undigested dry matter (g per kg) in the feed.

Furthermore, the variation in digestibility of different samples of a feedstuff is analysed with good accuracy by the two *in vitro* methods simulating organic matter digestibility at *ileal* and faecal level, respectively. Thus, the difference of digestible organic matter obtained by these two methods is also a reliable estimate for the fraction of fermentable carbohydrates (Boisen, 2003a).

The two *in vitro* methods have been implemented by scientists in many other countries throughout the world (Boisen, 2002). Several studies have demonstrated that variations in the *in*

vivo digestibility within feedstuffs could be precisely described by the developed *in vitro* methods (e.g. Beames et al., 1996; Chen et al., 1996; Pujol et al., 2001; Swiech & Buraczewska, 2005).

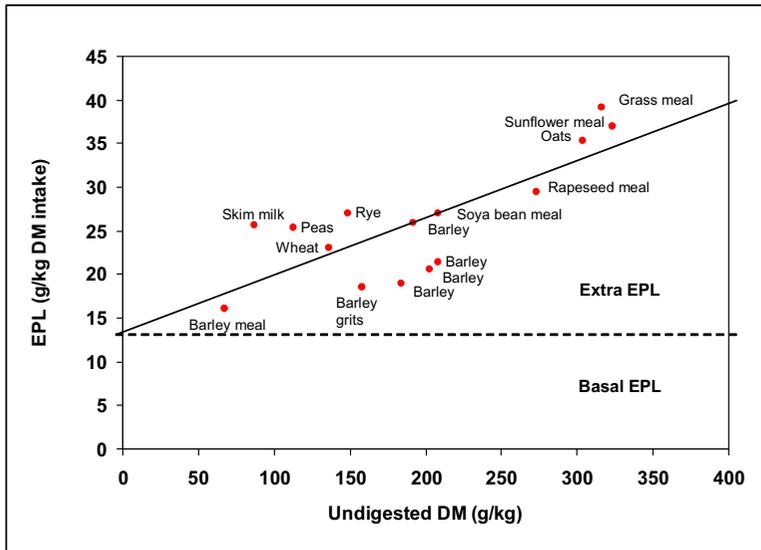


Figure 5. The relationship between calculated values of endogenous protein loss (EPL) and *in vitro* undigested DM, corresponding to enzyme indigestible dry matter at *ileal* level (EIDMi). Basal EPL corresponds to the intercept (at EIDMi = 0), whereas extra EPL is proportional to EIDMi (Boisen & Fernandez, 1995).

Thus, these studies have documented that the developed laboratory methods, with *in vitro* incubations of natural digestive enzymes, are able to analyse nutrient digestibility with similar results than direct determinations in the animals. Furthermore, *in vitro* analyses of digestibility are generally performed with a considerably lower variation than results obtained from animal studies.

Standardised digestible amino acids

The *in vivo* digestibility of protein and lipids is influenced by endogenous losses of protein and lipids, respectively, and corresponds to the apparent digestibility. The endogenous losses are correlated to dry matter intake and can be considered to consist of two fractions, *i.e.*:

- 1) a basal loss related to the amount of ingested feed, and which can be considered to be included in the maintenance requirements for the animal
- 2) an extra loss, which is specific for the feed and, therefore, should be debited on the feed itself. The extra losses are mainly caused by dietary fibre and can be related to undigested dry matter at *ileal* level. (However, in some feedstuffs ANF's may increase these losses considerably!).

Because results of *in vitro* digestibility are not influenced by endogenous losses they correspond to the real digestibility. Standardised digestibility of protein, as well as of lipids, is obtained when *in vivo* results are corrected for the basal endogenous loss.

Standardised digestibility of protein (and amino acids) can, alternatively, be calculated from the *in vitro* enzyme digestibility of protein (EDN) after correction for the extra protein loss (Figure 6) - or amino acid losses, which is calculated from *in vitro* enzyme undigested dry matter at ileal level (EUDMI). Protein digestion and digestibility was recently described in detail (Boisen, 2004).

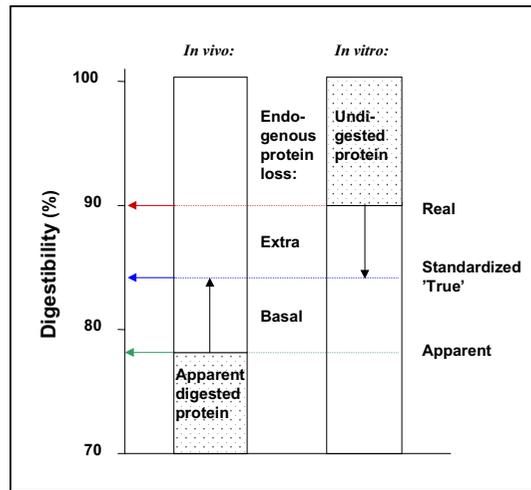


Figure 6. Calculation of standardised digestibility of protein (and amino acids) from *in vivo* and *in vitro* analyses, respectively (Boisen, 1998)

Calculation of standardised digestibility of crude protein and amino acids in feedstuffs, based on *in vitro* analyses are given in the Appendix. According to the calculation formula, the real digestibility of all amino acids is assumed to be identical with the real digestibility of crude protein. However, this may not always be correct. *E.g.* in cereals, endosperm proteins are highly digestible and relatively low in lysine. Thus, the real digestibility of lysine may be slightly lower than that of crude protein. Furthermore, due to the free amino group, lysine is more sensitive to chemical reactions (*e.g.* Maillard reaction) in improperly heat-treated feedstuffs. Consequently, the calculated values for standardised digestibility of lysine may be overestimated in such feedstuff batches.

All calculations are based on the official Danish table values for feedstuffs given in Table 1A, 2A and 3A, respectively, in the Appendix. The obtained results for some of the most common feedstuffs in Danish pig production are given in Table 2 and compared with standard values from published tables in the literature.

According to Table 2, most of the *in vitro* based digestibility data for barley, wheat, maize and rapeseed meal are in good agreement with those given in the published tables. However, calculated values for lysine are generally higher than those obtained from *in vivo* experiments. This indicates a generally lower real digestibility of lysine, than of the other amino acids *in vivo*.

Table 2. Standardised digestibility (%) of crude protein and essential and semi-essential amino acids in common feedstuffs used for pig diets. Results calculated from *in vitro* digestibility¹ compared with table values based on *in vivo* experiments with growing pigs

Feedstuff	CP	Lys	Thr	Met	Cys	Trp	Ile	Leu	Val	His	Phe	Tyr
Barley												
<i>in vitro</i>	79	81	76	84	82	79	82	84	82	83	85	83
Pedersen & Boisen (2002)	80	75	76	84	81	79	81	82	80	82	84	81
INRA (2002)	75	75	75	84	84	79	81	83	80	81	84	83
CVB (1999)	80	76	80	82	80	77	82	82	81	83	84	-
NRC (1998)	-	79	81	86	86	80	84	86	82	86	88	87
Wheat												
<i>in vitro</i>	87	86	83	88	83	87	88	89	88	89	89	89
Pedersen & Boisen (2002)	89	83	84	90	89	89	89	90	86	90	91	90
INRA (2002)	84	81	83	89	91	88	89	90	86	90	91	90
CVB (1999)	89	84	86	90	87	88	91	90	89	91	91	-
NRC (1998)	-	81	90	90	84	90	89	89	86	89	91	89
Maize												
<i>in vitro</i>	84	84	82	87	86	78	86	89	86	87	86	87
Pedersen & Boisen (2002)	86	77	81	89	86	77	86	90	84	86	89	89
INRA (2002)	88	80	83	91	89	80	88	93	87	89	91	90
CVB (1999)	83	76	80	87	81	76	86	89	86	86	88	86
NRC (1998)	-	78	82	90	86	84	87	92	87	87	90	89
Soybean meal												
<i>in vitro</i>	92	93	92	91	91	92	93	93	93	93	93	93
Pedersen & Boisen (2002)	87	88	85	91	85	88	88	87	88	91	90	90
INRA (2002)	87	90	92	86	87	89	90	89	88	91	91	92
CVB (1999)	87	89	90	86	86	87	88	88	87	90	89	-
NRC (1998)	-	89	91	84	85	87	88	88	86	90	88	90
Rapeseed meal												
<i>in vitro</i>	80	83	80	83	82	80	82	83	82	83	81	82
Pedersen & Boisen (2002)	76	77	76	87	81	75	78	81	77	83	81	79
INRA (2002)	75	75	75	87	81	80	78	82	77	84	83	80
CVB (1999)	76	80	78	84	70	81	80	87	80	82	82	78
NRC (1998)	-	78	76	86	83	75	78	81	77	85	82	79

¹ See Appendix for calculations and *in vitro* data

Furthermore, for soybean meal the *in vitro* based digestibility data are, generally, higher for all amino acids compared to the table values. However, the comparisons between *in vivo* and *in vitro* digestibility in Table 2 are not based on analysis of identical feed samples. The difference is, therefore, a consequence of the relatively high table value of 95 for EDN (see Appendix Table 1A). Thus, EDN analyses in samples of SBM obtained from the feed industry during the last ten years have varied from 91 to 96. The high values calculated from *in vitro* analyses of present samples in Table 2 may, therefore, also indicate that the quality of soybean meal have improved since the results from animal experiments were obtained.

In the new Danish feed evaluation system the table values for standardised digestibility of amino acids are based on the values given by Pedersen & Boisen (2002), except for cereals and

cereal by-products. For these feedstuffs, table values are corrected annually according to the actual analyses for chemical composition and *in vitro* digestibility analyses. The excellent agreement, generally obtained, between *in vivo* and *in vitro* digestibility values corresponding to *ileal* and faecal level, respectively, demonstrates the potential for reliable estimates of the digestibility of the different nutrients in actual feed samples.

Consequently, future needs for animal experiments for determining digestibility in feedstuffs and diets, can be reduced considerably. Furthermore, table values for digestibility should only be considered as a common guideline for the actual digestibility, whereas fast and reliable laboratory analyses should be performed for a direct measurement of digestibility in the actual feed samples.

Ideal protein

Dietary proteins are composed of 20 different amino acids of which nine are essential and two are semi-essential, *i.e.* they can be synthesised from essential amino acids, and the rest are non-essential, *i.e.* they can be synthesised from general metabolic compounds. Though, arginine should, theoretically, also be classified as a semi-essential amino acid, because the availability of *de novo* synthesised arginine from the urea cycle may be limited (Figure 7).

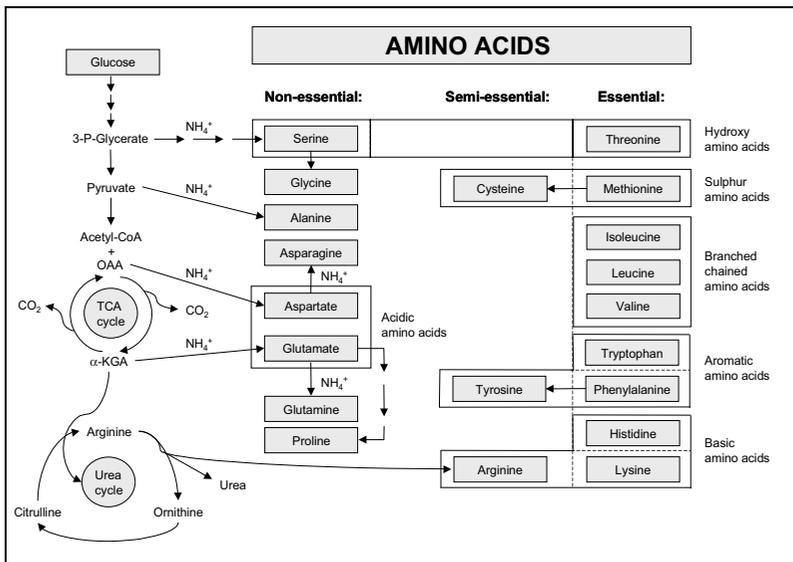


Figure 7. Essential amino acids and synthesis routes for semi-essential and non-essential amino acids (Boisen, 2003b).

The ideal protein for pigs corresponds to the amino acid composition of essential and semi-essential amino acids in the dietary protein. Though, generally only the essential amino acids need to be considered. According to their chemical property these amino acids can be grouped and further divided according to their abundance in primary and secondary limiting amino acids (Table 3).

Table 3. Essential amino acids according to their chemical properties and general order of limitation in common pig diets¹

Essential amino acid	Chemical property	Order of limitation
Lysine	Basic amino acid	Primary
Threonine	Hydroxy amino acid	
Methionine	Sulphur amino acids	
Methionine + Cystine		
Tryptophan	Indol amino acid	
Isoleucine	Branched chain amino acids	Secondary
Leucine		
Valine		
Histidine		
Phenylalanine	Aromatic amino acids	
Phenylalanine + Tyrosine		

¹Boisen (2003b)

Due to relatively low concentrations of lysine in some of the most important feedstuffs for pigs, e.g. wheat, maize and barley, lysine will, generally, be the first limiting amino acid in pig diets, whereas threonine, methionine, and tryptophan will, generally, be the next limiting amino acids.

Table 4. Amino acid composition (g per 160 g N) of sow's milk compared with the composition in whole body and deposited protein, endogenous protein loss, and hair, respectively

	Sow's milk ¹	Whole body ²	Deposited ³	Endogenous protein ⁴	Hair ³
<i>Essential and semi-essential amino acids:</i>					
Lysine	71	66	69	30	33
Threonine	39	39	38	45	59
Methionine	18	19	19	10	4
Cystine	13	11	10	16	134
Tryptophan	12	8	n.d.	12	n.d.
Isoleucine	41	35	40	25	35
Leucine	81	72	77	40	77
Valine	54	48	51	35	60
Histidine	25	29	32	15	11
Phenylalanine	39	39	37	30	23
Tyrosine	42	27	28	20	9

n.d. = not determined; ¹Mean of 32 samples (Boisen, 1997); ²Determined at 20 kg liveweight (Fuller, 1994); ³From 20 to 90 kg liveweight (Jørgensen et al., 1988); ⁴Mean of 36 determinations (Boisen & Moughan, 1996a).

The ideal amino acid pattern can be expected being reflected in sow's milk (Table 4), which is also closely related to the composition of the whole body. On the other hand, maintenance requirements, which mainly include endogenous protein losses and hair, also influence the ideal amino acid composition, in particular in slowly growing animals.

The requirements for essential amino acids are, in the literature, often related to the requirements of lysine and, thus, expressed relative to lysine. However, for characterising the protein quality, the requirements of all amino acids should, preferably, be related to the protein requirement. On the other hand, a precise definition for the ideal amino composition in pig diets is difficult to establish due to a large number of influencing factors on the actual experimental conditions and production results.

For suckling piglets, the ideal amino acid pattern can be expected being reflected in the composition of sow's milk, due to the general concept of evolution. In growing pigs, the amino acid requirements for deposition dominate the total amino acid requirements. Thus, the composition of deposited protein is comparable to that of sow's milk, except for the large neutral amino acids (LNAA), *i.e.* tryptophan, tyrosine and the branched chained amino acids. The relatively lower deposition of these amino acids can be explained by their use for other purposes, *e.g.* syntheses of hormones.

However, despite a continuous intensive research and updating of recommendations the ideal amino acid composition, according to national recommendations, still vary considerably (Table 5).

Table 5. Proposals for amino acid composition (g per 160 g N) of ideal protein for growing pigs

	A	B	C	D	E ¹	F ¹	G
<i>Primary limiting amino acids:</i>							
Lysine	70	65	59	81	70	70	70
Threonine	42	47	44	53	46	42	45
Methionine	18	-	16	25	-	-	18
Met + Cys	35	41	35	49	35	39	36
Tryptophan	10	12	11	15	13	13	12
<i>Secondary limiting amino acids:</i>							
Isoleucine	38	39	36	49	35	38	40
Leucine	70	72	65	81	70	71	80
Valine	49	49	44	55	49	48	52
Histidine	23	-	-	26	23	22	25
Phenylalanine	34	-	35	41	-	-	40
Phe + Tyr	67	78	72	77	70	65	80

A: ARC (1981); B: Wang & Fuller (1989); C: Fuller et al. (1989); D: Calculated from Chung & Baker (1992); E: Cole & Lunen (1994); F: NRC (1998); G: Boisen et al. (2000).

¹Literature values, where amino acids are given relatively to lysine = 100, recalculated on the assumption that lysine is 70g per 160 g N.

Protein quality of feedstuffs

The protein value of common feedstuffs and other protein sources has traditionally been related to the biological value (BV). However, this definition relates only to the first limiting amino acid and is, therefore, of limited value.

A more useful characterisation of the protein value of individual protein sources is obtained when all essential amino acids, contributing to the ideal protein for the specific animal category, is described (Boisen, 2003b). This can be obtained from the information given in the Appendix on

crude protein and amino acid composition in the feedstuffs (Table 3A), and those on standardised digestibility of crude protein and the individual amino acids (Table 7A), with the ideal amino acid pattern given in column G in Table 5.

i.e. for barley the lysine value will be: $3.6/7.0 * 81/79 * 100 = 52.7 = 53$;

whereas for threonine the value will be: $3.4/4.5 * 76/79 * 100 = 72.7 = 73$

In the Appendix (Table 8A), the protein quality, according to this definition and from these calculations, is given for the different feedstuffs. The values given in Table 8A demonstrate that in common Danish diets for growing pigs, based on cereals and soybean meal, supplementation of industrial amino acids will, generally, be sufficient after supplementation of the primary limiting amino acids given in Table 3.

Energy evaluation of the major components in feedstuffs and pig diets

Starch as energy reference for other nutrient fractions

Starch is considered as a pure energy source without any additional physiological effects. Furthermore, starch is generally the dominant energy source in pig diets. Starch is generally highly digestible (though in some cases only after proper heat treatment). Starch consists of macromolecules with glucose as the only carbohydrate monomer. The utilisation of glucose for ATP is precisely described. Thus, the potentially available energy of digested starch is precisely defined, and corresponds to 67% of the gross energy. Consequently, the energy value of starch is the obvious reference for the other nutrient fractions.

The energy value of other nutrient fractions is determined by their specific effect on the energy value in the diet when they substitute starch. However, this substitution effect has only a consequence for the fraction of digestible lipids in diets for growing pigs, because the dietary lipids save costs for alternative syntheses of deposited lipids from starch (via glucose and AcCoA). These costs are, therefore, credited the dietary lipid in order to maintain the same energy value in the diet when substituting dietary starch with lipids.

Ileal digestible carbohydrates

Starch, mono-saccharides (*e.g.* glucose), disaccharides (*e.g.* sucrose and lactose) and oligo-saccharides (raffinose, stachyose and verbascose) will all be measured as *ileal* digestible carbohydrates by the present analysis method for *ileal* digestible carbohydrates.

However, oligosaccharides may also be fermented because they cannot be degraded completely by the animal's own enzymes (only the linkage between glucose and fructose is susceptible for the animal enzyme, sucrase). Similarly, lactose may often be fermented because the pancreatic lactase activity is rapidly decreasing after weaning. Furthermore, starch may be partly resistant to pancreatic amylases and, therefore, partly fermented (dependent on origin and processing) and even not degraded totally at faecal level.

The energy value of ileal digestible carbohydrates is based on a mean value for typical diets for growing pigs (However, the practical analysis method for determining this fraction by routine in the actual feed samples is not yet available).

Ileal digestible lipids

The composition of crude fat is generally more heterogeneous than of most carbohydrates. On the other hand, more than 90% of crude fat is mainly composed of long-chained fatty acids with an utilisation of 67% of the gross energy (Table 1).

The digestibility of lipids cannot be determined in actual samples by simple *in vitro* digestibility methods. On the other hand, the digestibility of dietary lipids may be predicted from the fatty acid composition. Thus, fatty acids are, in principle, 100 % available if their composition in the diet is optimised according to the specific absorption process for lipids. However, the digestibility of lipids is influenced by the composition of saturated and unsaturated fatty acids, as well as the ratio of mono-acylglycerols. The lipids are emulsified with bile salts and lecithin in organised micelles, which diffuse through the unstirred water layer to the membrane of the brush border where they are absorbed. Consequently, the digestibility of lipids may be based on the contribution of fatty acids and glycerol in the crude fat fraction if the diet has been optimised with respect to the composition of crude fat.

The dietary supply of lipids is considered being directly transferred to the developing tissues and fat depots. Thus, digested lipids are supposed to avoid the general metabolism and are not actually used for energy generation. Because the dietary supply is generally lower than the deposited lipids in growing pigs, the energy value of dietary lipids should account for the saved costs for the alternative synthesis from glucose. Consequently, the energy value of dietary lipids relative to starch is the sum of the PPE of the *ileal* digestible lipids + the saved costs for the alternative synthesis of lipids. This supplemental energy value of lipids is also relevant for lactating sows.

The energy value of ileal digestible lipids is based on the contribution of fatty acids and glycerol in the dietary crude fat and the costs for their alternative synthesis from glucose.

Ileal digestible protein

Amino acids are, like fatty acids, primarily meant for building stones in tissues in the growing pig. The actual energy generation from digested protein is, therefore, mainly related to the surplus amino acids. Generally, the energy utilisation of protein is reduced because of the ammonia produced from nitrogen, and which need to be removed after energy requiring synthesis of urea.

Due to the varying amounts of nitrogen in amino acids and the different metabolic routes for the degradation of the twenty amino acids, generally contributing to proteins, the potential physiological energy value of protein is influenced by the amino acid composition of the digested dietary protein.

However, for a practical feed evaluation system, a mean value should be established. Because the composition of dietary protein ideally should reflect body protein this composition could also be a logical definition for a standard protein. Furthermore, this composition is close to that for the essential amino acids in the ideal protein, which reflect the amino acid requirements for growing pigs.

Because the nutritional value of the dietary protein for the growing pig is totally dominated by its supply of essential amino acids, the value of physiologically available energy from dietary protein is generally of little importance for the growing pig. Furthermore, the potential physiological energy value of the digested protein is integrated in the recommendations for amino acids and energy, respectively.

The potential physiological energy calculated from the composition of amino acids in body protein corresponds to about 49% of gross energy. However, amino acids account, generally, for only 85% of crude protein (N x 6,25), which also contains nucleic acids and other N-compounds. This may explain the lower literature value of 44% of gross energy in crude protein compared with that of body protein.

Ideally, the actual potential energy value of *ileal* digestible protein should be related to the digested surplus protein in relation to the actually required ideal protein. Furthermore, because the amino acid composition of this fraction may vary, the energy value of this fraction should be calculated for the specific optimisation of the actual diet. This would give the correct estimate for the energy value of this fraction relative to starch.

The energy value of ileal digestible protein is based on the general amino acid composition in deposited protein in growing pigs because this fraction contributes to a dominating portion of the ileal digested protein. An exact measure for energy value of this fraction is of minor importance because the dominating property is the contribution of amino acids. Moreover, surplus dietary protein, e.g. from imbalanced protein, should be reduced to a minimum in the feed optimisation.

Fermentable carbohydrates

Different plant cell structures, based on non-starch polysaccharides (NSP), which cannot be degraded by the enzymes in the small intestine, may be utilised after fermentation by micro-organisms, primarily located in the hindgut. The fermentation products, short-chained fatty acids (SCFA) can be utilised energetically by the host animal and, thus, represent an additional energy source. Obviously, this energy value can vary depending on the actual conditions and, furthermore, the degree of actual degradation may vary to some extent. However, this fraction may also have several additional physiological effects, *i.e.* increased metabolism and enlargements of the intestinal wall. Finally, the degree of utilisation is generally increased with age.

A general mean value corresponding to 60% of the energy value of starch has been used. The additionally physiological effects corresponding to increased metabolism and developments in the intestinal tissues was not considered in the present evaluation system.

Energy costs of other components in pig diets

Enzyme indigestible dry matter at ileal level (EIDMi)

EIDMi is energetically a negative property of the feed. Although this fraction includes the fermentable fibre fraction as a proportion of the total fibre fraction it is a general indication of the costs for the digestive processes in the small intestine. These specific costs include extra synthesis and secretion of enzymes and loss of epithelial cells together with extra re-absorption of the partly digested secretions. Furthermore, viscosity occurring from NSP's (*e.g.* arabino-xylans in wheat) may generally reduce the digestibility of nutrients, particularly in piglets.

The direct costs for extra syntheses of amino acids and fatty acids can be estimated to 1.4 MJ per kg EIDMi based on stoichiometric equations. In the new Danish system this is considered to account for 50% of the total extra costs. Thus, the correction for EIDMi is 2.8 MJ per kg EIDMi.

Surplus protein

Surplus protein increases the general metabolism and, thus, the energy costs for the actual performance. The available energy for production is, therefore, reduced.

Though, the negative effect of surplus protein on the energy value is not debited directly on the diet in the present practical feed evaluation. Due to the use of linear programming in feed optimisation the general energy value of protein is, alternatively, reduced according to an estimated mean effect in typical diets for growing pigs.

Anti-nutritional factors (ANF's)

Anti-nutritional factors (ANF's) represent a large number of different compounds, mainly from seeds and grains. ANF's have many different specific effects in the digestive tract or in other tissues after absorption from the intestine. Their presence in the feed may reduce the digestibility of the diet, increase the endogenous losses during digestion of the feed, and damage the gut wall, as well as internal organs, resulting in a general reduction of the performance of the animal.

Protease inhibitors, lectins and tannins are widely distributed in seeds, in particular from legumes and cereals. The inhibitory effect may vary significantly in different animal species. Thus, the trypsin inhibitor activity in different cereals and legumes were demonstrated to be different in assays using trypsin from different animal species, *e.g.* the inhibiting effect on the activity of porcine trypsin was, generally, considerably higher compared with the effect on the commonly used commercial preparation of bovine trypsin (Boisen, 1988). Protease inhibitors are proteins, whereas lectins are glycoproteins. Both groups of inhibitors are relatively compact molecules with many stabilising disulphur bridges and, therefore, often very stable towards heat treatments as well as degradation by digestive enzymes. However, efficient heat treatments can reduce most of their activity. Thus, proper heat treatment of soybean meal is essential for reducing the anti-nutritional effects of trypsin inhibitors and lectins.

Phytic acid is in most seeds a storage component for phosphorous, which is liberated after hydrolysis of phytase during germination (Boisen, 1987). However, phytic acid also complex with a variety of minerals as well as of dietary proteins and the digestive enzymes in the digestive tract and may, generally, reduce the digestibility of nutrients in the feed. On the other hand, endogenous phytase activity, or supplemented industrial phytase to the diet (Johansen, 2002), may be able to degrade the phytic acid in wet feeding systems, as well as in the digestive tract and, thus, improve the utilisation of phytic acid phosphorus, protein and other dietary nutrients.

Glucosinolates are the most important ANF's in rapeseed and are specific for seeds from the *Crucifera* family. These compounds do not interfere directly with the digestion processes but have a negative effect on the palatability and, thus, on the feed intake. Furthermore, they may cause serious lesions in the liver and kidney. However, during breeding glucosinolates in rapeseeds have been reduced considerably.

Faba beans and lupin are, together with peas, commonly used, in particular in organic farming, as alternative protein sources for imported soybean meal. However, these protein sources may also cause problems due to their relatively high contents of ANF's. Thus, vicine and convicine are glycosides that are primarily found in faba beans. These compounds are hydrolysed by the intestinal microflora to different degradation products, which may result in reduced reproductive performance in pigs. Alkaloids are compounds with a hetero-cyclic ring containing nitrogen and are generally basic and with a bitter taste. These compounds are, in particular, found in high levels in lupins.

Recently, a comprehensive review of the significance of ANF's in feedstuffs for monogastric animals was given by de Lange *et al.* (2000).

In general, heat treatments, *e.g.* expanding or extrusion, improve the digestibility of pig diets due to a general reducing of ANF's as well as a destruction of the starch matrix (by gelatinising).

The enzymatic determination of UDMI (undigestible dry matter at *ileal* level) may be considered as an unspecific indicator for ANF's in the feed. On the other hand, the surplus of enzyme activity in the *in vitro* assays are generally sufficient to overcome the effects from these compounds and, furthermore, these compounds are generally low in Danish feedstuffs although, for some feedstuffs, only after proper heat treatments.

At present, no general control of ANF's, as well as of toxins from possible contaminated fungi, is performed by routine in the actual batches of feedstuffs and diets. Consequently, reduced production results, compared with the expected results from general feed analyses and feed optimisation, may also indicate contaminations of these compounds.

A better control of the actual feed batches for specific properties of the ANF's should be performed, and more specific knowledge about the practical consequences is needed.

The practical performance of the new Danish feed evaluation system

Basal chemical analyses, factors and equations

The basic chemical analyses, factors and calculations of crude protein, crude fat and organic matter, respectively, are given in Table 6. Values of standardised digestibility of protein can be calculated from *in vitro* digestibility after correction for specific endogenous protein loss (see Figure 6).

Feed optimisation is based on the contributions of SDAA and digestible PPE corrected for the specific extra energy costs of EIDMi from the single feedstuffs. Thus, the feed specific costs of protein and energy for digestion of the feed are covered by the feed itself, whereas the maintenance requirements for protein and energy are integrated in the requirements of the pig.

Table 6. Basal chemical analyses, factors and equations for characterising nutrient digestibility of feed samples

	Protein	Lipids	Organic matter
Analyses	N	Crude fat (FA) ¹	Ash
Calculations	N x 6.25	FA x 1,04	DM - ash
Real digestibility	EDN (<i>in vitro</i>)	90	EDOM (<i>in vitro</i>)
Specific endogenous loss ²	0.066 x EIDMi	0.025 x EIDMi	0.091 x EIDMi
Basal endogenous loss ²	13.2	9.0	22.2

¹Fatty acids (FA) in feedstuffs can be calculated from crude fat - see Table 4A in the Appendix; ²g per kg DM intake

In many feedstuffs, the standardised digestibility of protein and amino acids are relatively constant. Therefore, it is not, generally, necessary to analyse the actual batches for *in vitro* digestibility of protein. However, in cereals the standardised digestibility of protein and amino acids varies, mainly due to the relatively high variations in crude protein contents (*e.g.* from 9 to 16% of dry matter). On the other hand, the variations in digestibility, which are mainly caused by changes in the composition of the highly digestible endosperm proteins relative to the less digestible proteins in the aleurone layer and embryo tissues, are predictable from the protein level in the sample. Table values on standardised digestibility can, therefore, be corrected directly according to the protein level in the actual batches of cereals (see Appendix).

Calculation of energy value (PPE)

The energy value of feedstuffs and diets, *i.e.* the potential physiological energy (PPE) from the different nutrient fractions, were given in Table 1.

The real digestibility of crude protein (RDCP) corresponds to the *in vitro* digestibility (EDN - Table 7) and is typically very high, about 91%, in most feedstuffs. Commonly, values of EDN are based on table values. However, for feedstuffs with a more variable protein digestibility, they may also be based on direct analyses of *in vitro* digestibility.

The real digestibility of crude fat (RDCF) is also generally very high but may be influenced by the fatty acid composition in the final diet. Thus, in feed optimisation a suitable fatty acid composition should be considered which allow for the assumption of a general value of 90%. The energy value take into account the spared energy for the alternative lipid synthesis from glucose.

The nutrient fraction, enzyme digestible carbohydrates (EDC) is calculated as the residue from *ileal* digestible organic matter corrected for the sum of real digestible crude protein and real

digestible crude fat, respectively (Table 7). It follows, that this fraction corresponds to the sum of starch + sugars + a possible supplementing organic residue.

Table 7. Calculation of energy value for slaughter pig diets in the new Danish feed evaluation system

Nutrient fraction	Calculation of fractions (g/kg)	Energy factor (kJ/g)
RDCP	CP x EDN ¹ /100	9.9
RDCF	CF x 0,9/100	31.7
EDC	OM ² x EDOMi/100 - (RDCP + RDCF)	11.7
FERMC ³	OM x (EDOM - EDOMi)/100	7.0 ⁴
EIDMi ⁵	OM x (100 - EDOMi)/100 + 0,3 x ash	- 2.8 ⁶

¹Enzyme digestibility of N; ² Organic matter, *i.e.* DM - ash; ³Fermentable carbohydrates; ⁴Energy value of absorbed SCFA from fermented organic matter (mainly carbohydrates); ⁵Enzyme indigestible dry matter at *ileal* level; ⁶Estimated energy costs for extra losses of protein and lipids throughout the digestive tract. For other abbreviations – see text.

In many common feedstuffs, in particular cereals, the EDC fraction is mainly composed by starch, whereas in other common feedstuffs, *e.g.* protein-rich extracted oilseeds, the EDC fraction is mainly composed by different sugars. Because the energy concentration, as well as utilisation, is different for these sub-fractions, the energy factors are also different. Consequently, the different feedstuffs have different energy coefficients for the EDC fraction. However, for typical diets for slaughter pigs, in which starch is the dominating sub-fraction, the factor 11.7 is used.

The fraction of fermentable carbohydrates (FERMC) is calculated from the difference between the two *in vitro* analyses of EDOM and EDOMi, respectively.

Finally, the feed specific extra energy costs for digestion are debited the energy value of the feed. These costs are based on analyses for enzyme indigestible dry matter at *ileal* level (EIDMi), calculated from the formula given in Table 7.

One feed unit for pigs (1 FUp) in the new Danish system corresponds to 1 FUp in a standard diet for slaughter pigs in the former Danish system. For obtaining this, 1 FUp corresponds to 7.38 MJ PPE, whereas 1 FUp corresponded to 7.88 MJ NE in the former system. Thus, according to Table 7, 1 FUp is calculated from the equation:

$$FUp = (9.9 \times RDCP + 31.7 \times RDCF + 11.7 \times EDC + 7.0 \times FERMC - 2.8 \times EIDMi) / 7.38$$

Standard values for chemical composition, values of *in vitro* digestibility, and energy coefficients of common feedstuffs are all given in the Appendix (Table 1A), whereas standard values for nutrient fractions and energy value of the same feedstuffs are given in Table 2A.

Generally, sows have a more efficient fermentation of undigested carbohydrates (and of organic matter) according to the general improvement of the capacity in the hindgut (Fernandez, 1986). This was taking into account by a higher energy value of fermentable carbohydrates, *i.e.* 9,0 kJ per g. Furthermore, the satisfying effect of soluble dietary fibre (corresponding closely to fermentable carbohydrates) reduces the maintenance energy requirements due to a reduced activity of the sows.

Standardised digestible amino acids (SDAA) in feedstuffs and diets

As discussed above, the real digestibility of protein within feedstuffs vary generally only little between different batches of most feedstuffs. Furthermore, analyses of *in vitro* digestibility of N are slightly more difficult to reproduce in commercial laboratories. Therefore, table values for SDAA of feedstuffs (Appendix, Table 6A) are presently used for production of diets.

The table values were established from literature data on apparent *ileal* digestibility of crude protein and amino acids as well as of measurements of basal endogenous protein and amino acid losses. Table values for standardised digestibility of crude protein and amino acids were calculated from statistical analyses of all available information on methods for their determination, including all influencing factors, *e.g.* type of cannula, composition of diets etc. (Pedersen & Boisen, 2002).

On the other hand, as already discussed in the basic principles, the analysis method for *in vitro* digestibility of crude protein (EDN) has the potential for a reliable determination of the variation in amino acid digestibility within actual samples of feedstuffs and diets. Calculation formula for standardised digestibility of crude protein (SDCP) and amino acids (SDAA), respectively, have been published (Boisen, 1998) and are specified in the Appendix. Thus, from the tabulated values of basic chemical analyses given in Table 1A, 2A and 3A, respectively, the corresponding values for SDCP and SDAA, based on *in vitro* analyses, can be calculated as given in Table 7A.

Amino acid recommendations

During growth the general amino acid requirements, relative to the energy requirement, are continuously decreasing (Boisen, 1993; NRC, 1998). These changes in requirements during the growing period are often not considered in experimental research as well as in the opportunity for reducing surplus nutrients (N and P) in the practical production. However, today is it possible to follow these changes closely by the use of two feeding tubes with complete diets corresponding to the nutrient requirements at the beginning and finishing of the period, respectively, and continuous mixing of these two diets throughout the growing period. By this procedure, undersupply in the first period and an increasing oversupply of nutrients in the finishing phase can be avoided. This feeding technique will, generally, minimise excretion of surplus nutrients, *e.g.* N and P as well as microminerals, *e.g.* Cu and Zn.

It is well known that castrates have a slightly lower protein deposition capacity during the finishing phase of the slaughter pig period as indicated in Figure 8. However, in modern lean genotypes this difference has become small and may be of little practical consequence. On the other hand, feed restriction or, alternatively, energy dilution in the diets for finishing castrates can result in a higher meat percentage at slaughter.

Figure 8 illustrates the reduced requirements for lysine and threonine throughout the growth period for slaughter pigs. However, in practical feeding in Denmark it is still relatively common to use only one diet, prepared according to the recommendations, throughout the whole period from 30 to 100 kg live weight. On the other hand, the Danish Advisory Centre also gives a number of recommendations for shorter periods. In Table 8 the specific recommendations for the initial period (from 20 to 45 kg live weight) and the finishing period (from 65 to 110 kg live weight) are given.

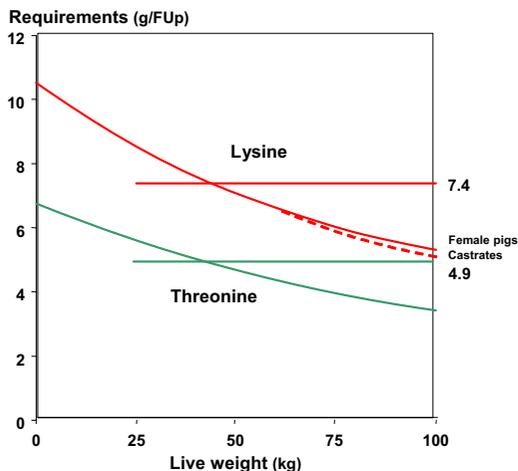


Figure 8. Estimated requirements for lysine and threonine for growing/finishing pigs compared with the official recommendations for the same diet throughout the growing/finishing period from 25 to 100 kg live weight. A slightly reduced lysine requirement for castrates, due to a higher fat deposition, is indicated as discussed in the text.

According to Table 8, essential amino acid recommendations, relative to lysine, are, generally, slightly increasing during growth. This is mainly caused by the increasing contribution of requirements for maintenance protein, which is low in lysine compared with deposited protein. The table also demonstrates small variations in some of the experimentally determined requirements for essential amino acids.

Table 8. Recommendations for amino acids (relative to lysine) for pigs at different periods of growth (kg live weight) compared with sow's milk and proposals for ideal protein¹

	Milk	20-45	30-100	65-110	A	B	C	D
<i>Primary limiting amino acids:</i>								
Lysine	100	100	100	100	100	100	100	100
Threonine	55	63	66	69	75	65	64	60
Methionine	25	30	30	30	27	31	26	27
Met + Cys	44	56	58	59	59	60	52	55
Tryptophan	17	18	19	19	19	18	17	18
<i>Secondary limiting amino acids:</i>								
Isoleucine	58	57	58	59	61	60	57	54
Leucine	114	103	109	111	110	100	114	102
Valine	76	73	73	73	75	68	74	68
Histidine	35	34	36	38	32	32	35	32
Phenylalanine	55	59	61	61	59	51	57	60
Phe + Tyr	114	115	116	119	122	95	114	93

¹From Boisen (2003). A: Fuller et al. (1989); B: Chung & Baker (1992); C: Boisen (1997); D: NRC (1998).

On the other hand, it is remarkable that when comparing the proposals for ideal amino acid composition, relative to lysine, only threonine and sulphur amino acids appear to be significantly different from the composition in sow's milk.

Feed optimisation based on SDAA and PPE

As illustrated in Fig. 1 the dietary supply of amino acids has a dominating influence on pig growth and, consequently, on feed utilization. These effects are mainly caused by associated water retention, and to a much smaller degree, by the associated skeletal development. Thus, the deposition of 1 kg of protein results in about 4.4 kg body weight gain. On the other hand, surplus dietary protein has to be avoided due to negative environmental effects. Therefore, the dietary protein supply should be minimised without negative effects on growth.

It follows, that the optimal composition of the feed is basically related to a correct proportion between physiologically available amino acids and physiologically available energy, *i.e.* SDAA and PPE, respectively.

The basic principles for feed optimisation in the new system are illustrated in Fig. 9. In the example, the complete diet is optimised on the basis of the contribution of standardised digestible nutrients from three different feedstuffs (A, B and C), which should fulfil the requirements for individual SDAA relative to PPE for the actual production.

The contributions from the individual feedstuffs are corrected for the specific endogenous losses caused by the particular feedstuff when using values of standardised digestibility, whereas the basal endogenous losses, which are minimal costs for digestion, are integrated in the pig's maintenance requirements.

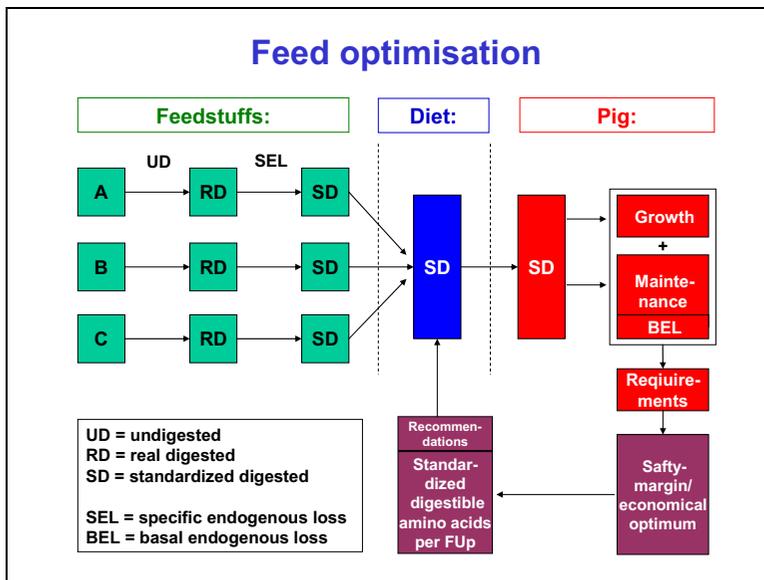


Figure 9. Feed optimisation based on standardised digestible nutrients in the feedstuffs and recommendations, which in turn is based on requirements for growth and maintenance, which also include basal endogenous losses of protein and lipids (Boisen, 2003a).

The estimated requirements for growth and maintenance for amino acids are supplemented with a safety margin in the recommendations. For the actual diet optimisation economical aspects, with respect to actual costs for a range of relevant feedstuffs and their effect on production results, are also included.

In the new system one feed unit for pigs (FUp) has, for practical reason, been adjusted to equalise the former FUp for a standard diet for slaughter pigs. Thus, the recommendations for digestible amino acids, relative to energy, have been adjusted according to the general changes in energy and protein evaluation, *i.e.* from NE and apparent faecal N digestibility, used for digestibility of all amino acids, in the individual feedstuffs, in the former Danish system, to PPE and SDAA in the new system.

The new feed evaluation system, together with the new *in vitro* digestibility analysis methods for predicting nutrient digestibility (at *ileal* and faecal level, respectively) in actual feedstuff samples, has resulted in nutritionally more uniform diets, which more precisely correspond to the requirements of the pig in the actual situation. Thus, the new system will, generally, reduce surplus N by more precise amino acid composition in the diets and also reduce costs for supplementation of industrial amino acids, due to the opportunity for reducing safety margins for recommendations for essential amino acids. Furthermore, a generally improved production economy can be expected.

The new Danish feed evaluation system for pigs has been voluntary for a period of one and a half year. During this period, the practical experience with the new system, including all available and relevant data were collected at The Agricultural Advisory Centre in Denmark and analysed statistically. The new system became the official Danish feed evaluation system, April 2004.

In Denmark, the national authority, placed at the Danish Plant Directorate, controls all commercially produced diets regularly. The actual feed batches are controlled for their contents of nutrient fractions as well as their energy value according to the official feed evaluation system. This control also includes the results obtained from the two *in vitro* digestibility analysis methods and which may have a major impact on the energy value in the actual feed batches.

The new Danish feed evaluation system compared with other systems

As briefly described in the introduction, the classical feed evaluation systems have been developed from systems based on digestible energy and metabolisable energy, respectively, to several different systems based on net energy (NE). Thus, the general development for definition of the energy value has moved towards more and more dependency of the animal factors, as well as other influencing factors related to the actual experimental conditions.

Nevertheless, the appropriateness of using NE as a suitable basis for husbandry animal feed evaluation is still debated (*e.g.* de Lange & Birkett, 2005). During the last two decades developments within pig feed evaluation have, in particular, been influenced by the work of Noblet and co-workers in France. These developments have focussed on experimentally determinations of NE for feedstuffs and diets.

As discussed above, the main purpose for feed evaluation is to produce optimised diets from available batches of feedstuffs and for a specific purpose in the actual animal production. In this process, contributions of digestible amino acids and energy, respectively, from the actual batches of different available feedstuffs should be additive in the final optimised diet. Furthermore, recommendations for digestible amino acids, relative to the energy value of the feed for the actual feeding purpose, play an important and principal role. Thus, the specific recommendations are strongly influenced by the feed evaluation system, which is used for the practical feed optimisation.

Consequently, the most important factor for feed evaluation is the relative energy contributions from the different nutrient fractions. Furthermore, because starch can be considered as a pure energy source and, moreover, is the dominant contributor of energy in pig diets, this nutrient is the obvious energy reference for the other nutrient fractions. The practical importance of different feed evaluation systems should, therefore, be evaluated on the basis of differences in the relative contributions of energy from the individual nutrient fractions. Some examples are given in Table 9.

According to Table 9 the former Danish NE system was quite different from the Dutch and French versions. This was mainly caused by the assumption that NE could be calculated directly from experimentally determined values of ME by the general equation given in the footnote.

Table 9. Energy value (in MJ/kg and relative to starch, respectively) of digested main nutrient fractions in different feed evaluation systems for growing pigs based on net energy (NE), ATP energy from potentially retained nutrients (NER), and potentially available physiological energy (PPE).

	NE			NER	PPE	
	Former Danish (1982) ¹	Dutch (1993) ²	French (2000) ³	Rostock (2003) ⁴	General (2000) ⁵	New Danish (2006) ⁶
Starch	10.7 (100)	13.5 (100)	14.4 (100)	12.7 (100)	11.7 (100)	11.7 (100)
FMC	10.7 (100)	9.5 (70)	12.1 (84)	- ⁷	7.0 (60)	7.0 (60)
Crude protein	13.2 (123)	10.8 (80)	11.3 (78)	11.0 (87)	10.4 (92)	9.9 (85)
Crude fat	23.4 (219)	36.1 (267)	35.0 (243)	27.0 (213)	26.1 (223)	31.7 (271)
EIDMi	-	-	-		-	- 2.8 (- 24)

¹Just (1982): NE, MJ/kg DM = 0,75 x ME, MJ/kg DM - 1,88 MJ/kg DM; ²Blok (1993); ³Noblet & van Milgen (2000); ⁴Jentsch et al. (2003); ⁵Boisen & Versteegen (2000). ⁶Tybirk et al. (2006); ⁷Corresponding fraction calculated from the equation: 12 - 0,14 * (80 - dE); dE: faecal digestibility of energy.

The practical result of this calculation was a system, which was more comparable to systems based on ME, as the present German system. Thus, with respect to the relative energy values for the

different nutrient fractions, the former Danish system can be considered more as a ME system than a NE system (Boisen & Verstegen, 2000).

Generally, systems based on digestible energy (DE) and ME are fundamentally different from systems based on NE (Boisen & Verstegen, 1998), mainly due to the differences between the relative energy values of the different nutrient fractions. The main argument for using a system based on NE rather than DE and ME is also that the latter systems systematically overestimate the energy content of protein and fibre rich feeds and underestimate the energy value of starch- and fat-rich feeds. Therefore, a new proposal for an improved ME system, in which these overestimations are corrected, has recently been developed (Susenbeth, 2005).

Interestingly, the present Dutch and French NE systems are comparable to the new Danish feed evaluation system based on potential physiological energy (PPE) when considering the relative energy values for the different nutrient fractions (Table 9). However, due to the different principles the absolute energy values are generally different (Boisen & Verstegen, 2000; Noblet & van Milgen, 2004). Generally, the energy values in NE systems will be higher than energy values in the PPE system because systems based on NE consider the deposited energy as gross energy and, consequently, operate with two different energy forms.

In the new proposal for feed evaluation from Rostock (Jentsch et al., 2003), the relative energy values based on the ATP energy from the potentially retained nutrient fractions (Chudy, 2000) are also close to the relative values for potential physiological energy (PPE) in the new Danish system. The main difference between the two systems is the higher energy value of fat and the negative value for indigestible dry matter in the Danish system. Furthermore, the Rostock proposal for energy evaluation is based on NE values obtained from specific experimental conditions used in animal studies.

However, as already discussed, the combination of two different energy forms into one property of the feed appears not to be suitable for a general energy evaluation system. Generally, the NE of the feed may be highly influenced by the actual use of the feed, as well as the actual feed intake and production potential of the animal. Furthermore, estimates of NE for single feedstuffs are artificial values and, finally, all diets, limiting in one single nutrient for the actual purpose, will overestimate the actual NE!

In conclusion, NE of feeds obtained in the actual production under a variety of production conditions can be highly variable and may, furthermore, be very different from the experimentally determined NE. Consequently, the principle of NE is unsuitable for general feed evaluation.

On the contrary, PPE is directly based on the properties of the feed itself and is, therefore, independent of its actual use. Thus, PPE is a precise definition for feed value and can be used directly for any purpose in a feed evaluation system. The optimal utilisation of the prepared diet is basically dependant on the actual use of the feed for the specific production, which can influence the utilisation of the feed considerably. Therefore, precise and detailed recommendations for the different productions are needed for an optimal utilisation of the actual feed.

Because the relative energy values of the main nutrient fractions are comparable in energy evaluation systems based on NE and PPE, respectively, the general practical consequences may be of less importance. Thus, a number of other factors in the specific production may influence the actual utilisation of the feed, as well as the general performance of the pig, considerably.

In fact, the present practical performance of feed evaluation is still rather premature. However, the potential for improvements is considerable. Obviously, the efficiency in the developments of most of these improvements may depend on the use of a correct feed evaluation system.

A feed evaluation system based directly on the feed itself focus on the variation in the properties of the feed, *i.e.* detailed chemical composition and potential digestibility of the different nutrients.

Furthermore, the contribution and potential effects of a number of different anti-nutritional factors should be included in the characterisation and evaluation.

The new Danish feed evaluation system is unique in using *in vitro* digestibility analyses for describing the potential digestibility of the different feedstuffs and for controlling the actual feed batches - by feed producers as well as by the official feed control. The two *in vitro* analysis methods for measuring the potential digestibility of organic matter at *ileal* and faecal level, respectively, have proved to be robust and give reliable measures for the variability of the potential digestibility in the different feedstuffs as well as in complete diets. Thus, the feed industry can analyse the actual batches of feedstuffs, as well as produced pre-mixes and complete diets, for controlling the actual production. Furthermore, the official authority can control the produced commercial diets.

The new Danish system for energy evaluation includes, also, a negative component, *i.e.* the extra costs for digestion of the feed. This cost is based on the enzymatic determined *ileal* indigestible fraction of dry matter, and is estimated to be twice the direct costs for the extra losses of protein and lipids during digestion.

Although the relative energy values of the main nutrients are, generally, comparable between the two different principles, NE and PPE, future developments within general feed science, as well as international scientific cooperation and feed trade, will benefit considerable by the agreement on a common international system, which is based directly on present scientific knowledge.

Obviously, a general international system needs to be based on the present scientific knowledge on nutrient metabolism and utilisation in the target animals. Furthermore, it is necessary to realise that the feed value can only be related to the potentials for the feed itself. These potentials can be analysed directly by the feed industry and controlled by the national authoritative. Recommendations for the specific production in different countries can then be related directly to the specific universal (international) properties of the feed.

Further developments and improvements in feed evaluation and pig production

The basis for the potential feed value is the chemical composition of the actual feed samples. At present this is described by simple routine analyses of the main components. However, most common feedstuffs have a much more complex and variable composition, which may influence the properties of the actual diet. In particular, dietary fibre and anti-nutritional factors (ANF's) can have considerable effects. Therefore, improved knowledge to these compounds, and how their negative effects can be reduced during processing, is needed.

Furthermore, fast and reliable analysis methods for a general control in the actual feed batches should be developed and implemented. Thus, the general tendency to change from laborious chemical methods to fast and reliable physical methods, for screening the nutritional value of actual feed batches, will most probably be an efficient tool for controlling the feed quality in on line feed production of optimised diets.

Feed evaluation and optimisation of diets in practise

Feed evaluation should be performed in three steps according to:

A. Potential feed value based on the information of single feedstuffs:

1. Detailed nutrient composition (including amino acids, fatty acids, sugars, starch and dietary fibre) and *in vitro* digestibility of organic matter corresponding to *ileal* and faecal level, respectively
2. Effects of processing (*e.g.* milling and storing, respectively, on negative properties (*e.g.* ANF's and mycotoxins)
3. Basal properties *i.e.* potential physiological energy value (PPE) and standardised digestible amino acids (SDAA)

B. Potential production value based on the information of complete diets:

1. Precise information about diets (as given in A)
 1. Effects of processing (*e.g.* heating, pelleting, enzyme treatments) and storing
 2. Basal properties (PPE, standardised digestible ideal protein (SDIP), and surplus N and P

C. Actual production value of complete diets:

1. All available information according to B
2. Composition of diet relative to the actual requirements of the animal (category, weight, and health status)
3. Production conditions (temperature, stocking density, feeding strategy...)

All evaluation data according to step A and B are related directly to the feed itself and are based on adequate analyses of the actual feed sample and, consequently, not influenced by actual production data. The data according to step B are related to the actual production conditions, which may have considerable influence on the actual feed value. These effects may be calculated from simple specific equations but should preferably be based on computer simulations, which will be able to include an increasing number of influencing factors. Furthermore, developments of proper computer models offer the optimal opportunity for systematic collection and utilisation of all relevant data from specific experiments and practical results.

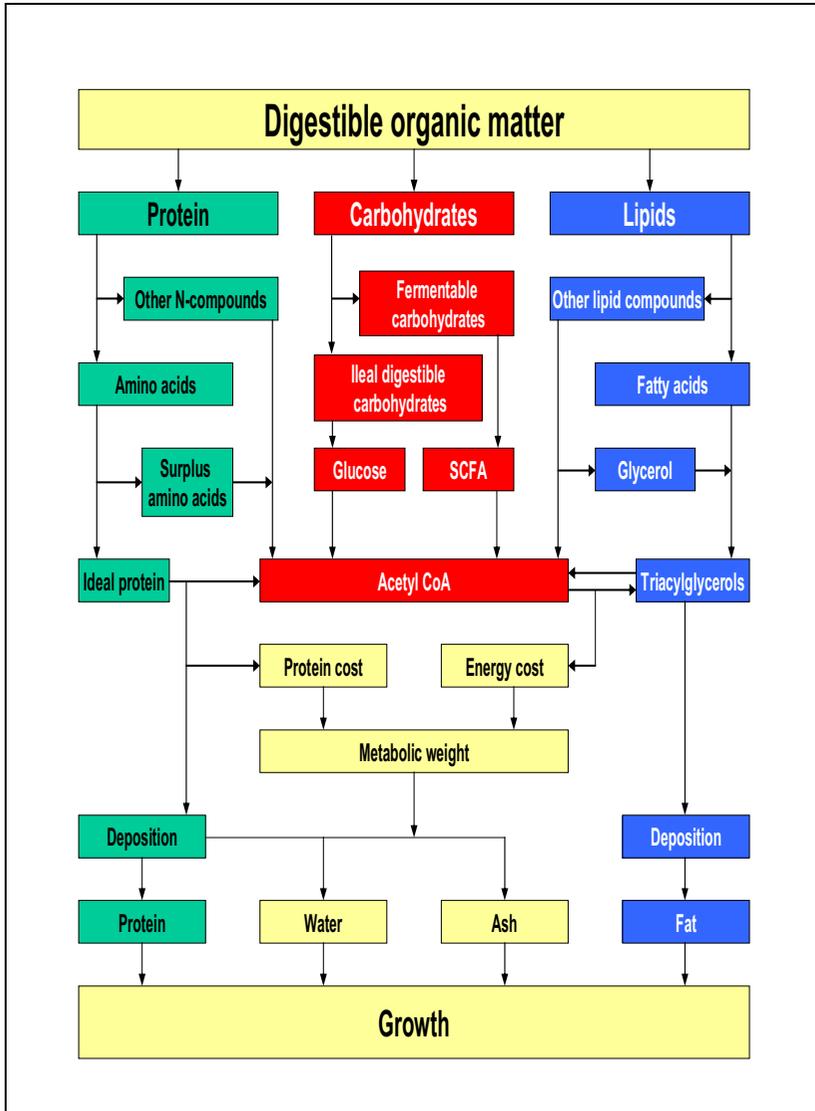


Figure 10. Flow diagram of an integrated model for feed characterisation and the transformation of feed components into physiological energy and body components. SCFA: Short chained fatty acids; CoA: coenzyme A (Boisen, 2000b).

A simple description of the relation between the properties of the feed, *i.e.* digestible main nutrient fractions and the growth of the pig is illustrated in Figure 10. The optimal performance of the pig for the specific production requires an optimal composition of the diet, basically relating to standardised digestible amino acids and potential physiological energy.

Feeding techniques and feeding strategy

Optimal pig production with minimal surpluses of nutrients, *e.g.* N and P, demands precise supplies of all essential nutrients throughout the production.

Modern technology allows for advanced feeding techniques for optimising the actual production of husbandry animals. Suitable feeding robots can, automatically, perform the gradual changes in the optimal nutrient composition for slaughter pig production, as indicated in Figure 8. Thus, a feeding robot, placed in specialised feeding boxes and connected to a weighing machine, identifies the individual pigs from a microchip placed in the ear, and determines the actual weight of the pig. Based on these data the optimal proportion of two diets supplied from different feeding tubes is calculated, automatically, and delivered together with a calculated amount of water to give a suitable resulting slurry. The two diets are optimised according to the initial and final weight, respectively, of the pigs according to the intended feeding period, *e.g.* from 30 to 100 kg live weight.

From current registrations of all relevant data including feed composition, dietary intake and live weight gain, respectively, the optimal diet can be currently adjusted. Thus, according to the identity of the pig, expected growth potential, eating behaviour, and other relevant information, respectively, the optimal feed composition, meal size, and feeding conditions for the individual pig throughout the feeding period can be defined and performed. It follows, that this new technique has the potential for several general improvements in pig production, *i.e.* a more efficient production in practise; systematic verification and documentation of specific effects of different feed components and their combinations and, furthermore, optimal feeding strategy according to production efficiency as well as animal health and welfare. Moreover, multi-phase feeding systems will, generally, have the potential for securing minimal surpluses of nutrients, *e.g.* N and P.

A detailed description of the N-flow in the pig is given in Figure 11.

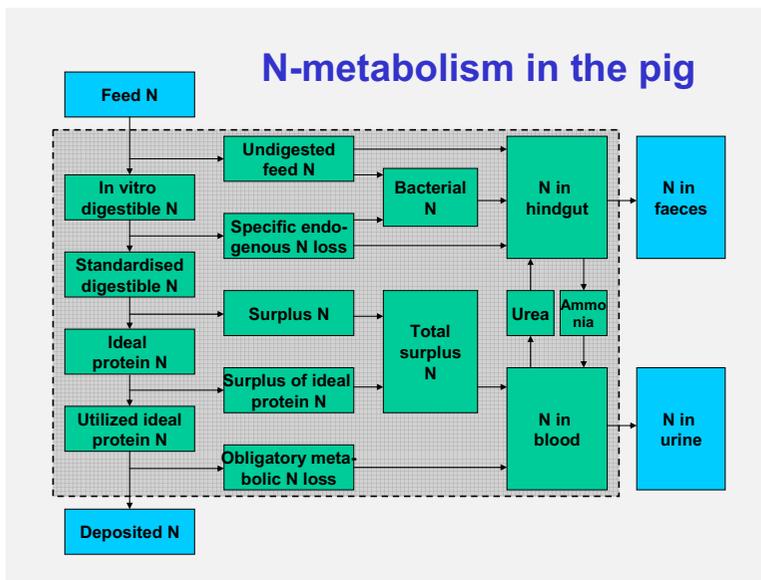


Figure 11. Flow diagram for the main routes in nitrogen metabolism in the pig (Boisen, 2000b).

The importance of protein deposition for the weight gain of growing pigs was illustrated in Figure 1. Thus, the figure indicated that the deposition of 1 kg protein results in 4.4 kg live weight gain, mainly caused by the associated water retention. Figure 12 is based on the results from a previous experiment with growing pigs and illustrates the relationship between average daily feed intake and daily gain in pigs from 20 to 50 kg live weight. The figure also illustrates the potential for a highly efficient feed utilisation under optimal production conditions.

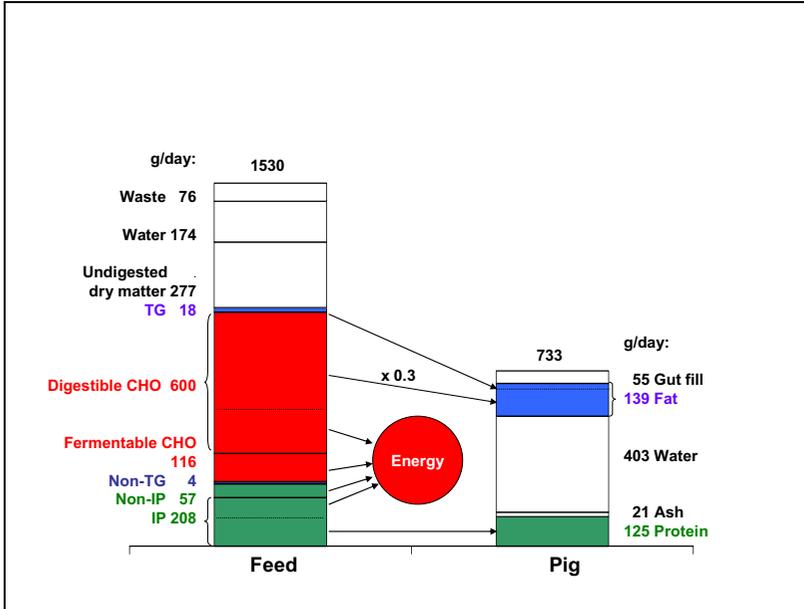


Figure 12. Relationships between average daily feed intake and daily gain in pigs from 20 to 50 kg live weight. IP: ideal protein; TG: tri-glycerides; CHO: carbohydrates (Boisen, 2000b)

General principles for a step-wise feed evaluation based on the new Danish system

Feed evaluation is a step-wise process according to laboratory analyses of nutrients and their digestibility, and followed by measurements of the animal responses (Figure 13).

The first step is the outcome of basic chemical analyses, which include crude protein ($N \times 6.25$), crude fat (lipids) and ash, whereas crude carbohydrates are calculated as a difference between dry matter and the sum of other analyses (ash + crude protein + crude fat). From these analyses of single feedstuffs the actual contributions of amino acids, starch and sugars, and fatty acids, respectively, in the diets can be calculated.

In the second step, organic matter digestibility at *ileal* and faecal level, respectively, is predicted from *in vitro* digestibility analyses of feedstuffs and complete diets. From these analyses, fermentable carbohydrates are calculated, whereas digestible protein and digestible amino acids are based on table values for protein and amino acid digestibility in feedstuffs. Furthermore, calculation

of digestible crude fat are based on the composition of fatty acids from table values of lipid sources, and, finally, digestible carbohydrates are calculated as a difference from digestible organic matter and the sum of digestible protein + digestible lipids (tri-acylglycerols) + fermentable carbohydrates.

In the third step, the potential physiologically available energy (PPE) from the four nutrient fractions is calculated. In feed formulation the contribution of PPE and standardised digestible amino acids (SDAA) from actual batches of individual feedstuffs are additive and result in the optimised diet for the specific purpose.

In the fourth step, the optimal composition can be defined from actual data on feed intake and expected protein deposition capacity (Pd_{max}) for the specific pig category and weight range. Finally, the surplus N (and P) for the specific production can be calculated. For these purposes, the development of computer models for the different pig categories is necessary (see Appendix).

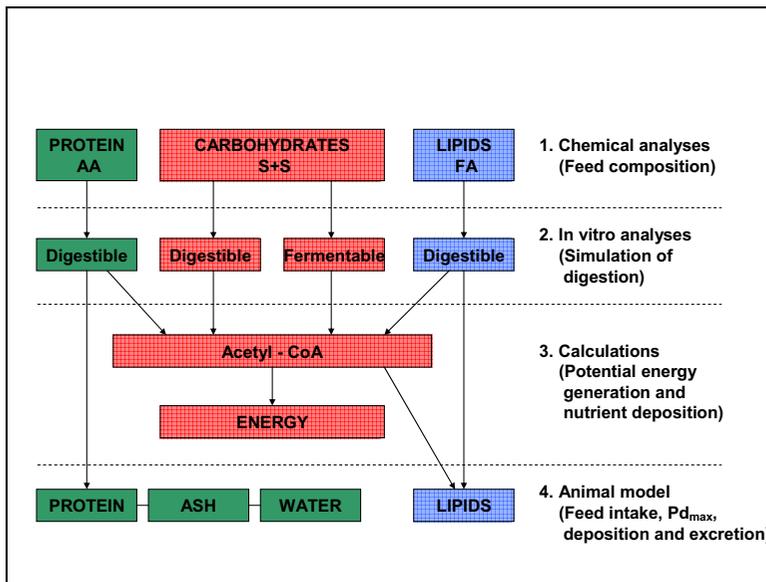


Figure 13. A step-wise characterisation of the feed value of complete diets. The first three steps lead to a characterisation of the potential feed value for use in recommendations and prize setting. The last step predicts the actual feed value in relation to the specific feeding situation. AA: amino acids; S+S: sugar and starch; FA: fatty acids; CoA: coenzyme A (Boisen, 2000a).

General discussion

The principles for feed evaluation have been discussed intensively among scientists within animal nutrition. The discussion has mainly focussed on the appropriateness of different systems based on NE. During the symposium on protein and energy evaluation held in Rostock in 2003, different national systems for pigs and ruminants was presented and discussed and it was decided to follow up with workshops in the following EAAP meetings. Furthermore, a proposal has been prepared for a standard method for determining nutrient digestibility in the pig (Wenk et al., 2004). So far, this seems to be the only outcome of the efforts for harmonising evaluation of pig feeds in Europe.

On the other hand, throughout the world a lot of efforts and resources have already been spent on animal experiments for determining the digestibility of nutrients in actual batches of feedstuffs. Thus, the level and variation in nutrient digestibility for the individual feedstuffs has already been intensively studied.

At Danish Institute of Agricultural Sciences, more than 800 batches from pig digestibility experiments performed during the last three decades have been stored in the freezer. Based on these samples two *in vitro* methods for determining the digestibility corresponding to the degradation at *ileal* and faecal level, respectively, have been developed. The two methods have been demonstrated to give reliable results for *in vivo* digestibility across the different feedstuffs and, moreover, the methods are also able to describe precisely the variation in digestibility in different batches of the same feedstuff.

Therefore, the need for most of these animal studies can be questioned, and efforts should much more focus on reliable laboratory methods.

Obviously, digestibility is not an exact property of the feed as is the case for a chemical analysis. The digestibility of a feed is influenced by many different factors, including processing, feeding conditions, feeding strategy and animal category. Furthermore, the artificial experimental conditions may influence the digestibility, as well as the utilisation, of the feed.

On the other hand, a proper and well-documented laboratory method for analysing the potential digestibility of the different nutrients in the feed may offer the optimal basis for a correct feed evaluation in practise.

A feed evaluation system based directly on the properties of the feed would be the obvious choice for a future common international system. In the new Danish feed evaluation system, the energy value is based directly on the potential physiological energy, which is independent of the actual use of the feed. Furthermore, the protein value is based on standardised digestible essential amino acids, contributing to the ideal protein for the specific pig category.

On this basis, recommendations for digestible amino acids, relative to those for energy, are formulated according to expected requirements for the different categories of pigs.

It is generally agreed that the actual production value of the feed is influenced by a number of different factors relating to the feed, as well as to the animals, in the specific feeding situation. The present characterisation of the feed is still based on the main nutrient components, although many secondary compounds may influence and, in particular, reduce the utilisation of the feed.

It follows, that more detailed and systematic investigations are needed for evaluating the nutritional effects of the composition of dietary fibre as well as of ANF's. Furthermore, possible specific effects of many different possible combinations of fatty acids and other lipids are not yet investigated.

The contribution of digestible essential amino acids from the feed plays a central role for optimal feed utilisation as well as the production value of the feed. Thus, for a general reduction in the dietary protein, the first limiting amino acids are commonly supplemented. These are often considered to be 100% available. However, several investigations indicate that the availability of

these amino acids is not higher than that of protein-bound amino acids, and may also be even lower (van Meulen *et al.* 1998). This could be explained by a higher susceptibility to microbial degradations or by a less efficient transport through the epithelial layer, because the common degradation products from dietary proteins are di- and tripeptides.

Furthermore, complete diets with supplemented amino acids, may often be re-mixed during transport from the manufacturers, as well as in the feeding tubes at the farm. Consequently, temporary, or more permanent, undersupply of essential ingredients, and reduced gain for the pigs, may often be the result.

The importance of a sufficient supply of all essential amino acids contributing to the ideal protein for the specific pig category was illustrated in Figure 1. The figure showed that deposition of one kg protein in growing pigs is followed with 4.4 kg live weight gain due to the associated water retention and bone formation.

It follows, that a correct supply of all essential nutrients is necessary for optimising the actual pig production. Consequently, current knowledge to the different properties of the actual feed is fundamental for a correct feed optimisation. Furthermore, the ingredient contribution of standardised digestible amino acids, together with the potential physiological energy from the digestible nutrient fractions, should be optimised according to the actual requirements for the specific production. Finally, feeding strategy should be optimised in order to meet the potential for the specific production.

In conclusion, classical feed evaluation systems based on animal experiments, performed under artificial experimental conditions, is no longer acceptable as a suitable basis for feed evaluation. Modern animal production should basically be optimised according to the properties of the feed, characterised by modern analysis methods, and supplied with recommendations for the optimal dietary composition of available amino acids and physiological energy.

Future research should, therefore, focus on the effects of those dietary compounds and specific production conditions, which can be positive or negative in the actual production. International agreement on a common feed evaluation system, based directly on the feed itself, would offer the optimal basis for concerted actions on systematic improvements in the characterisation of the specific properties of the feed, as well as of the general feeding conditions in the practical pig production.

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Appendix:

Table 1A. Table values for chemical composition (% of the feedstuff), *in vitro* digestibility (%) and energy coefficient of carbohydrates (EC_{carb}) of common feedstuffs for pig diets¹

Feedstuff	DM	Ash	CP	CF	EDOM	EDOMi	EDN	EC _{carb}
Barley	85	1.9	9.2	2.6	84	79	90	11.6
Wheat	85	1.6	9.9	2.1	91	87	93	11.6
Rye	85	1.5	8.1	1.8	90	83	90	11.1
Triticale	85	1.8	8.9	2.1	91	85	93	11.6
Oats	85	2.2	8.3	4.6	68	65	91	11.6
Corn	88	1.3	8.4	4.0	90	88	91	11.6
Wheat bran	87	5.1	15.9	4.0	63	51	83	11.0
Corn gluten feed	89	6.2	20.4	4.0	67	60	89	11.0
Peas	85	3.2	20.5	1.9	91	79	96	11.0
Skim milk powder	96	8.1	35.4	0.5	99	98	99	11.1
Fish meal, standard	91	14.4	70.5	7.6	95	95	95	9.9
Fish meal, LT	92	13.7	70.6	9.1	93	93	93	9.9
Potato protein concentrate	90	2.1	77.3	2.0	90	90	90	9.9
Soya protein,HamletHP300	92	7.0	55.4	2.4	94	85	96	9.0
Soybean meal	88	6.5	42.7	2.5	91	72	95	9.0
Soybean meal, dehulled	87	6.6	46.8	2.5	95	77	96	9.0
Sunflower meal, p.dehulled	90	6.7	37.2	2.9	76	65	92	9.0
Sunflower cake, p.dehulled	92	5.9	29.8	10.2	66	52	90	9.0
Rapeseed meal	88	7.4	34.2	3.7	79	58	86	9.0
Rapeseed cake, 9% fat	89	6.2	31.1	9.3	81	60	86	9.0
Tapioca	87	5.4	2.8	0.7	90	80	66	11.6
Beet pellets	74	6.2	8.5	1.1	88	37	83	9.3
Beet pellets, molassed	73	10.0	8.8	1.1	90	50	83	9.3
Molasses, sugar beet	88	9.4	9.6	0.1	100	98	90	9.3
Molasses, sugar cane	91	15.5	5.1	0.2	99	97	90	9.3
Grass pellets	91	9.1	14.8	2.8	51	39	77	9.0
Whey powder, sweet	97	8.2	12.5	1.3	100	98	94	11.1
Whey, Category A	6	0.5	0.7	0.2	99	98	98	10.1
Whey, Category B	7	0.7	0.3	0.1	99	98	98	10.1
Yeast, Novo	12	1.2	6.1	0.5	91	77	95	9.0
Yeast, Ethanol	21	1.8	9.4	1.3	84	66	94	9.0
Fish pulp, H.pro.	41	4.7	30.5	5.8	100	99	97	9.9

¹Official general values in 2005 from Danish Agricultural Advisory Centre, Skejby, Denmark. The data are currently updated. CP = crude protein; CF = crude fat; EDOM = enzyme digestibility of organic matter (at faecal level); EDOMi = enzyme digestibility of organic matter at ileal level; EDN = enzyme digestibility of nitrogen (at ileal level); EC_{carb} = energy coefficient for digestible carbohydrates

Table 2A. Nutrient fractions (%) of feedstuffs contributing to the energy value in diets for growing pigs (FUp per kg)¹

Feedstuff	RDCP	RDCF	EDC	FERMC	EIDMi	FUp
Barley	8.3	2.3	55.0	4.2	18.0	1.05
Wheat	9.2	1.9	61.5	3.3	11.3	1.16
Rye	7.3	1.6	60.4	5.8	14.6	1.08
Triticale	8.3	1.9	60.6	5.0	13.0	1.14
Oats	7.6	4.1	42.1	2.5	29.6	0.85
Corn	7.6	3.6	65.1	1.7	10.8	1.26
Wheat bran	13.2	3.6	25.0	9.8	41.7	0.64
Corn gluten feed	18.2	3.6	27.9	5.8	35.0	0.74
Peas	19.7	1.7	43.2	9.8	18.1	1.01
Skim milk powder	35.0	0.5	50.6	0.9	4.2	1.24
Fish meal, standard	65.9	6.8	0.0	0.0	8.2	1.15
Fish meal, LT	64.6	8.2	0.0	0.0	9.6	1.18
Potato protein concentrate	69.6	1.8	7.7	0.0	9.4	1.08
Soya protein, HamletHP300	53.2	2.2	9.3	14.5	22.5	0.97
Soybean meal	40.6	2.3	15.9	15.5	24.8	0.89
Soybean meal, dehulled	44.9	2.3	14.7	14.5	20.5	0.94
Sunflower meal, p.dehulled	34.2	2.6	17.3	9.2	31.2	0.75
Sunflower cake, p.dehulled	26.8	9.2	8.8	12.1	43.1	0.81
Rapeseed meal	29.4	3.3	14.0	16.9	36.1	0.73
Rapeseed cake, 9% fat	26.7	8.4	14.6	17.4	35.0	0.93
Tapioca	1.8	0.6	62.8	8.2	17.9	1.05
Beat pellets	7.1	1.0	17.0	34.6	44.6	0.51
Beat pellets, molassed	7.3	1.0	23.2	25.2	34.5	0.54
Molasses, sugar beet	8.6	0.1	68.3	1.6	4.4	0.98
Molasses, sugar cane	4.6	0.2	68.5	1.5	6.9	0.92
Grass pellets	11.4	2.5	18.0	9.8	52.7	0.37
Whey powder, sweet	11.8	1.2	74.1	1.8	4.2	1.32
Whey, Category A	0.7	0.2	4.5	0.1	0.3	0.08
Whey, Category B	0.3	0.1	5.8	0.1	0.3	0.09
Yeast, Novo	5.8	0.5	2.1	1.5	2.8	0.13
Yeast, Ethanol	8.8	1.2	2.7	3.5	7.1	0.21
Fish pulp, H.pro.	29.6	5.2	1.1	0.4	1.8	0.63

¹Calculated from Table 1A (see text)

RDCP = real digestible crude protein; RDCF = real digestible crude fat; EDC = enzyme digestible carbohydrates;

FERMC = fermentable carbohydrates; EIDMi = enzyme indigestible dry matter at *ileal* level;

FUp = Feed units for growing pigs.

Table 3A. Crude protein (CP) and essential amino acid composition in common feedstuffs for pig diets^{1,2}

Feedstuff	CP		Essential amino acids (% of crude protein)									
	%	Lys	Thr	Met	Cys	Trp	Ile	Leu	Val	His	Phe	Tyr
Barley	9.2	3.6	3.4	1.7	2.3	1.2	3.5	7.0	4.9	2.3	5.1	3.1
Wheat	9.9	2.8	2.9	1.6	2.2	1.	3.3	6.6	4.2	2.4	4.6	2.9
Rye	8.1	4.1	3.6	1.8	2.6	1.0	3.6	6.5	5.1	2.6	4.9	2.7
Triticale	8.9	3.6	3.4	1.8	2.5	1.0	3.6	6.8	4.8	2.5	4.8	2.9
Oats	8.3	4.2	3.5	1.7	2.9	1.2	3.8	7.3	5.2	2.4	5.2	3.3
Corn	8.4	3.0	3.6	2.1	2.3	0.7	3.4	12.3	4.8	3.1	4.9	3.9
Wheat bran	15.9	4.1	3.3	1.6	2.1	1.4	3.1	6.2	4.6	2.8	4.0	2.8
Corn gluten feed	20.4	2.7	3.5	1.6	2.1	0.6	3.1	9.0	4.5	2.9	3.7	3.0
Peas	20.5	7.2	3.8	1.0	1.5	0.9	4.1	7.2	4.7	2.6	4.7	3.4
Skim milk powder	35.4	7.7	4.4	2.5	0.8	1.4	5.1	9.8	6.3	2.8	4.9	5.0
Fish meal, standard	70.5	7.6	4.2	2.8	0.9	1.0	4.1	7.2	4.8	2.8	4.0	3.2
Fish meal, LT	70.6	7.6	4.2	2.8	0.9	1.0	4.1	7.2	4.8	2.8	4.0	3.2
Potato protein concentrate	77.3	7.9	5.8	2.3	1.6	1.4	5.5	10.3	6.6	2.3	6.6	5.8
Soya protein,HamletHP300	55.4	6.1	3.8	1.3	1.5	1.2	5.0	7.6	5.2	2.7	5.0	3.9
Soybean meal	42.7	6.1	3.8	1.3	1.5	1.2	5.0	7.6	5.2	2.7	5.0	3.9
Soybean meal, dehulled	46.8	6.1	3.8	1.3	1.5	1.2	5.0	7.6	5.2	2.7	5.0	3.9
Sunflower meal, p.dehulled	37.2	3.5	3.7	2.2	1.8	1.2	4.1	6.3	5.0	2.4	4.5	2.6
Sunflower cake, p.dehulled	29.8	3.5	3.7	2.2	1.8	1.2	4.1	6.3	5.0	2.4	4.5	2.6
Rapeseed meal	34.2	5.5	4.4	2.0	2.5	1.3	3.9	7.1	5.2	2.8	4.0	3.0
Rapeseed cake, 9% fat	31.1	5.5	4.4	2.0	2.5	1.3	3.9	7.1	5.2	2.8	4.0	3.0
Tapioca	2.8	3.8	3.4	1.3	1.4	1.0	3.3	5.9	4.2	2.2	4.3	-
Beet pellets	8.5	5.9	4.3	1.9	1.3	0.8	3.7	6.0	5.9	3.7	3.4	4.5
Beet pellets, molassed	8.8	5.1	3.8	1.7	1.2	0.7	3.4	5.4	5.2	3.2	2.9	4.2
Molasses, sugar beet	9.6	1.5	0.6	0.2	0.6	0.8	2.6	2.6	1.8	0.5	0.5	2.7
Molasses; sugar cane	5.1	0.4	1.4	0.4	0.9	0.2	0.9	1.3	3.0	0.3	0.6	1.3
Grass pellets	14.8	4.0	4.3	1.5	1.1	0.6	3.9	7.0	5.4	2.0	4.1	2.7
Whey powder, sweet	12.5	7.4	6.0	1.4	1.9	1.0	5.3	8.9	5.0	1.9	3.0	2.1
Whey, Category A	0.7	7.0	5.3	1.3	1.8	1.4	-	-	-	-	-	-
Whey, Category B	0.3	3.6	2.4	0.7	0.7	0.7	-	-	-	-	-	-
Yeast, Novo	6.1	5.8	4.4	1.1	0.8	1.2	-	-	-	-	-	-
Yeast, Ethanol	9.4	7.0	4.7	1.2	1.0	1.2	-	-	-	-	-	-
Fish pulp, H.pro.	30.5	6.5	4.2	2.8	1.0	1.0	4.0	6.7	4.7	2.0	3.9	2.7

¹Degussa (1996); ²Bedriftsløsning (2004)

Table 4A. Crude fat (CF) and fatty acid composition in common feedstuffs for pig diets

Feedstuff	CF		Fatty acids (% of total fatty acids)							
	%	< C ₁₂	C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	Others
Barley	2.6	-	-	-	20	1	12	58	9	-
Wheat	2.1	-	-	-	21	2	14	58	5	-
Rye	1.8	-	-	-	18	1	19	52	6	3
Triticale	2.1	-	-	1	11	1	7	42	4	-
Oats	4.6	-	-	-	19	1	33	44	3	-
Corn	4.0	-	-	-	13	2	33	50	2	-
Wheat bran	4.0	-	-	-	21	2	14	58	5	-
Corn gluten feed	4.0	-	-	-	13	2	33	50	2	-
Peas	1.9	-	-	-	13	4	25	47	10	3
Skim milk powder	0.5	9	3	11	31	11	27	2	1	-
Fish meal, standard	7.6	-	-	6	13	2	15	3	-	61 ¹
Fish meal, LT	9.1	-	-	6	13	2	15	3	-	61 ¹
Potato protein concentrate	2.0	-	-	-	18	4	2	29	21	2
Soya protein,HamletHP300	2.4	-	-	-	10	2	26	54	8	-
Soybean meal	2.5	-	-	-	10	2	26	54	8	-
Soybean meal, dehulled	2.5	-	-	-	10	2	26	54	8	-
Sunflower meal, p.dehulled	2.9	-	-	-	8	5	23	63	1	-
Sunflower cake, p.dehulled	10.2	-	-	-	8	5	23	63	1	-
Rapeseed meal	3.7	-	-	-	5	1	58	21	12	3 ²
Rapeseed cake, 9% fat	9.3	-	-	-	5	1	58	21	12	3 ²
Tapioca	0.7	1	4	2	32	3	35	16	8	-
Beet pellets	1.1	-	-	-	21	1	10	58	11	-
Beet pellets, molassed	1.1	-	-	-	21	1	10	58	11	-
Molasses, sugar beet	0.1	-	-	-	-	-	-	-	-	-
Molasses, sugar cane	0.2	-	-	-	-	-	-	-	-	-
Grass pellets	2.8	-	-	-	18	2	4	20	55	-
Whey powder, sweet	1.3	9	1	9	34	12	22	4	1	-
Whey, Category A	0.2	-	-	-	-	-	-	-	-	-
Whey, Category B	0.1	-	-	-	-	-	-	-	-	-
Yeast, Novo	0.5	-	-	-	-	-	-	-	-	-
Yeast, Ethanol	1.3	-	-	-	-	-	-	-	-	-
Fish pulp, H.pro.	5.8	-	-	6	25	4	12	2	2	36 ¹
<i>Pure fat sources:</i>										
Palm kernel oil	100	9 ³	50	15	7	2	15	1	-	-
Coconut oil	100	15 ³	47	18	9	2	7	1	-	-
PFAD ⁴	100	-	-	1	42	10	42	10	-	-
<i>Pig fat</i>										
<i>Sow's milk</i>										
		2	-	1	29	12	48	6	-	-
		-	-	1	25	23	25	8	-	19

Data from Jakobsen (1999) and Sauvart et al. (2004). ¹main components: C_{20:5}, C_{22:6}; ²main components: ¹C_{20:1}, C_{22:1}; ³main components: C_{8:0}, C_{10:0}; ⁴Palm fatty acid distillate.

Table 5A. Carbohydrate composition (% of the feedstuff)¹ and calculated values for the fermentable carbohydrate fraction (FMC) from *in vitro* digestibility (%) analyses corresponding to ileal and faecal level, respectively, of common feedstuffs for pig diets

Feedstuff	EDC ²	Starch	Sugar	Oligo-S ³	S-NCP ⁴	I-NCP ⁵	FMC ⁶
Barley	55	52	2	1	5	11	4
Wheat	62	59	1	2	2	8	3
Rye	60	52	4	4	4	9	6
Triticale	61						
Oats	42	40	1	1	3	16	3
Corn	65	62	2	1	1	8	2
Wheat bran	25	17	4	4	5	30	10
Corn gluten feed	28	24	3	1	3	28	6
Peas	43	38	3	2	5	11	10
Skim milk powder	51						1
Fish meal, standard	0	-	-	-	-	-	0
Fish meal, LT	0	-	-	-	-	-	0
Potato protein concentrate	8	1	1				0
Soya protein,HamletHP300	9						15
Soybean meal	16	3	7	6	6	14	16
Soybean meal, dehulled	15						15
Sunflower meal, p.dehulled	17	4	8	4	10	18	9
Sunflower cake, p.dehulled	9	2	4	2	5	9	12
Rapeseed meal	14	2	6	2	5	16	17
Rapeseed cake, 9% fat	15	2	7	2	4	9	17
Tapioca	63	67	2	0	2	5	8
Beet pellets	17	0	2	0	30	13	35
Beet pellets, molassed	23						25
Molasses, sugar beet	68						2
Molasses, sugar cane	69						2
Grass pellets	18	2	5	1	3	17	10
Whey powder, sweet	74						2
Whey, Category A	5						0
Whey, Category B	6						0
Yeast, Novo	2						2
Yeast, Ethanol	3						4
Fish pulp, H.pro.	1	-	-	-	-	-	0

¹Bach-Knudsen, 1997; ²Enzyme digestible carbohydrates; ³Oligo-saccharides; ⁴Soluble non-cellulose poly-saccharides; ⁵Insoluble non-cellulose poly-saccharides; ⁶Fermentable carbohydrates.

Table 6A. Standardised digestibility (%) of crude protein and essential and semi-essential amino acids in common feedstuffs for pig diets calculated from *in vivo* investigations¹

Feedstuff	CP	Lys	Thr	Met	Cys	Trp	Ile	Leu	Val	His	Phe	Tyr
Barley	80	75	76	84	81	79	81	82	80	82	84	81
Wheat	89	83	84	90	89	89	89	90	86	90	91	90
Rye	77	73	71	81	83	76	77	79	76	79	82	77
Triticale	86	84	81	90	89	85	87	87	86	89	90	87
Oats	75	76	70	84	75	78	80	82	79	85	85	81
Corn	86	77	81	89	86	77	86	90	84	86	89	89
Wheat bran	72	72	74	79	75	75	77	78	75	83	81	82
Corn gluten feed	69	66	71	83	67	66	80	85	77	75	84	83
Peas	81	84	76	80	71	73	80	80	77	84	82	81
Skim milk powder	91	97	93	97	95	94	93	97	92	96	98	94
Fish meal, standard	88	91	90	92	86	88	91	92	91	89	90	90
Fish meal, LT												
Potato protein concentrate	89	88	86	90	71	71	86	88	87	87	89	85
Soya protein,HamletHP300												
Soybean meal	87	88	85	91	85	88	88	87	88	91	90	90
Soybean meal, dehulled												
Sunflower meal, p.dehulled	82	81	82	90	83	83	84	85	82	84	85	87
Sunflower cake, p.dehulled												
Rapeseed meal	76	77	76	87	81	75	78	81	77	83	81	79
Rapeseed cake, 9% fat												
Tapioca												
Beet pellets	50	52	30	61	22	41	60	59	42	59	51	52
Beet pellets, molassed												
Molasses, sugar beet												
Molasses; sugar cane												
Grass pellets												
Whey powder, sweet												
Whey, Category A												
Whey, Category B												
Yeast, Novo												
Yeast, Ethanol												
Fish pulp, H.pro.												

¹Pedersen & Boisen, 2002

Table 7A. Potential standardised digestibility (%) of crude protein and essential and semi-essential amino acids in common feedstuffs for pig diets calculated from *in vitro* analyses¹

Feedstuff	CP	Lys	Thr	Met	Cys	Trp	Ile	Leu	Val	His	Phe	Tyr
Barley	79	81	76	84	82	79	82	84	82	83	84	83
Wheat	87	86	83	89	88	87	88	89	88	89	89	89
Rye	80	83	77	84	84	78	83	84	83	84	84	83
Triticale	85	86	82	88	88	83	87	88	87	88	88	87
Oats	71	77	65	79	80	71	78	80	76	79	79	79
Corn	84	84	82	87	86	78	86	89	86	87	86	87
Wheat bran	68	72	63	74	72	70	71	73	72	75	72	72
Corn gluten feed	79	78	76	83	81	69	81	85	81	84	81	82
Peas	91	94	90	91	91	89	93	93	92	93	93	93
Skim milk powder	98	99	98	99	98	98	99	99	99	99	99	99
Fish meal, standard	94	95	94	95	94	94	95	95	95	95	95	95
Fish meal, LT	92	93	92	93	92	92	93	93	92	93	92	93
Potato protein concentrate	89	90	89	90	89	89	90	90	90	90	90	90
Soya protein,HamletHP300	94	95	93	94	93	94	95	95	94	95	95	95
Soybean meal	92	91	92	91	92	93	93	93	93	93	93	93
Soybean meal, dehulled	93	95	93	94	93	94	95	95	94	95	95	95
Sunflower meal, p.dehulled	87	88	86	90	88	87	89	89	89	89	89	88
Sunflower cake, p.dehulled	81	83	79	86	82	81	85	84	84	85	84	83
Rapeseed meal	80	83	80	83	82	80	82	83	82	83	81	82
Rapeseed cake, 9% fat	79	82	79	83	82	80	82	82	82	82	81	82
Tapioca	29	37	18	38	24	22	38	41	35	41	40	-
Beet pellets	57	70	56	69	52	45	66	66	68	73	60	72
Beet pellets, molassed	64	72	61	72	58	51	69	69	70	74	63	74
Molasses, sugar beet	87	84	68	75	82	86	87	85	88	82	72	88
Molasses; sugar cane	82	55	76	78	82	65	78	76	85	67	67	83
Grass pellets	56	61	55	63	46	34	63	65	63	61	61	61
Whey powder, sweet	92	93	92	93	92	91	93	93	93	92	92	92
Whey, Category A	98	98	98	98	98	-	-	-	-	-	-	-
Whey, Category B	98	97	97	97	97	-	-	-	-	-	-	-
Yeast, Novo	95	95	95	94	95	-	-	-	-	-	-	-
Yeast, Ethanol	94	93	93	92	93	-	-	-	-	-	-	-
Fish pulp, H.pro.	97	97	97	97	97	97	97	97	97	97	97	97

¹ Values from Table 1A and 3A, respectively, and calculations according to the equations given p. 47.

Table 8A. Protein quality (composition of standardised digestible essential amino acids relative to ideal protein) of common feedstuffs for pig diets¹

Feedstuff	%	Relative to the ideal protein										
		CP	Lys	Thr	Met	M+C	Trp	Ile	Leu	Val	His	Phe
Barley	9.2	53	73	100	117	100	91	93	98	97	136	108
Wheat	9.9	39	61	91	107	100	84	85	82	98	117	96
Rye	8.1	61	77	104	128	85	93	85	100	109	128	98
Triticale	8.9	53	73	103	122	81	92	88	94	103	123	98
Oats	8.3	65	71	105	142	100	104	102	107	104	144	118
Corn	8.4	43	78	120	100	54	87	160	94	128	124	101
Wheat bran	15.9	62	67	97	109	120	81	81	93	124	104	89
Corn gluten feed	20.4	38	75	93	106	44	80	118	89	122	95	87
Peas	20.5	106	83	56	69	73	105	92	91	106	120	103
Skim milk powder	35.4	111	98	140	92	117	129	124	122	113	124	125
Fish meal, standard	70.5	110	93	158	103	83	104	91	93	113	101	91
Fish meal, LT	70.6	110	93	158	103	83	104	91	93	113	101	91
Potato protein concentrate	77.3	114	129	129	108	117	139	130	128	93	166	156
Soya protein, Hamlet HP300	55.4	86	84	71	78	100	125	95	100	109	126	112
Soybean meal	42.7	86	84	71	78	100	125	95	100	109	126	112
Soybean meal, dehulled	46.8	86	84	71	78	100	125	95	100	109	126	112
Sunflower meal, p.dehulled	37.2	51	81	125	112	100	105	81	98	98	115	90
Sunflower cake, p.dehulled	29.8	51	81	125	112	100	105	81	98	98	115	90
Rapeseed meal	34.2	82	98	114	128	108	100	92	102	115	101	90
Rapeseed cake, 9% fat	31.1	82	98	114	128	108	100	92	102	115	101	90
Tapioca	2.8	69	47	94	62	63	109	105	98	124	149	-
Beet pellets	8.5	103	95	128	81	53	108	87	135	190	89	115
Beet pellets, molassed	8.8	82	80	106	73	46	92	73	109	148	72	103
Molasses, sugar beet	9.6	21	23	10	25	67	65	33	35	20	10	39
Molasses, sugar cane	5.1	4	29	21	36	13	21	15	60	10	12	24
Grass pellets	14.8	62	95	90	59	30	110	102	117	87	112	92
Whey powder, sweet	12.5	107	133	79	92	82	134	112	97	76	75	65
Whey, Category A	0.7	100	118	72	86	117	-	-	-	-	-	-
Whey, Category B	0.3	51	53	39	39	58	-	-	-	-	-	-
Yeast, Novo	6.1	83	98	61	53	100	-	-	-	-	-	-
Yeast, Ethanol	9.4	99	103	66	61	100	-	-	-	-	-	-
Fish pulp, H.pro.	30.5	93	93	156	106	83	100	84	90	80	98	83

¹Calculated from Table 5 in the text and from Table 3A and 7A in the Appendix.

See calculation example in the text p. 18.

CALCUATION OF STANDARDISED DIGESTIBILITY OF CRUDE PROTEIN AND AMINO ACIDS IN FEEDSTUFFS FOR GROWING PIGS

1. Real digestible crude protein (RDCP):

$$\text{RDCP (g/kg DM)} = 10 * \text{CP} * \text{EDN/DM}$$

2. Specific endogenous crude protein losses (SECPL):

$$\text{SECPL (g/kg DM intake)} = 10 * 0.066 * \text{EIDMi}$$

3. Standardised digestible crude protein (SDCP):

$$\text{SDCP (g/kg DM)} = \text{RDCP} - \text{SECPL}$$

4. Standardised digestibility (%) of crude protein (SDCP, %)

$$\text{SDCP, \%} = 0,1 * \text{DM} * \text{SDCP/CP}$$

5. Lysine (LYS)

$$\text{LYS (g/kg DM)} = 10 * \text{LYS(\% of CP)} * \text{CP/DM}$$

6. Real digestible lysine (RDLYS):

$$\text{RDLYS (g/kg DM)} = \text{LYS(g/kg DM)} * \text{EDN/100}$$

7. Specific endogenous lysine losses (SELYSL):

$$\text{SELYSL(g/kg DM intake)} = 0.188 * \text{SECPL (g/kg DM intake)/6.25}$$

8. Standardised digestible lysine (SDLYS):

$$\text{SDLYS (g/kg DM)} = \text{RDLYS} - \text{SELYSL}$$

9. Standardised digestibility of lysine (SDLYS,%):

$$\text{SDLYS, \%} = 100 * \text{SDLYS/LYS (g/kg DM)}$$

Similar calculations for all other essential amino acids (AA).

- The specific factor (0.188) for lysine correspond to the factors for
- threonine (0.281), methionine (0.063), cystine (0.100), tryptophan (0.075), isoleucine (0.156), leucine (0.250), valine (0.219), histidine (0.094), phenylalanine (0.188), and tyrosine (0.125).

AA = Lys, Thr, Met, Cys, Trp, Ile, Leu, Val, His, Phe, Tyr (Table 3A).

METHOD FOR ANALYSIS OF ENZYME DIGESTIBLE ORGANIC MATTER AT FAECAL LEVEL (EDOM_f) IN FEEDSTUFFS FOR GROWING PIGS

1. Purpose and type of samples:

Determination of the enzyme digestible organic matter in feedstuffs and pig diets corresponding to the faecal digestibility in pigs. The results contribute to the calculation of the energy value in pig feeds.

2. Principle:

The feed sample is incubated with pepsin for 75 minutes, followed with pancreatin for three and a half hour, and finally with Viscozyme for about 18 hours (overnight). Unsolubilised sample materials are collected after filtration and then dried and finally ashed. Based on the results from determined dry matter and ash in the sample and residue, respectively, enzyme digestibility of organic matter is calculated.

3. Reagents:

- 3.1. Acetone, BBB 10010
- 3.2. Celite (545, Tecator), BBB 12120
- 3.3. Chloramphenicol, ICN no. 190321.
- 3.4. Disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), Merck art no. 6580
- 3.5. EDTA (Titriplex III), Merck art no. 8418
- 3.6. Ethanol($\text{CH}_3\text{CH}_2\text{OH}$), 96%.
- 3.7. Acetic acid (CH_3COOH), 100%, 17.4 mol/L, Merck art no. 63
- 3.8. Sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$), Merck art no. 6345
- 3.9. Sodium hydroxyde (NaOH), Merck art no. 6498
- 3.10. Pancreatin (Porcine pancreas grade VI), Sigma no. p-1750.
- 3.11. Pepsin (2000 FIP U/g), Merck art no. 7190.
- 3.12. Hydrochloric acid (HCl), conc. 37%, 12.08 mol/L, Merck art no. 317
- 3.13. Viscozyme L, Novo-Nordisk, A/S. Stored in refrigerator. Stable for one year.
- 3.14. Chloramphenicol-solution, 0.1% i ethanol:

- 0.2 g Chloramphenicol (3.3) solubilised in 200 ml 96% Ethanol (3.6). Stored in freezer.
- 3.15. Phosphate buffer A, 0.1 mol/L, pH 6.0:
1.98 g disodium hydrogenphosphate (3.4) and 29,44 g sodium dihydrogenphosphate (3.8) are solubilised in about 1.5 L de-mineralised water in a beaker. pH is controlled and adjusted, if necessary, with 1 mol/L sodium hydroxyde (3.17) or 1 mol/L hydrochloric acid (3.22). The solution is transferred to a 2 L measuring flask and filled up with demineralised water.
- 3.16. Phosphate buffer B, 0.2 mol/L, pH 6.8 :
19.30 g disodium hydrogenphosphate (3.4) and 45,48 g sodium dihydrogenphosphate (3.8) are solubilised in ca. 1.5 L de-mineralised water in a beaker. The pH is controlled and adjusted if necessary with 1 mol/L sodium hydroxide (3.17) or 1 mol/L hydrochloric acid (3.22). The solution is transferred to a 2 L measuring flask and filled up with de-mineralised water.
- 3.17. Sodium hydroxide (NaOH), 1 mol/L:
40 g sodium hydroxide (3.9) is solubilised in de-mineralised water *ad* 1000 mL.
- 3.18. Sodium hydroxide (NaOH), 0.6 mol/L:
24.0 g sodium hydroxyde (3.9) is solubilised in de-mineralised water *ad* 1 L.
- 3.19. Pancreatin solution, 0.10 g/mL:
3.000 g pancreatin (3.10) is solubilised with magnetic stirring in 30 ml of phosphate buffer B (3.16) for ca. 15 min. Non-solubilised material is removed by centrifugation (3000 rpm/min). The solution is prepared shortly before use.
- 3.20. Pepsin solution, 0.025 g/mL:
0.750 g pepsin (3.11) is solubilised in 30 ml of a 0.2 mol/L Hydrochloric acid (3.21).
- 3.21. Hydrochloric acid, 0.2 mol/L:
200 ml of a 1 mol/L hydrochloric acid (3.22) is diluted with de-mineralised water *ad* 1 L.
- 3.22. Hydrochloric acid, 1 mol/L:
83.5 ml of conc. hydrochloric acid, 37% (3.12) or 88.3 ml conc. Hydrochloric acid 35% (3.12) is diluted with de-mineralised water *ad* 1000 mL.
- 3.23. EDTA, 0.2 mol/L:
74.48 g EDTA (3.5) opløses i demineraliseret vand *ad* 1 l.
- 3.24. Acetic acid (CH₃COOH), 30%, 5.8 mol/L:
Concentrated Acetic acid (3.7) is diluted 1:2 with de-mineralised water and stored safely (in room 3022).

4. Special equipment

- 4.1. Conical flasks (100 ml)
- 4.2. Small magnets
- 4.3. Magnetic stirrer (general)
- 4.4. pH-meter (PHM 83, Autocal, Radiometer)
- 4.5. Electrode (GK 2401C, Radiometer)
- 4.6. Rubber stoppers (Diameter: 3 cm)
- 4.7. Magnetic stirrers (Multipoint HP 15, Variomag)
- 4.8. Heating chamber (Thermocenter, Salvas), 40°C +- 1°C
- 4.9. Glass filter crucibles (diameter: 3 cm, pore size: P2 (40-90 mikrons))
- 4.10. Apparatus for fiber analysis (Fibertec system M, Tecator)
- 4.11. Water pressure pump
- 4.12. Heating chamber (general, 100°C +- 1°C)
- 4.13. Dessiccator
- 4.14. Cold extraction unit (Tecator)
- 4.15. Ashing oven
- 4.16. Analysis weight; 0-200 g; accuracy 0.002 g
- 4.17. Multipipette, Eppendorf

5. Performance of analysis

Dry matter and ash content in the sample is determined (for later calculation).

- 5.1. Approximately 0.5 g of feed is weighed with 1 mg accuracy in a 100 ml conical flask.
- 5.2. One blank without sample and three reference samples are also included in the series.
- 5.3. The samples are mixed carefully with 25 mL of a phosphate buffer (3.15) to a slurry.
- 5.4. To the slurry is added 10 mL of a 0.2 mol/L hydrochloric acid (3.21) and 1 mL of a pepsin solution (3.20). Thereafter, the slurry is adjusted to pH 2.0 with a 1 mol/L hydrochloric acid (3.22) and eventually using a 1 mol/L sodium hydroxide solution (3.17).
- 5.5. Then, 0,1 mL chloramphenicol solution (3.14) is added. The flask is closed with a rubber stopper and the sample is incubated in a heating chamber at 40°C for 75 minutes with constant magnetic stirring.
Note! The incubation time is from the time when the temperature in the slurry has reached 40°C.
- 5.6. After incubation 5 mL of 0.6 mol/L sodium hydroxide (3.18) and 10 mL of phosphate buffer B (3.16) are added and then the slurry is adjusted to pH 6.8 with a 1 mol/L hydrochloric acid (3.22) or a 1 mol/L sodium hydroxide (3.17).

- 5.7. Then 1 mL of a pancreatin solution (3.19) is added. The flask is closed with a rubber stopper and the sample is incubated under constant magnetic stirring in a heating chamber at 40°C for three hours and thirty minutes.
- 5.8. Sample is added 10 mL of a 0,2 mol/L EDTA (3.23) and then the pH is adjusted with acetic acid (3.24) to pH 4.8 in the sample.
- 5.9. Sample is added 1.0 mL Viscozyme (3.13).
- 5.10. The sample is incubated, under constantly magnetic stirring, in a heating chamber at 40°C for 17.5 hours (overnight).
- 5.11. Glass filter crucibles (5.9) are added ca. 0.4 g Celite (3.2) and rinsed three times with warm water in a fibre analysis apparatus (5.10). Then, the crucibles are dried at 100°C for at least four hours and weighed after cooling in a dessiccator. Then, the crucibles are placed in a carefully cleaned fibre analysis apparatus (5.10). The samples are filtrated when assuring all materials are carefully transferred with demineralised water. Then, the samples are rinsed further with 2 x 10 mL ethanol (3.6) and sucked (with the water pump) to be as dry as possible.
- 5.12. The sample is placed in a cold extraction unit (5.10) and rinsed with 2 x 10 mL of Aceton (3.1), leaving the sample for about 3 minutes in the rinsing fluid after each rinsing. The magnetic rod used during the incubation is removed after carefully rinsing all adhering material down into the crucible (with water or eventually acetone). All Aceton is collected in a special container in the fume cobbard
- 5.13. Crucibles with undigested materials are dried at 100°C overnight. Then, the crucibles are cooled in a dessiccator and weighed.
- 5.14. Crucibles are placed in an ashing oven (5.15) and the content is ashed at 525°C for about four hours. After ashing, the crucibles are cooled in a dessiccator and weighed.

6. Calculations:

Sample (g) = a

Dry matter factor = b

Ash (g/100g DM) = c

Enzyme digestible organic matter (EDOM):

g weighed dry matter = (axb)/100 = A

g weighed ash = Axc/100 = E

Sample: Tara + undigested dry matter (6.13) = C

sample: Crucible + Celite + undigested ash (6.14) = D

Blank: Tara + undigested dry matter (6.13) = C_b

Blank: Crucible + Celite + undigested ash (6.14) = D_b

g/100g EDOM = (1 - (C -D -(C_b - D_b))/A -E) * 100

7. Traceability

For control of the method, relevant samples with known EDOM values are used as internal reference samples.

<i>Quantification limit:</i>	25 g/100 g organic matter
<i>Repeatability:</i>	2 g/100 g organisk matter, absolute
<i>Reproducibility:</i>	2.5 g/100 g organisk stof absolute val.

8. References:

- Boisen, S. and Fernandez, J.A. 1992. Ny metode til bestemmelse af energiværdien i foderblandinger til svin. 825. Meddelelse fra Statens Husdyrbrugsforsøg. (Boisen, S. and Fernandez, J.A. 1992. *New Method for Estimating the Energy Value of Feeds to Pigs*. 825th Communication from Danish Institute of Agricultural Sciences).
- Boisen, S. and Fernandez, J.A. 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. *Animal Feed Science Technology* 68, 277-286.

METHOD FOR ANALYSIS OF ENZYME DIGESTIBLE ORGANIC MATTER AT *ILEAL* LEVEL (EDOM_i) IN FEEDSTUFFS FOR GROWING PIGS

1. Purpose and type of samples:

Determination of the enzyme digestible organic matter in feedstuffs and pig diets corresponding to the *ileal* digestibility in pigs. The results contribute to the calculation of the energy value in pig feeds.

2. Principle:

The feed sample is incubated with pepsin for 75 minutes, followed with pancreatin for 18 hours (overnight). Solubilised, but incompletely degraded protein is precipitated with sulphosalicylic acid. Insolubilised and precipitated materials are collected after filtration and then dried and finally ashed. Based on the results from determined dry matter and ash in the sample and residue, respectively, enzyme digestibility of dry matter and organic matter is calculated.

3. Reagents:

- 3.1. Acetone, BBB 10010
- 3.2. Celite (545, Tecator), BBB 12120
Ashed at 475-500⁰C, 4-6 hours (can be performed in suitable big portions)
- 3.3. Chloramphenicol, ICN no. 190321.
- 3.4. Disodium hydrogen phosphate (Na₂HPO₄,2H₂O), Merck art no. 6580
- 3.5. Ethanol (CH₃CH₂OH), 96%
- 3.6. Sodium dihydrogen phosphate (NaH₂PO₄,2H₂O), Merck art no. 6345
- 3.7. Sodium hydroxyde (NaOH), Merck art no. 6498
- 3.8. Pancreatin (Porcine pancreas grade VI), Sigma no. p-1750.
- 3.9. Pepsin (2000 FIP U/g), Merck art no. 7190.
- 3.10. Hydrochloric acid (HCl), conc. 37%, 12.08 mol/L, Merck art no. 317
- 3.11. Sulphosalicylic acid (C₇H₆O₆S, 2H₂O), Merck art 691
- 3.12. Chloramphenicol-solution, 0.05% in ethanol:
0.1 g Chloramphenicol (3.3) solubilised in 200 ml 96%
Ethanol (3.6). Stored in freezer.

- 3.13. Phosphate buffer A, 0.1 mol/L, pH 6.0:
1.98 g disodium hydrogenphosphate (3.4) and 29,44 g sodium dihydrogenphosphate (3.8) are solubilised in about 1.5 L de-mineralised water in a beaker. pH is controlled and adjusted, if necessary, with 1 mol/L sodium hydroxyde (3.17) or 1 mol/L hydrochloric acid (3.22). The solution is transferred to a 2 L measuring flask and filled up with demineralised water.
- 3.14. Phosphate buffer B, 0.2 mol/L, pH 6.8 :
19.30 g disodium hydrogenphosphate (3.4) and 45,48 g sodium dihydrogenphosphate (3.8) are solubilised in ca. 1.5 L de-mineralised water in a beaker. The pH is controlled and adjusted if necessary with 1 mol/L sodium hydroxide (3.17) or 1 mol/L hydrochloric acid (3.22). The solution is transferred to a 2 L measuring flask and filled up with de-mineralised water.
- 3.15. Sodium hydroxide (NaOH), 1 mol/L:
40 g sodium hydroxide (3.9) is solubilised in de-mineralised water *ad* 1000 mL.
- 3.16. Sodium hydroxide (NaOH), 0.6 mol/L:
24.0 g sodium hydroxyde (3.9) is solubilised in de-mineralised water *ad* 1 L.
- 3.17. Pancreatin solution, 0.10 g/ml:
3.000 g pancreatin (3.10) is solubilised with magnetic stirring in 30 ml of phosphate buffer B (3.16) for ca. 15 min. Non-solubilised material is removed by centrifugation (3000 rpm/min). The solution is prepared shortly before use.
- 3.18. Pepsin solution, 0.025 g/ml:
0.750 g pepsin (3.11) is solubilised in 30 ml of a 0.2 mol/L Hydrochloric acid (3.21).
- 3.19. Hydrochloric acid, 0.2 mol/L:
200 ml of a 1 mol/L hydrochloric acid (3.22) is diluted with de-mineralised water *ad* 1 L.
- 3.20. Hydrochloric acid, 1 mol/L:
83.5 ml of conc. hydrochloric acid, 37% (3.12) or 88.3 ml conc. Hydrochloric acid 35% (3.12) is diluted with de-mineralised water *ad* 1L.
- 3.21. Sulphosalicylic acid, 20%
200 g sulphosalicylic acid (3.11) *ad* 1 L de-mineralised water
- 3.22. Sulphosalicylic acid, 1%
100 ml 20% sulphosalicylic acid (3.21) *ad* 2 L de-mineralised water

4. Special equipment

- 4.1. Conical flasks (100 ml)
4.2. Small magnets
4.3. Magnetic stirrer (general)

- 4.4. pH-meter (PHM 83, Autocal, Radiometer)
- 4.5. Electrode (GK 2401C, Radiometer)
- 4.6. Rubber stoppers (Diameter: 3 cm)
- 4.7. Magnetic stirrers (Multipoint HP 15, Variomag)
- 4.8. Water bath, 40⁰C +/- 1⁰C.
Alternatively a heating chamber (Thermocenter, Salvas), 40⁰C +/- 1⁰C
- 4.9. Glass filter crucibles (diameter: 3 cm, pore size: P2 (40-90 mikrons)
Should be discarded and replaced with new crucibles after 25 runs!
See also preparation and pre-treatments before use (5.8).
- 4.10. Apparatus for fiber analysis (Fibertec system M, Tecator)
- 4.11. Cold extraction unit (Tecator)
- 4.12. Water pressure pump
- 4.13. Heating chamber (general, 103⁰C +/- 1⁰C)
- 4.14. Dessiccator
- 4.15. Analysis weight; 0-200 g; accuracy 0.002 g
- 4.16. Multipipette, Eppendorf
- 4.17. Ashing oven

5. Performance of analysis

Dry matter and ash content in the sample is determined (for later calculation).

- 5.1. Approximately 0.5 g of sample, grinded with a 1mm sieve, is weighed with 1 mg accuracy in a 100 ml conical flask (4.1). One blank without sample and three reference samples are also included in the series.
- 5.2. Samples are mixed carefully with 25 ml of a phosphate buffer (3.13) to a slurry.
- 5.3. The slurry is added 10 ml of a 0.2 mol/L hydrochloric acid (3.19) and 1 ml of a pepsin solution (3.18). Thereafter, the slurry is adjusted to pH 2.0 with a 1 mol/L hydrochloric acid (3.20) and eventually using a 1 mol/L sodium hydroxide solution (3.15).
- 5.4. Furthermore, 0,5 ml chloramphenicol solution (3.12) is added to the slurry. Then, the flask is closed with a rubber stopper and the sample is incubated in a heating chamber, at 40⁰C, for 75 minutes with constant magnetic stirring.
Note! The incubation time is from the time when the temperature in the slurry has reached 40⁰C.
- 5.5. After incubation, 5 ml of 0.6 mol/L sodium hydroxide (3.16) and 10 ml of phosphate buffer B (3.14) are added, and then the slurry is adjusted to pH 6.8 with a 1 mol/L hydrochloric acid (3.20) or a 1 mol/L sodium hydroxide (3.15).
- 5.6. Then 1 ml of a pancreatin solution (3.17) is added. The flask is closed with a rubber stopper and the sample is incubated under constant magnetic stirring in a heating chamber at 40⁰C for about 18 hours (overnight).
- 5.7. Next morning, 5 ml of a 20% sulphosalicylic acid (3.21) is added to the solution,

which is stirred for 30 minutes at 40⁰C.

- 5.8. *Previously, glass filter crucibles (4.9) are added ca. 0.4 g Celite (3.2) and rinsed three times with warm water in a fibre analysis apparatus (4.10). Then, the crucibles are dried at 100⁰C for at least four hours and weighed after cooling in a dessiccator.*

The crucibles are placed in a carefully cleaned fibre analysis apparatus (4.10). The samples are filtrated when assuring all materials are carefully transferred with demineralised water. Then, the samples are rinsed further with 2 x 10 ml ethanol (3.6) and sucked (with the water pump) to be as dry as possible.

- 5.9. The sample is placed in a cold extraction unit (4.11) and rinsed with 2 x 10 ml of Aceton (3.1), leaving the sample for about 3 minutes in the rinsing fluid after each rinsing. The magnetic rod used during the incubation is removed after carefully rinsing all adhering material down into the crucible (with water or eventually acetone). All acetone is collected in a special container in the fume cobbard.
- 5.10. Crucibles with undigested materials are dried at 103⁰C overnight. Then, the crucibles are cooled in a dessiccator and weighed.
- 5.11. Crucibles are placed in an ashing oven (5.15) and the content is ashed at 475-500⁰C for about four hours. After ashing, the crucibles are cooled in a dessiccator and weighed.

6. Calculations:

a = sample, g

b = dry matter factor

c = ash, g/100g DM

A = g sample DM = (a x b)/100

E = g ash = (A x c)/100

or, alternatively, directly from the sample: E = (a x ash% in sample)/100

B = crucible + Celite, g

C = crucible + Celite with undigested DM, g

D = crucible + Celite with undigested ash, g

Bb = crucible + Celite, g (Blank)

Cb = crucible + Celite with undigested DM, blank, g

Db = crucible + Celite with undigested ash, blank, g

EDOMi = 100 x (1 - (C - D - (Cb - Db))/ A - E)

EDDMi = 100 x (1 - (C - B - (Cb - Bb))/ A)

EIDMi = 100 - EDDMi

7. Traceability

For control of the method, relevant samples with known EDOM values are used as internal reference samples.

<i>Quantification limit:</i>	25 g/100 g organic matter
<i>Repeatability:</i>	2 g/100 g organisk matter, absolute value
<i>Reproducibility:</i>	2.5 g/100 g organisk stof, absolute value

8. Comments to the analysis procedure

It is important that the filtration velocity is reasonable fast (max 15 min). Otherwise, it may be difficult to remove some of the solubilised material. Normally, changing to new crucibles filtration problems with specific samples can be solved. Sand (Merck no. 7712), preliminary ashed and rinsed with acids, may also facilitate the filtration.

9. References:

Boisen, S. and Fernandez, J.A. 1995. Prediction of the apparent digestibility of protein and amino acids in feedstuffs and mixtures for pig diets by in vitro analyses. *Animal Feed Science Technology* 51, 29-43.

Feed quality is of significant importance in husbandry animal production. This is due to the influence on animal health and welfare as well as the sustainability of the production according to environmental influences and production economy. The basic principles for feed evaluation have developed during the last century. However, feed evaluation in most countries is still dominated by classical principles and either based on digestible, metabolizable or net energy, respectively. The new Danish feed evaluation system for pigs arises on new principles where the feed value is based on the potential physiologically available energy related directly to the nutrient composition of the feed itself and described by modern analysis methods. Feed evaluation in the new system is a step-wise process in which the actual value of single feedstuffs depends on its contribution to the complete diet and, moreover, the value of the diet depends on its actual use in the production according to the requirements and feed intake of the animals.

PLANT SCIENCE



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