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Für meine Familie und Freunde

SUMMARY

This thesis combines three different topics that are seemingly unconnected but inherently linked. The first part deals intensively with co-products from biofuel production on a European level. There are numerous co-products which are all suitable as protein supplements for ruminants, pigs and poultry. The results of a number of experiments with lactating dairy cows and fattening bulls suggest that distillers grains, rapeseed meal and rapeseed cake as the main protein source may support a high productive performance. Pigs would particularly benefit from breeding or production progress in further reduction of glucosinolate levels of rapeseed, whereas in cattle, a safer quality assessment of the rapeseed cake is needed. Another fundamental co-product of the biodiesel production is glycerine. In ruminant diets, glycerine at different purities may help to stabilise the hygienic quality of pelleted compound feeds without compromising physical quality of pellets. The efficient utilisation of biofuel co-products is a key tool towards more sustainable biofuel production. Future research should quantify all expenditures on the processing of biofuel co-products in order to be able to evaluate meaningful carbon footprints.

The second part of the thesis draws attention to the question of whether it makes sense to use equations based on feed and intake characteristics to estimate methane (CH_4) emissions from dairy cows. Nine CH_4 prediction equations were applied to five typical Central European diets in order to compare their applicability. As a result, smallest differences to mean values were observed with equations using neutral detergent fibre, while standard deviations were highest, and therefore showed the best capability to differentiate between diets, when using equations that operated with forage proportion and dry matter intake. The differences in levels of CH_4 estimates show that the equations are still inaccurate and may only serve as implications to locate trends. It should be taken into consideration to expand datasets, involving future CH_4 measurements, on animal and herd level, feeding typical up to date regional diets in order to get more precise equations, suitable for a greater range of estimations. To ease and simplify the future applications, the prediction equations could be classified into groups, clearly stating by which data they were derived, for example regional origin and diet composition.

In the third part of this study, 33 samples with main focus on unprotected or rumen-

protected protein supplements, were analysed using an enzymatic *in vitro* procedure (EIVP) in order to determine intestinal crude protein (CP) digestibility (IPD) of ruminally undegraded CP (RUP). Results of this study showed that the EIVP seems to be an adequately working, simple and reliable method to estimate IPD of RUP in concentrate feeds. This method in its current, strictly standardized form can be applied to develop a database which can be used for protein evaluation systems for establishing tabular values of IPD. However, future studies may be constricted since sufficient reference values are missing.

In conclusion it can be stated that there is still research needed to improve existing systems in order to optimise feeding strategies to meet the animals' nutrient requirements as well as minimising greenhouse gas (GHG) emissions and energy loss in agricultural production systems. This reseach should include the improvement of GHG estimation systems towards a more differentiated view to regional conditions and resources as well as an improvement of the protein evaluation system with standardised, easy to apply laboratory methods to estimate nutrient requirements for a more efficienct usage of local resources and co-products.

ZUSAMMENFASSUNG

Potenziale und Grenzen zur Verbesserung der Energie- und Sticktoffnutzungseffizienz in der Ernährung landwirtschaftlicher Nutztiere

Die vorliegende Arbeit beinhaltet drei verschiedene Themen, deren Zusammenhänge auf den ersten Blick nicht sofort erkennbar, dennoch aber stark miteinander verknüpft sind. Der erste Teil beschäftigt sich mit der Biokraftstoffproduktion und deren Koppelprodukten in der EU. Diese Koppelprodukte eignen sich als Proteinergänzungsfutter für Wiederkäuer, Schweine und Geflügel. Ergebnisse aus Versuchen mit laktierenden Kühen und Mastbullen zeigen, dass Nass- und Trockenschlempen, Rapsexktraktionsschrot und Rapskuchen als alleiniges Proteinergänzungsfutter durchaus die hohe Leistung der Tiere fördern. Trotz alledem müssen, vor allem in der Schweinefütterung, die Glucosinolatkonzentrationen bei Rapsprodukten durch verarbeitungstechnischen und züchterischen Fortschritt noch verringert werden. Dies ist unkritisch für Wiederkäuer, allerdings sollte eine bessere Qualitätsprüfung für Rapskuchen gewährleistet werden. Ein weiteres Koppelprodukt aus der Biodieselproduktion ist Glycerin. In Wiederkäuerrationen kann es in unterschiedlichen Reinheitsgraden zu einer besseren hygienischen Qualität von Mischfutter beitragen, ohne die physikalische Eigenschaften der Pellets zu beeinträchtigen. Die effiziente Nutzung der Biokraftstoff-Koppelprodukte trägt zu einer nachhaltigen Kraftstoffproduktion bei. Allerdings sollten zukünftige Recherchen alle Aufwände quantifizieren, die mit der Biokraftstoffproduktion und deren Koppelprodukten zusammenhängen, um eine präzisere CO_2-Bilanz ermitteln zu können.

Im zweiten Teil dieser Arbeit wird die Frage untersucht, ob es sinnvoll ist, Schätzgleichungen auf Basis von Futtermittel- und Futteraufnahmekenngrößen zu verwenden, um die Methanemissionen bei Wiederkäuern zu ermitteln. Um ihre praktische Eignung vergleichen zu können, wurden neun Schätzgleichungen auf fünf typische mitteleuropäische Rationen angewendet. Die kleinsten Unterschiede zum Mittelwert wurden bei den Schätzgleichungen festgestellt, welche die Neutral-Detergenzien-Faser als Variable benutzen. Die Standardabweichungen waren am höchsten in Gleichungen, die die Trockenmasseaufnahme als Variable benutzen. Somit waren diese Gleichungen am besten in der Lage, zwischen verschiedenen Rationen zu differenzieren. Die zum Teil großen Unterschiede in den Ergebnissen zeigen jedoch auch, dass bisherige Gleichungen ungenau sind. Generell sollte man eine Erweiterung der Datenbasis, mit deren Hilfe

Schätzgleichungen und Modelle gebildet werden, in Betracht ziehen. Dies sollte vor allem neue Messungen mit einbeziehen, sowohl am Einzeltier als auch auf Herdenniveau, bei denen typische, aktuelle und lokale Rationen gefüttert werden. Um die Anwendung von Schätzgleichungen in Zukunft einfacher zu gestalten, könnten diese zum Beispiel in Gruppen eingeteilt werden, welche die Herkunft der Daten und Rationsgestaltung genauer definieren. Im dritten Teil der Arbeit wurden 33 Futtermittel, mit einem Schwerpunkt auf ungeschützten und pansengeschützen Proteinergänzungsfuttermitteln mit einem enzymatischen *in vitro*-Verfahren (EIVP) analysiert, um die Dünndarmverdaulichkeit des Rohproteins zu bestimmen. Die Ergebnisse dieser Studie zeigen, dass das EIVP eine verlässliche und einfach anwendbare Methode ist, die sich besonders für verschiedene Konzentratfutter eignet. Diese Methode kann angewandt werden, um eine Datenbank zu schaffen, mit deren Hilfe Proteinbewertungssysteme verbessert und weiterentwickelt werden können. Es fehlen jedoch bei dieser Vorgehensweise noch Referenzwerte aus *in vivo*-/*in situ*-Versuchen.

Zusammenfassend kann festgestellt werden, dass Fütterungsstrategien noch weiter optimiert werden sollten, um den Bedarf der Tiere genauer zu decken und somit Treibhausgasemissionen und Energieverluste weiter zu verringern. Um dies bewerkstelligen zu können, ist es erforderlich, dass die Schätzung von Treibhausgasemissionen in der Landwirtschaft durch Gleichungen und Modelle noch genauer auf regionale Bedingungen eingeht. Proteinbewertungssysteme könnten durch einfachere und besser standardisierte Methoden noch genauere Empfehlungen geben, um regionale Ressourcen und Koppelprodukte effizienter nutzen zu können.

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ABBREVIATIONS

ADF	Acid detergent fibre expressed inclusive of residual ash
ADFom	Acid detergent fibre expressed exclusive of residual ash
BG	Brewers grain
CA	Crude ash
CF	Carbon footprint
CH_4	Methane
CL	Crude lipids
CO_2	Carbon dioxide
CP	Crude protein
DDGS	Dried distillers grain with solubles
DGS	Distillers grain with solubles
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
EMS	Ear-maize silage
EIVP	Enzymatic in vitro procedure
GHG	Greenhouse gas
GS	Grass silage
IPCC	Intergovernmental panel on climate change
IPD	Intestinal crude protein digestibility
ISIVP	In situ in vitro procedure
MBT	Mobile bag technique
ME	Metabolisable energy
MS	Maize silage
MUFA	Monounsaturated fatty acid
N	Nitrogen
NA	Not analysed
ND	Not detected
NDF	Neutral detergent fibre expressed inclusive of residual ash
NDFom	Neutral detergent fibre expressed exclusive of residual ash

NE	Net energy
NEL	Net energy for lactation
NPN	Non-protein Nitrogen
NSP	Non-starch polysaccharide
PUFA	Polyunsaturated fatty acid
RCP	Residual crude protein
RUP	Ruminally undegradable crude protein
RSC	Rapeseed cake
RSM	Rapeseed meal
SBM	Soybean meal
SFA	Short chain fatty acid
TMR	Total mixed ration
TP	True protein
uCP	Utilisable crude protein at the duodenum

1. GENERAL INTRODUCTION

Environmental issues and agriculture are two subjects that are closely related to each other. In a world where the population is growing and demand for food increases there is a need to use resources efficiently and at the same time keep the impact on the environment as low as possible. One possible option to achieve this is the use of by-products of different commodities that are produced for human needs. These by-products can be used as feedstuffs for several farm animals. As the populations grows the need for transportation increases as well. Road transport fuels are considered to contribute about 18% of Greenhouse Gas (GHG) emissions in the European Union (EEA, 2008; The Royal Society, 2008; Pinkney, 2009). Politicy makers considered the use of biofuels as an essential element to reduce the emissions from fossil fuel and to decarbonise transport fuels with a GHG reduction potential of at least 50% when compared to fossil fuel emissions (CONCAWE, EUCAR, JRC, 2007; RFA, 2008). Nevertheless, the use of biofuels is still a controversial issue. There is a public debate about pressure on land use and the competition between feed, food and fuel. The CO_2-saving effect of biofuel of the first generation, with by-products such as glycerine, oilseed meals and cakes, and distillers grains with solubles, depends on many factors, like processing, manufacturing and using appropriate feedstock (Windhorst, 2008; Fischer, 2009; Pinkney, 2009). When a by-product of sufficient quality is obtained it is well suited as an alternative to conventional feedstuffs. Another important issue that is of high interest is the contribution of methane (CH_4) from (dairy) cattle into the atmosphere. Methane is one of the major GHG which may contribute greatly to global warming. Globally, 1.3 billion cattle produce approximately 80 million tonnes of CH_4 a year, accounting for around one third of anthropogenic emissions of CH_4 (Jentsch et al., 2009). Cattle lose approximately 2-10% of their ingested energy as eructated CH_4, depending mainly on diet quality and feed intake level (Johnson and Johnson, 1995). Through optimised feeding strategies it may be possible to decrease CH4 emissions and energy loss. However, this is only one fraction of the answer to the problem – an optimised and efficient production cycle with high performance has the most potential to mitigate GHG emissions worldwide. In order to use feeds more efficiently standardised methods are needed to analyse nutrients and furthermore give precise advice for the animals' requirements. For example, crude protein (CP) values of feeds do not supply precise information about the protein that flows into and may actually be digested in the small

intestine of ruminants. The CP reaching the small intestine consists of both, the ruminally synthesized microbial CP as well as the feed CP that escaped ruminal degradation. In the ideal case, the animal is neither undersupplied nor oversupplied. Nitrogen losses through faeces and urine – after conversion outside the animal's body – contribute to environmental pollution, either as ammonia, nitrous oxide, N oxides in air, or as nitrate in soil and ground water. To meet the animal's requirements it is important to know the intestinal digestibility of the ruminally undegraded CP of the respective feedstuffs. There are several techniques that include *in vivo* and *in vitro* methods, and differ highly in complexity, cost and effort. The challenge is to find a simple, cheap and easy to standardise method that serves all demands and is helpful to support efficient feeding strategies for high performing animals. Growing agricultural production, high demand for food, food security, the emerging biofuel development and climate change are all linked to each other and in the future will all have a significant impact on the world food system.

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2. SCOPE OF THE THESIS

This is a cumulative thesis composed of three papers directly or indirectly addressing topics that are related to environmental aspects and feed efficiency in regard to farm animals and particularly their nutrition. The three main chapters (3 - 5) compile manuscripts that are formatted according to the regulations of the journal chosen for submission.

The third chapter, in a comprehensive review, adresses co-products from biofuel production from a European perspective. There are numerous co-products which are all suitable as protein supplements for ruminants, pigs and poultry. The objective of this chapter is to analyse and summarize results of studies dealing with by-products from biofuel production in farm animal nutrition under European conditions.

Chapter four of the thesis has a more theoretical approach. The most commonly used equations to estimate Methane emissions from dairy cows based on feed and intake characteristics are applied to five typical Central European diets. The general question is raised if it makes sense to use equations to estimate emissions. Most equations are imprecise and there is a high risk of getting lost in over- or underestimations. The objective of this study is to compare and interpret the equations' applicability in regard to dietary measures to mitigate CH_4 production and energy loss in dairy cattle.

Chapter five is a laboratory-based approach to study to estimate intestinal protein digestibilities (IPD) of the ruminal undegraded protein of several protein supplements. The application of a new enzymatic in vitro procedure lends hope to a more standardized and easy to execute method. This method in its current, strictly standardized form can be applied in order to develop a database which can be used for protein evaluation systems for establishing tabular values of IPD. A second objective of this study was to evaluate relationships and interactions between calculated IPD values and analysed chemical variables of feedstuffs.

3. BY-PRODUCTS FROM BIOFUEL PRODUCTION FOR FARM ANIMALS – AN EU PERSPECTIVE

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Hippenstiel, F., Südekum, K.-H., Meyer, U., Flachowsky, G. 2012. By-products from biofuel production for farm animals – an EU perspective. In Makkar, H.P.S., ed. Biofuel Co-Products as livestock feed opportunities and challenges, pp. 209-227. Rome, Italy, Food and Agriculure Organisation of the United Nations.

ABSTRACT

In the first part of this chapter, a brief history of by-products from bioethanol production is presented. By-products, like distillers grains are well known for their beneficial nutrient composition and have been used in animal nutrition already since the end of 19th century. Recent animal trials have shown that wheat-based dried distillers grains with solubles (DDGS) may replace protein supplements like soybean or rapeseed meals in dairy cow diets up to about 200 g/kg dry matter Other than maize-based DDGS in North America which are higher in fat, European wheat-based DDGS has not influenced milk fat content negatively. Moreover, trials with fattening bulls exhibited that DDGS as a main protein source is able to sustain a high productive performance. Trials with grower-finisher pigs suggested that DDGS up to 200g/kg diet did not influence the growth performance, fattening and slaughtering variables. Similarly, laying intensity of hens as well as egg quality and health were not affected by inclusion levels ranging from 150 g/kg to 300 g/kg diet. Trials with broilers suggest that diets that contain more than 100 g/kg DDGS may lower performance. Hence, it is recommended to add non-starch polysaccharide (NSP)-degrading enzymes (e.g., xylanase or xylanase mixed with other enzymes) to poultry diets rich in DDGS.

In the second part, a brief review and summary of data is presented on the use of glycerol for farm animals with emphasis on ruminants which will encompass the following topics: quality criteria for glycerol, rumen events and effects on feed intake and performance of dairy cows. For the benefit of a fail-safe usage of glycerol in diets of all farm animals, methanol should be removed from the glycerol as far as technically possible. Glycerol at different purities may help to stabilise the hygienic quality of pelleted compound feeds without compromising physical quality of pellets. Glycerol is a versatile feedingstuff in particular for ruminants. Data on ruminal turnover of glycerol would suggest that it should replace rapidly fermentable carbohydrates and thus, is not a direct competitor of propylene glycol. Previous studies have shown that glycerine may help to prevent ketoacidosis in high yielding dairy cows by increasing glucose precursors. Mature cattle can consume considerable quantities of glycerol (1 kg/day). However, greater dry matter intakes by cows supplemented with glycerine often did not result in increased milk or milk component yields. Further labour is thus required to fully explore the potential of glycerol in dairy cow diets but type of diet and route of glycerol administration seem to play important roles.

In the third part, again putting an emphasis on ruminants, the feeding value of rapeseed products such as rapeseed meal (solvent-extracted) and rapeseed cake (mechanically extracted) is reviewed. Rapeseed meal compare well with soybean meal for dairy cows if fed on an isonitrogenous basis. Milk and milk component yields were similar for diets containing soybean meal or rapeseed meal. The value of rapeseed cake would benefit from a standardization of the composition, because varying crude fat and crude protein concentrations makes the feeding value difficult to predict and could also affect storage stability of the cake. Even though the amino acid composition in rapeseed products is quite well balanced and favourable to non-ruminant animals, the sensitive reaction of pigs and poultry to glucosinolates in rapeseed meal and cake are still of concern. Therefore, it is recommended to add iodine, since glucosinolates act as antagonists. However, if glucosinolates are present in high concentrations, the negative effects may not be compensated, even if iodine is supplemented in high amounts. Concluding, it becomes evident that a more widespread use of rapeseed meal and rapeseed cake in diets for pigs and poultry requires further reduction of glucosinolate levels.

Finally, energy utilisation efficiency and sustainability of by-products from biofuel are addressed. Up to this day, no definite regulations exist in order to assign emissions either to the main product or the by-product(s). When considering the causation principle, the producer or the responsible party should be accountable for all emissions. However, drying of DGS is only of interest if the products will be utilized as feedstuffs for animals and thus emissions associated with processing of by-products are not of interest or necessity for biofuel producing companies.

Key words: feeding value, DDGS, glycerine, rapeseed cake, rapeseed meal, pig, poultry, ruminants

3.1. Introduction

Road transport fuels are considered to contribute about 18% of Greenhouse Gas (GHG) emissions in the EU (EEA, 2008; The Royal Society, 2008; Pinkney, 2009) with a consistent increase of about 1.6% per year (IEA, 2008a). Apart from more efficient cars and new transportation technologies, politics considered the use of biofuels as an essential element to reduce the emissions from fossil fuel and to decarbonise transport fuels. Some expert groups assessed the GHG reduction potential of biofuel being at least 50% of fossil fuel emissions (e.g., CONCAWE, EUCAR and JRC, 2007; RFA, 2008). Estimations by IEA (2008a) expect an increase in world biofuel consumption from 24.4 million tonnes oil equivalents (Mtoe) in 2006 to 94 Mtoe in 2020; 125 Mtoe in 2030 and approximately about 210 Mtoe in 2050 (about 6% of the global need; IEA, 2008a). In 2020 about 55 Mtoe of biofuel will be consumed in the United States and the EU.

Fischer (2009) analysed the relationships of emerging biofuel development, food security and climate change, concluding that the additional non-food use of crops will have a significant impact on the world food system. Therefore, higher plant yields and the continuous development of the second generation of biofuels, produced from woody or herbaceous non-food plant materials will receive increasing interest in the future (IEA, 2008b).

The CO_2-saving effect or the carbon footprints (CF) of biofuel of the first generation depends on many factors such as proper manufacturing, using the most appropriate feedstock, efficiency of feed production for fermentation, processing of by-products (e.g., drying), further use of by-products. The utilisation of by-products from biofuel production of the first generation such as glycerine, oilseed cakes and meals and distillers grains with solubles in wet (DGS) or dry (DDGS) form is an important controversial issue (see Windhorst, 2008; Fischer, 2009; Pinkney, 2009) that encompasses

- contribution in the reduction of GHG emissions,

- pressure on land use,

- competition between feed, food and fuel for crop yields.

By-products may contribute to mitigate this conflict. They contain less fat and starch than oilseeds and cereal grains, respectively but more fibre, proteins and minerals. The crude

protein (CP) concentration of the by-products varies between 300 and 400 g/kg dry matter (DM) and is similar to some traditional feed protein sources. All environmental and nutritional aspects and calculations (e.g., CF) should consider the whole processing chain and all final products. Crutzen et al. (2008) estimated the N_2O release from agro-biofuel production without considering by-products and their utilisation. They concluded that use of cereal grains and rapeseed for biofuel production is a very ineffective and environmental unfriendly way. However, in a more recent publication on this subject the same authors performed a life-cycle analysis and came to a similar conclusion, namely that biofuel production may trigger a net increase in global warming (Mosier et al., 2009).

The objective of this chapter is to analyse and summarize results of studies dealing with by-products from biofuel production in farm animal nutrition under European conditions.

3.2. By-products from bioethanol production

3.2.1. History

Distillers grains with solubles in wet and dry form are the most important by-products of alcohol production from cereal grains. The starch of the raw material is mainly fermented to alcohol. The by-product comprises of all the other components of the original substrate such as CP, ether extract, fibre and ash as well as the CP from yeast used for fermentation. Traditionally, DGS at DM concentrations at 40 - 90 g/kg has been fed to ruminants, horses and pigs in close proximity to the distilleries.

At the end of the 19th century many data about the composition and the feed value of distillers grain were available (e.g., Schulze and Maerker, 1872; Behrend and Morgan, 1880; both in Kellner, 1905). Already at that time it was known that the raw materials had the ability to influence the composition of DGS, Maerker (1908) described that the fermentation of cereal grains resulted in by-products (DGS) with the highest concentration of nutrients, and those from molasses with the lowest nutritive value, On the basis of the composition of the original substrate and the alcohol output the same author calculated the composition of DGS. In his famous textbook “The Nutrition of Domestic Animals”, Kellner (1905) summarized the composition (Table 3.1), digestibility (Table 3.2) and starch units for different by-products of ethanol production.

Table 3.1. Composition (g/kg dry matter unless stated) of distillery by-products (fresh and dried) of various origins (Kellner, 1905).

Source of by-product	Water (g/kg)	Crude protein	Crude fat (Ether extract)	Crude fibre	N-free extractives	Ash
Cereal grains, unspecified, dried	75	235	75	134	415	66
Maize grain, fresh	913	20	9	8	45	5
Dried	86	285	107	102	401	22
Molasses, fresh	922	19	-	-	40	19
Rye grain, fresh	922	17	4	7	46	4
Dried	100	165	82	162	478	13
Potatoes, fresh	943	12	1	6	31	7
Dried	100	243	37	95	408	117

Table 3.2. Mean digestibility coefficients (ranges in parentheses) of distillery by-products for ruminants and pigs (Kellner, 1905).

Source of by-product	Organic matter	Crude protein	Crude fat (Ether extract)	N-free extractives	Crude fibre
Ruminants					
Cereals grains, general	0.710 (0.600-0.810)	0.640 (0.490- 0.800)	0.940 (0.920- 0.940)	0.800 (0.540-0.850)	0.610 (0.410-0.920)
Maize grain	0.690 (0.660-0.720)	0.640 (0.610- 0.670)	0.930 (0.910- 0.950)	0.700 (0.700-0.710)	0.670 (0.640-0.700)
Rye grain	0.570 (0.450-0.680)	0.590 (0.520- 0.650)	0.620 (0.600- 0.640)	0.490 (0.440-0.540)	0.500 (0.370-0.620)
Pigs					
Cereal grains, general	0.580	0.780	0.560	0.510	0.360

Developments in distilling technology with consequences on composition and nutritive value of DGS during the last century were reported in several scientific publications (e.g., Naesi, 1985; Askbrant and Thomke, 1986), in animal feeding (e.g., Jensenet al.,1974; Firkins et al., 1985), as substrate for ensiling (e.g., Abrams et al., 1983, Flachowsky et al., 1990) and were summarized in various textbooks in Germany (e.g., Kling, 1928; Nehring, 1949; Becker and Nehring, 1967; Kling and Wöhlbier, 1983; Menke and Huss, 1987; Jeroch et al., 1993).

Due to the high demand of liquid fuels throughout Europe and the decreasing disposability

of fuels from fossil sources, the production of biofuel including bioethanol has gained more importance. The increased production capacity and the ascending number of large biofuel plants resulted in large amounts of DGS. It is unrealistic to distribute large amounts of DGS in nearby areas of the biofuel plant. Due to the short shelf-life of DGS, a large proportion is dried and used as DDGS. The nutritional quality of DGS and DDGS varies remarkably caused by the variability of the feedstock, the diversity of the production process and the proportion of solubles which are included in the final commodity (Belyea et al., 2004; Losand et al., 2009; Zijlstra and Beltranena, 2009). Intensive research on the use of mostly maize-based distillers grains in livestock has been conducted in North America over the past years (reviewed by e.g., Klopfenstein et al., 2008; Schingoethe et al., 2009). However, experiments that examine the nutritional value of DDGS common in Europe based on wheat, barley or rye grains, or mixtures of these grains are rare (Franke et al., 2009; Aldai et al., 2010; Meyer et al., 2010).

3.2.2. Nutritive value and feeding to ruminants

The chemical composition and energy concentration of DGS and DDGS from different grains are presented in Table 3.3. Distillers grains with solubles are high in CP with a considerable variation between the different types of grains used in the production process. The highest average CP content of 370 g/kg DM was reported for DDGS produced from a mix of 90% wheat and 10% barley (Franke et al., 2009, Losand et al., 2009, Meyer et al., 2010). Mustafa et al. (2000) reported that the ruminal escape of CP was lower for wheat- than barley-based DGS (490 versus 415 g/kg CP). Generally, distillers grains have a relatively high fibre concentration, with highest cell-wall (neutral detergent fibre, NDF) values found for barley-based distillers grains likely due to a greater hull proportion of grain DM.

Nutrient digestibility coefficients can be used to calculate metabolisable energy (ME) for ruminating animals (GfE, 1995). Therefore a number of experiments were carried out with adult wethers in order to evaluate the nutrient digestibility of rye DGS as well as wheat- or wheat/barley-based DDGS. The experimental diets consisted of grass hay, grass silage or straw supplemented with DDGS ranging from 15 to 75% of diet DM. The apparent total tract digestibility of organic matter, ether extract, crude fibre, NDF and acid detergent fibre (ADF) is shown in Table 3.4.

Table 3.3. Chemical composition and net energy (NE) concentration (g/kg of dry matter unless stated) of distillers grains with solubles in wet (DGS) or dry (DDGS) form from various sources. (unsp., unspecified; n.a., not analysed; NDF, neutral detergent fibre, ADF, acid detergent fibre)

		Mustafa et al. (2000)	Schingoethe et al. (2009)	Franke et al. (2009)	Losand et al. (2009)	Engelhard (2011)	Meyer et al. (2010)
Grain source		Barley, wheat and rye/triticale	Wheat	Wheat and barley	Wheat and barley	Rye	Wheat and barley
		DGS	unsp.	DDGS	DDGS	DGS	DDGS
Dry matter (DM)	g/kg	289	n.a.	923	934	n.a.	923
Crude protein		154	362	367	370	153	367
Ether extract		60	67	62	50	67	64
Ash		42	54	58	54	28	58
NDF		743	414	496	305	n.a.	490
ADF		311	173	159	155	n.a.	162
Starch		110	n.a.	n.a.	n.a.	54	n.a.
Sugar		n.a.	n.a.	n.a.	n.a.	45	n.a.
Calcium		n.a.	3.0	n.a.	n.a.	n.a.	n.a.
Phosphorus		n.a.	10.5	n.a.	n.a.	n.a.	n.a.
Sodium		n.a.	2.3	n.a.	n.a.	n.a.	n.a.
Magnesium		n.a.	6.0	n.a.	n.a.	n.a.	n.a.
Sulfur		n.a.	5.7	n.a.	n.a.	n.a.	n.a.
NE maintenance	MJ/kg	n.a.	9.13	n.a.	n.a.	n.a.	n.a.
NE gain	MJ/kg	n.a.	6.28	n.a.	n.a.	n.a.	n.a.
NE lactation	MJ/kg	n.a.	8.46	n.a.	n.a.	n.a.	n.a.
NE lactation	MJ/kg DM	n.a.	n.a.	n.a.	7.3	n.a.	n.a.

Table 3.4. Digestibility coefficients of nutrients measured in sheep according to GfE (1991) and estimated concentrations of metabolisable energy (ME) of distillers grains with solubles in wet (DGS) or dry (DDGS) form from rye, wheat or wheat/barley.

Authors	Alert et al. (2007)[1]	Losand et al. (2009)[1]	Meyer et al. (2010)[2]
Grain source + supplement	Rye + DGS	Wheat or wheat and barley + DDGS	Wheat and barley +DDGS
n	6	15	4
Organic matter	0.568 (±0.038)	0.758 (±0.048)	0.780 (±0.021)
Ether extract	0.598 (±0.302)	0.839 (±0.107)	0.914 (±0.010)
Crude fibre	0.515 (±0.100)	0.517 (±0.259)	
n		4	
NDF[3]		0.650 (±0.131)	
ADF[4]		0.544 (±0.110)	
ME (MJ/kg DM)	9.1	12.1	12.6

[1]Means with standard deviation in parenthesis
[2]Least squares means with standard error in parenthesis
[3]NDF, neutral detergent fibre
[4]ADF, acid detergent fibre

The digestibility of ether extract and fibre fractions showed the highest variation. When compared with rapeseed meal wheat- and barley-based DDGS had similar organic matter and ether extract digestibilities (Meyer et al., 2010). Organic matter digestibility of the rye-based DGS was notably lower and ranged from 0.531 to 0.619 (Alert et al., 2007). This reflects in a lower concentration of ME of rye DGS for which no obvious explanation exists. The ME concentration of wheat- and barley-based DDGS compared well with ME of rapeseed meal (RSM; Meyer et al., 2010).

Table 3.5 shows results of experiments with lactating dairy cows conducted in Germany and Austria that compared DDGS or DGS (mainly based on wheat) with other protein supplements like RSM or soybean meal (SBM). The aim of these studies was to investigate whether the different kinds of distillers grains can adequately replace RSM or SBM in diets of high yielding cows. Most of the rations comprised a considerable portion of grass silage and maize silage. The proportion of distillers grains in the diets ranged from 50 g (Urdl and Gruber, 2011) to 170 g/kg DM (Franke et al., 2009). The feed intake in all experiments varied between 21 and 24 kg DM/day and was not influenced by protein source. Mean milk yield and milk fat concentration across studies ranged from 26 to 43 kg/day and from 33 to 45 g/kg milk. However, no significant differences were detected within the experiments. Only one study showed a lower milk protein concentration yet no lower protein yield for cows fed

DDGS compared with RSM (Franke et al., 2009). In accordance with recommendations of Schingoethe et al. (2009) the outcome of the different experiments suggest that distillers grains can replace other protein supplements up to about 200 g/kg DM in dairy cow rations.

The results of trials with male calves and fattening bulls are presented in Table 3.6. Primarily wheat-based DDGS replaces RSM or SBM in maize silage or maize silage- and hay-based rations. The animals were fed DDGS from 140 g (Ettle et al., 2009) up to 200 g/kg DM (Preißinger et al., 2009) of the diets. No differences between protein sources were detected in DM, CP and ME intake as well as in live weight gain in both experiments with Simmental calves (Preißinge et al., 2009). Due to the higher final live weight the mean feed intake of Simmental bulls (Ettle et al., 2009) was higher (9.4 versus 7.7 kg DM/day) than that of Holstein bulls (Meyer et al., 2010). Simmental and Holstein bulls showed a good growth performance and live weight gain averaged about 1.55 and 1.40 kg/day. However, live weight gain differed significantly within experiments. Ettle et al. (2009) found differences between bulls fed DDGS (1.49 kg/day) and SBM (1.60 kg/d) which might be a result of the higher energy concentration of SBM as DM intakes were not different across treatments. Feeding a mixture of DDGS and RSM resulted in the highest weight gain (1.46 kg/day) compared with SBM, RSM or DDGS (1.31 kg/day; Meyer et al., 2010). The results of the experiments with fattening bulls showed that DDGS as the main protein source compares well with other protein supplements and is able to sustain a high productive performance. This does also indicate that differences between CP sources regarding the amino acid pattern of the ruminally undegraded CP (RUP) was not a constraint for intensive growth.

Table 3.5. Comparative evaluation of distillers grains with solubles in wet (DGS) or dry (DDGS) form mainly from wheat fermentation in diets for lactating dairy cows. (unsp., unspecified; MS, maize Silage; GS, gras Silage, RSM, rapeseed meal; BG, brewers grain; SBM, soybean meal; RSC, rapeseed cake)

Location	FLI Braunschweig1		LLFG Iden[2]		TLL Jena[3]		HBLFA Irdning[4]		
Authors	Franke et al. (2009)		Engelhard (2011)		Dunkel (2011)		Urdl and Gruber (2011)		
Duration (days)	147		50		unsp.	unsp.	60		
Cows (n)	16		36		126	123	3		
Basal diet	MS, GS		MS, GS		MS, GS		MS, GS, Hay		
Protein supplement	DDGS (wheat)	RSM	DWG (rye)	BG	DDGS (wheat)	SBM, RSM	DDGS (maize)	DDGS (wheat)	SBM, RSC
(kg dry matter [DM]/day)	3.5	3.6	ca. 3.8	ca. 1.9	ca. 1.8	ca. 1.5	ca. 1.1	ca. 1.0	ca. 1.2
DM intake (kg/day)	20.8	21.9	ca. 24.0	ca. 23.6	unsp.	unsp.	20.8	20.9	20.9
Milk (kg/day)	34.9	34.0	42.1	42.5	35.8	37.0	26.4	25.9	26.2
Fat (g/kg milk)	32.6	35.3	38.9	39.7	41.0	42.0	44.6	44.8	44.3
Protein (g/kg milk)	31.1[a]	32.9[b]	32.3	32.4	35.1	35.3	33.3	33.4	33.9

[a,b]Different superscripts in one column within an experiment indicate significant differences ($P<0.05$)
[1]Institute of Animal Nutrition, Friedrich-Loeffler-Institut (FLI), Federal Institute for Animal Health, Braunschweig, Germany
[2]Centre for Livestock Husbandry and Equipment, Regional Institute for Agriculture, Forestry and Horticulture Saxony-Anhalt (LLFG), Iden, Germany
[3]Agricultural Research Centre of Thuringia (TLL), Jena, Germany
[4]Institute of Livestock Research, Agricultural Research and Education Centre Raumberg-Gumpenstein (HBLFA), Irdning, Austria

Table 3.6. Comparative evaluation of dried distillers grains with solubles (DDGS) in diets for bulls during the whole fattening period and growing male calves before the beginning of the fattening period.

Location	LfL Poing[1]			FLI Braunschweig[2]				LfL Poing			
Authors	Ettle et al. (2009)			Meyer et al. (2010)				Preißinger et al. (2009)			
Animals (n)	44		42	15		14	15	21			
Final live weight (kg)	710	712	720	556	560	557	558	162	164	153	157
Basal diet	MS[3]			MS				MS, Hay			
Protein supplement	DDGS	SBM[4]	RSM[5]	DDGS	SBM	RSM	RSM + DDGS	DDGS	RSM	DDGS	RSM
(kg dry matter [DM]/day)	ca. 1.3	ca. 1.0	ca. 1.4	1.44	0.96	1.30	0.72 +0.74	0.42	0.44	0.59	0.58
DM intake (kg/day)	9.37	9.37	9.51	7.66	7.54	7.59	7.97	2.4	2.4	2.9	3.0
Crude protein intake (kg/day)	1.110	1.116	1.102	1.118	1.103	1.078	1.155	0.412	0.423	0.469	0.476
Energy intake (MJ ME[6]/day)	108.3	109.3	111.0	86.2	84.9	84.7	89.3	31.0	30.3	35.5	36.2
Live weight gain (kg/day)	1.493[b]	1.602[a]	1.549[ab]	1.310[b]	1.390[ab]	1.440[ab]	1.460[a]	1.008	1.039	1.003	1.053

[1] Institute for Animal Nutrition and Feed Management, Bavarian State Research Centre for Agriculture (LfL), Poing, Germany
[2] Institute of Animal Nutrition, Friedrich-Loeffler-Institut (FLI), Federal Institute for Animal Health, Braunschweig, Germany
[3] MS, maize silage
[4] SBM, soybean meal
[5] RSM, rapeseed meal
[6] ME, metabolisable energy
[a,b] Different superscripts in one column within an experiment indicate significant differences ($P<0.05$)

3.2.3. Nutritive value and feeding to non-ruminants – pigs

By-products from biofuel production such as DDGS have been fed also to non-ruminant animals, particularly pigs (e.g., Lindermayer, 2004; Richter et al., 2006a; Berk, 2007; Hackl et al., 2007; Berk et al., 2008; Kluge and Kluth, 2008) and poultry (e.g., Damme and Pegeanova, 2006; Richter et al., 2006b; Trautwein et al., 2008). Patience et al. (2007) summarized mainly North American results from feeding studies with DDGS in pigs.

Some authors investigated the amino acid pattern of DDGS and their praecaecal digestibility in pigs (e.g., Richter et al., 2006a; Hackl et al., 2007; Hackl et al., 2007; Kluth et al., 2009). Hackl et al. (2007a) and Hackl et al. (2007b) studied a wheat-DDGS with 386 g CP per kg DM. Compared to wheat (32 g lysine per kg CP) DDGS contained only 17 g lysine per kg CP. The low concentration and the low praecaecal digestibility coefficient of lysine in wheat-DDGS (0.69 compared with 0.872 for wheat) underline the significance of lysine as the first-limiting amino acid in DDGS for pigs. Although DDGS contains about 2.5-3 times more CP than wheat, it only 1-1.5 times the concentration of praecaecally digestible lysine. Very low praecaecal digestibilities have been reported by Hackl et al. (2007a) and Hackl et al. (2007b) only for sulphur-containing amino acids (0.67 – 0.69), but not for most of the other essential amino acids. In broilers, however, Kluth et al. (2009) measured a praecaecal digestibility coefficient for lysine in DDGS of 0.79.

In a feeding trial with 80 growing-finishing pigs (40 females and 40 castrated males) from 35 kg initial live weight up to 115 kg slaughtering weight, Berk (2007) partially replaced SBM and/or RSM by DDGS or a DDGS/RSM mix (Table 3.7). The feed in mash form and drinking water were offered for ad lib intake. Feed intake, total weight and slaughtering results were not influenced ($P>0.05$) by protein source. From this data it can be concluded that DDGS can partially replace SBM in diets for growing-finishing pigs in intensive production systems.

Table 3.7. Protein sources (grower/finisher), feed intake, daily weight gain and some slaughtering data of pigs (Berk, 2007).

Protein source		Soybean	Soybean/rapeseed meal	Soybean/DDGS[1]	Soybean meal/ rapeseed meal/ DDGS
Soybean meal	Grower	15.0	6.0	8.0	6.0
	Finisher	11.0	(-)	5.0	3.0
Rapeseed meal	Grower	(-)	10.0	(-)	5.0
	Finisher	(-)	15.0	(-)	6.0
DDGS	Grower	(-)	(-)	8.0	5.0
	Finisher	(-)	(-)	10.0	6.0
Crude protein (g/kg dry matter)	Grower	178	176	178	175
	Finisher	163	166	166	169
Feed intake (kg/(animal x day))	total	2.83	2.81	2.83	2.76
Weight gain (g/(animal x day))		1010	959	998	940
Lean meat (%)		54.4	55.6	54.7	55.7
Backfat thickness (mm)		29.0	28.0	28.4	25.1
Backfat fatty acids					
SFA[2]		40.5	40.1	41.1	39.2
MUFA[3]	(% of total)	47.4	49.5	46.8	48.8.
PUFA[4]		12.1	10.4	12.0	12.4

[1]DDGS, dried distillers grains with solubles
[2]SFA, short-chain fatty acids
[3]MUFA, monounsaturated fatty acids
[4]PUFA, polyunsaturated fatty acids

Richter et al. (2006a) carried out four feeding trials with piglets (0 - 100 g/kg DDGS in the diet; Table 3.8) as well three trials with growing-finishing pigs (0 - 250 g/kg DDGS in the diets; Table 3.9). The authors concluded that piglets below 10 kg live weight should not consume DDGS and diets of heavier piglets may contain DDGS up to 100 g/kg diet.

Table 3.8. Average live weight gain (g/day) of piglets (18-65 animals per treatment; initial age: 28-48 days; final age: 70 days) fed with various amounts of wheat-based dried distillers grains with solubles (DDGS; Richter et al., 2006a).

DDGS (g/kg of diet) Trial	0	30	50	80	100
1	480[a]	440[bd]	448[bc]	417[d]	-
2	518	-	-	-	505
3	445[a]	-	408[ab]	-	346[c]
4	364	-	353	-	361

[a,b,c,d] different indices indicate significant differences (P<0.05)

Table 3.9. Average live weight gain (g/day) of pigs (15-36 animals per treatment; initial live weight: 27-32 kg; final live weight: 112-121 kg) fed with various amounts of wheat-based dried distillers grains with solubles (DDGS; Richter et al., 2006a).

DDGS (g/kg of diet) Trial	0	100	150	200	250
1	791	784	787	-	-
2	834a	-	827a	-	745b
3	932	905	-	939	-

[a,b] different indices indicate significant differences (P<0.05)

The results suggest that DDGS up to 200 g/kg diet in grower-finisher diets of pigs did not influence the performance. The lower recommended inclusion level for piglets is most likely due to the low lysine content of the DDGS. Hence, higher inclusion levels may be possible if lysine levels are adjusted as well. Kluge and Kluth (2008), Punz et al. (2010) and Schedle et al. (2010) replaced SBM in grower-finisher diets completely by DDGS and did not observe any adverse effect on fattening and slaughtering variables. Additional non-starch polysaccharide (NSP) enzyme supplementation did not improve animal performance.

Another important aspect of DDGS incorporation in pig diets is the P excretion, which is a major concern for the swine industry due to its potential impact on the environment. There are no European studies on this subject so far. A Canadian study evaluated the effect of wheat-based DDGS on P excretion patterns of grower-finisher pigs. Intake, excretion and retention of P were influenced by DDGS. Total tract P digestibility of DDGS was 40 percentage units higher than that of wheat. Similarly daily P excretion of pigs fed DDGS was higher than that of pigs fed the wheat control diet (Widyaratne and Zijlstra, 2007). In another study conducted in North America measured, among others, P in maize-based DDGS fed to growing pigs. Apparent total tract digestibility for P in DDGS was measured at 59.1% while the control group fed a maize-based diet had apparent total tract digestibility of 19.3%. It was concluded that with DDGS a greater proportion of the organic P will be digested and absorbed, hence, lowering the need to add inorganic P to pig diets (Pedersen et al., 2007).

3.2.4. Nutritive value and feeding to non-ruminants – poultry

Richter et al. (2006b) included up to 200 g/kg wheat-based DDGS to diets of chicks, pullets, laying hens and broilers. No effect of DDGS inclusion level on growth performance of chicks and pullets was observed (Table 3.10).

Table 3.10. Influence of dried distillers grains with solubles (DDGS) on live weight and feed conversion ratio (FCR) of chicks and pullets (average of two trials; 168 animals per treatment; Richter et al., 2006b).

DDGS (g/kg of diet)	0	50	100	150	200
Live weight (g)					
8 weeks	654	654	658	644	656
18 weeks	1432	1439	1448	1429	1435
FCR (kg/kg, feed/gain)					
0-8 weeks	3.16	3.18	3.17	3.17	3.16
0-18 weeks	5.12	5.13	5.08	5.09	5.10

Laying intensity of hens as well as egg quality were not affected (P>0.05) by 150 g/kg DDGS in diets of laying hens (Damme and Peganova, 2006; Richter et al., 2006b). Askbrant and Thomke (1986) did not observe any negative effect on egg yield and health of laying hens fed

diets with 300 g/kg DDGS.

Richter et al. (2006b) carried out three feeding studies with 276 broilers per treatment (unsexed). The diets contained 0, 50, 100, 150 or 200 g/kg DDGS and was offered in pelleted form from day 1-14; mash feed was fed from day 15-33. The final live weight of the broilers amounted to 1995, 1987, 1953, 1884 and 1842 g per animal for DDGS inclusion levels of 0, 50, 100, 150 and 200 g/kg, respectively. These results suggest that diets that contain more than 100 g/kg DDGS may lower performance, which is in agreement with Chidothe et al. (2002a), Chidothe et al. (2002b) and Trautwein et al. (2008).

Other authors added NSP-degrading enzymes (e.g., xylanase or xylanase mixed with other enzymes) to poultry diets rich in DDGS. In addition to an improved energy supply due to partial degradation of NSP and subsequent absorption of its constituent sugars (reviewed by Dänicke, 1999), the supplementation of xylanase is supposed to change the composition and metabolic potential of bacterial populations and may also influence fat absorption in younger animals (Hübner et al., 2002). Dalibard et al. (2008) added a NSP-enzyme produced by *Penicillium funiculosum* to diets of layers containing 100 or 200 g/kg maize-based DDGS. Enzyme supplementation did not increase nutrient digestibilities and energy concentration, but enzyme-supplementation of diets with 100 and 200 g/kg DDGS increased apparent ME concentration by 0.24 and 0.18 MJ/kg DM. Richter et al. (2006b) measured higher final live weight of chicks and pullets after enzyme supplementation to a diet with 150 g/kg DDGS. However, laying hens did not respond to enzyme supplementation. Chidothe et al. (2002a), Chidothe et al. (2002b) measured higher live weight gain of broilers fed with 100 and 200 g/kg enzyme-supplemented DDGS, but the gain was still below the level of the control group without DDGS. Similar results have been reported by Trautwein et al. (2008) after feeding diets with 100 g/kg DDGS.

Another important aspect which needs to be considered is the availability of P. Studies referring to wheat-based DDGS, the most common DDGS source in Europe, is reviewed in another chapter in this document, which provides a more in-depth account of wheat DDGS in poultry (Noblet et al., 2012). Studies on maize-based DDGS reported a substantial variability in relative P bioavailability among different batches, which seems mainly due to different heating conditions employed during processing. During the process of fermentation for bioethanol production, small quantities of phytase are produced by the yeast, converting the P into better available forms (Martinez Amezuca et al., 2004).

3.3. By-Products from Biodiesel Production

3.3.1. Glycerine

Biofuel production in the European Union is mainly based on rapeseed oil, basically in form of rapeseed oil methylester or biodiesel, leaving glycerine as a co-product. During biodiesel generation fatty acids are hydrolyzed from the glycerine backbone of the triglyceride molecule by a transesterification process using methanol. Subsequent to separation of the fatty acid esters, glycerine still contains methanol and salts from the reactions. Separation or purification of glycerine can be fluctuating depending on the plant and the applied process (Schröder and Südekum, 1999). Yield of glycerine from this process is approximately 1unit per 10 units of biodiesel produced (Friedrich, 2004).

Starting around 60 years ago, researchers have shown that glycerine may help prevent keto-acidosis in the high-yielding dairy cow by increasing glucose precursors (Forsyth, 1953; Johnson, 1954; Fisher et al., 1971; Fisher et al., 1973). Around 40 years ago, glycerine was registered as a feed additive (E 422) in the European Union (Anonymous, 1970) with no restrictions as to animal species and quantity added to feeds. Today, glycerine is listed as a feedstuff in the “Positive List” of authorized feed materials (Central Committee of the German Agriculture, Standards Commission for Straight Feeding Stuffs, 2011) while research expanded not only in dairy cattle but also in other farm animals to elucidate the conditions under which glycerine may be used advantageously. The reader is referred to two other chapters in this document which provide a more in-depth account of inclusion of glycerine in transition and lactating cow diets and of swine energy value, metabolism, contaminants, feeding levels, and performance and carcass composition .

3.3.3. Glycerine quality

Glycerine may be obtained with varying quality, depending on the degree of refinement. Schröder and Südekum (2002) analyzed the chemical composition of glycerine at different stages of the rapeseed oil methylester production process (Table 3.11). Important to notice is that the impure quality with elevated methanol concentrations (267 g/kg DM) was not a commodity but an intermediary product that was used for experimental purposes only. For the benefit of a fail-safe usage of glycerine in diets for all farm animals, methanol should be removed as far as technically possible.

Table 3.11. Chemical composition of glycerine representing different stages of the rapeseed oil methylester production process (Schröder and Südekum, 2002).

	Purity of glycerine		
Item	Low	Medium	High
Water (g/kg)	268	11	25
Dry matter composition (g/kg unless stated)			
Glycerine	633	853	998
Crude fat	7.1	4.4	NA
Phosphorus	10.5	23.6	NA
Potassium	22.0	23.3	NA
Sodium	1.1	0.9	NA
Lead (mg/kg)	3	2	NA
Methanol	267	0.4	NA

NA, not analysed; analyses were omitted because the glycerine content was close to 1000 g/kg

Table 3.12. Standardized composition (g/kg) of two different glycerine qualities according to the German "Positive List" (Central Committee of the German Agriculture, Standards Commission for Straight Feeding Stuffs, 2011).

Item	Glycerine	Glycerine, crude
Glycerine	Minimum 990	Minimum 800
Water	5 - 100	100 – 150
Ash	Maximum 1.0	Maximum 100
Methanol	ND	Maximum 2.0
Other	-	NaCl, K, P, S

ND, not detected

Table 3.12 presents two different glycerine qualities according to the German "Positive List" (Central Committee of the German Agriculture, Standards Commission for Straight Feeding Stuffs, 2011). Crude glycerine is the quality currently used in farm animal feeding and it is strongly recommended that at least the specifications listed should be declared on each batch of crude glycerine. Due to legal restrictions as to the use of animal products in farm animal feeding and because crude glycerine may contain some residual fat, the source of the glycerine must also be known and stated.

Südekum et al. (2008) investigated physical, chemical and hygienic quality characteristics of pelleted compound feeds with varying quality glycerine (Table 3.11) inclusion levels of 50,

100 and 150 g/kg concentrate DM. The quality of the concentrates was assessed under two environmental conditions (15 °C and 60% relative humidity; 20 °C and 70% relative humidity) and storage durations of four and eight weeks. The chemical composition was only slightly affected by concentration and purity of glycerine or by storage and duration influences. Moreover, the data indicated that glycerine of different purities had a preserving effect and the physical quality of the pellets was not affected by purity or concentrations of glycerine. However, Löwe (1999) noted that when pellets were produced with molasses and glycerine concentrations greater than 50 g/kg, pellets showed a rough and scaly surface. This author also remarked that when feeds are stored in meal form, concentrations greater than 50 g glycerine/kg may result in lump formation, and therefore suggested to restrict glycerine concentration in pelleted compound feeds to 60 - 70 g/kg based on general storage behaviour including storage in large silos.

In conclusion, glycerine of different purities as a by product from rapeseed oil methylester production may help stabilise the hygienic quality of pelleted compound feeds without compromising physical quality of the pellets.

3.3.4. Rumen events of feeding glycerine

Previous studies on ruminal metabolism of glycerine indicated that glycerine is rapidly and extensively fermented in the rumen with propionic acid as the major product of fermentation (Bergner et al., 1995; Kijora et al., 1998). However, there is controversial information regarding the exact biochemical pathway and the end products of glycerine fermentation by ruminal microbes. Ferraro et al. (2009) measured *in vitro* gas production from glycerine lucerne and maize silage. Results indicated that glycerine has a long lag time and a slow rate of degradation. Moreover, glycerine fermentation resulted in reduced acetate and increased butyrate concentration. Krueger et al. (2010) evaluated the *in vitro* effect of two levels of glycerine (20 or 200 g/kg) on their inhibitory effect against ruminal lipolysis by mixed rumen microbes as well as the effect of feeding various amounts of glycerine on fermentation kinetics of lucerne hay. They concluded that an inclusion rate of up to 200 g/kg decreased the rate of free fatty acid accumulation, decreased fermentation rate but appeared to have no negative effect on NDF digestibility. The authors suggested that utilizing glycerine as a short-term feed ingredient in cattle diets can potentially inhibit bacterial fat degradation.

Schröder and Südekum (2002) evaluated *in vivo* effects of glycerine in compound feeds on

nutrient turnover in the rumen and digestibilities in the whole tract of cattle. Four ruminally cannulated steers were used in a 4 x 4 Latin square design and received a mixed diet consisting of 400 g/kg DM forage and 600g/kg DM concentrate. Concentrate in pelleted form comprised either no glycerine or 150 g/kg glycerine of different purities (630, 850 or >995 g/kg glycerine). Feeding glycerine resulted in a slight shift towards a reduced ratio of acetic acid towards propionic acid. Rumen fill was slightly higher when diets contained glycerine. Furthermore, glycerine appeared to have an impact on water turnover since the proportion of bailable liquids of total ruminal contents was higher when diets contained glycerine irrespective of quality. No effect on fermentation of fibre components was observed *in vivo*, however, when glycerine was supplemented to a medium containing cellobiose as the sole energy source (Roger et al., 1992), it inhibited the growth and cellulolytic activity of two rumen cellulolytic bacterial species (*Ruminococcus flacefaciens, Fibrobacter succinogenes*). The growth of the anaerobic fungal species, *Neocallimasix frontalis*, was inhibited as well and its cellulolytic activity almost completely disappeared. Another study by Abo El-Nor et al. (2010) measured the effects of substituting maize grain with glycerine at different levels (36, 72, 108 g/kg DM) on deoxyribonucleic acid (DNA) concentration of selected rumen bacteria using continuous fermenters. The DNA concentration for *Butyrivibrio fibrisolvens* (fibre degradation) and *Selenomonas ruminantium* (starch and sugar degaradation) were reduced when glycerine at levels 72 and 108 g/kg DM was supplemented. However, implications derived from this data about the inhibition of bacterial and fungal growth could be caused by both, specific *in vitro* conditions such as the single species and sole substrate conditions.

The *in vivo* data indicated that there should be no negative effects on ruminal turnover and digestibilities of organic matter constituents in the total tract when glycerine is used as a substitute for rapidly-fermentable starch sources like wheat or maize grain. Further, possible effects of glycerine on rumen microbial protein metabolism may require more detailed investigations. Paggi et al. (1999) investigated the *in vitro* effect of increasing levels of glycerine (50, 100, 200, 300 mM) on the proteolytic activity of bovine rumen fluid and found that all concentrations of glycerine reduced proteolytic activity by 20%. Kijora et al. (1998) infused 400 g glycerine per day (corresponding to 100 g/kg DM intake) into the rumen of growing bulls which were fed a hay-grain diet. They observed lower concentrations of isobutyric and isovaleric acid in the rumen and concluded that fewer branched-chain amino acids had been degraded. A slower rumen microbial crude protein and amino acid degradation would primarily increase the protein value of fermented forages.

3.3.5. Dairy cow performance in response to glycerine

Previous studies have shown that glycerine may help to prevent ketoacidosis in high yielding dairy cows by increasing glucose precursors (Forsyth, 1953; Johnson, 1954; Fisher et al., 1971; Fisher et al., 1973; Sauer et al., 1973). In the majority of these trials glycerine was applied as an oral drench. Recent research has focussed on using glycerine either as a dietary supplement or as a partial replacement for starchy dietary ingredients.

Khalili et al. (1997) fed grass silage for ad libitum consumption and 7 kg per day of a barley based concentrate to mid lactating Friesian cows. Barley was partially replaced with either glycerine, a fractionated vegetable fatty acid blend or a 1:1 mixture of glycerine and free fatty acids. Glycerine intakes (150 g/day) had no effects on intake or performance, however the combination of glycerine and free fatty acids tended to increase milk yield. DeFrain et al. (2004) fed complete diets to Holstein cows from 14 days prepartum to 21 days postpartum. Diets were top-dressed with 860 g maize starch (control), 430 g maize starch and 430 g glycerine, or 860 g glycerine (day x cow). Rapidly fermentable glycerine replaced a slowly and incompletely fermentable carbohydrate source. Prepartum dry matter intake was greater for cows fed the control when compared with the two glycerine-supplemented diets. Rumen fluid collected postpartum from cows who received a glycerine supplemented diet had greater total volatile fatty acids, greater molar proportions of propionate and a decreased ratio of acetate to propionate. Furthermore, concentrations of butyrate seemed to be greater in rumens of cows fed glycerine-supplemented diets. Yield of energy-corrected milk during the first 70 days postpartum tended to be greatest for cows fed the control diet. Since the only observed effect of glycerine-supplemented diets prepartum was on dry matter intake the authors suggested that glycerine should be delivered as a drench in hypoglycaemic dairy cows and not fed as a component of transition dairy cow diets. Bodarski et al. (2005) observed an increase in β-hydroxybutyrate in blood serum as well after adding 500 mL glycerine per day for the first 70 days postpartum. However, glycerine supplementation decreased total non-esterified fatty acid levels when compared to the non-supplemented controls. Other than DeFrain et al. (2004), Bodarski et al. (2005) observed that cows which consumed the glycerine diet exhibited a higher dry matter intake and gave 13 to 18% more milk than the control groups.

Recently, two German groups investigated glycerine in diets for dairy cows in direct comparison with propylene glycol. Engelhard et al. (2006) supplemented the same calculated amounts per cow of both, glycerine and propylene glycol prepartum (150 g/day) and postpartum (250 g/day). Energy-corrected milk yields as well as concentrations of milk fat

and protein were not different between cows fed propylene glycol or glycerine. Nevertheless, the authors observed that older cows (> second lactation) which received the glycerine supplemented diet consumed more DM and thus energy. Blood levels of indices of ketosis such as β-hydroxybutyrate and non-esterified fatty acids were not different between groups.

3.3.6. Rapeseed meal and rapeseed cake – ruminants

Rapeseed meal is still considered to be an important source of high-quality protein for all farm animal species and especially for ruminants. Approximately 4.4 million tons of RSM were produced in Germany in the year 2008, from which 3 million tons were used for domestic consumption exclusively (Weiß and Schwarz, 2010). It can be assumed that the main part was utilized as protein supplements in ruminant nutrition. One of the main reasons for this may be the low cost of RSM in comparison to imported SBM. Moreover, techniques to extract RSM, including physical pressure and high temperatures, are responsible for an increased fraction of CP which is protected from ruminal degradation.

Protein values of SBM and RSM published in feeding value tables and research papers differ to great extents. The concentration of RUP is stated as 350 g/kg CP for SBM and 250 g/kg CP for RSM (Universität Hohenheim – Dokumentationsstelle, 1997). Similarily, mean values calculated from data reported in the feed composition table of the ARFC (1993) resulted in 280 g RUP/kg CP for RSM and 370 g RUP/kg CP for SBM at a rumen outflow rate of 5%/h.

However, more recent experiments indicate that the considerable differences between the tabulated ruminal degradability values of the two meals in favour of SBM no longer reflect the current situation. A cross-sectional study conducted by Südekum et al. (2003; Table 3.13) covered all oilmills processing rapeseed and soybean in Germany and in addition encompassed some imported SBM commodities.

Table 3.13. Protein value of contemporary qualities of rapeseed (RSM) and soybean (SBM) meals (Südekum et al., 2003) as compared with feeding table values

Item	RSM	SBM
Mean RUP[a], g/kg of crude protein	300	300
DLG Table (Universität Hohenheim – Dokumentationsstelle, 1997)	250	350
Mean uCP[b], g/kg dry matter	231	288
DLG Table (Universität Hohenheim – Dokumentationsstelle, 1997)	219	298 – 308

[a]RUP, ruminally undegraded crude protein

[b]uCP, utilisable crude protein at the duodenum (sum of microbial and ruminally undegraded crude protein)

A total of 15 studies published between 1983 and 1997 could be identified (Rooke et al., 1983; Mir et al., 1984; Voigt et al., 1990; Kendall et al., 1991; Tuori, 1992; Zinn, 1993; Khorasani et al., 1994; Liuet al., 1994; Moss and Givens, 1994; Vanhatalo et al., 1995; Stanford et al., 1995; Stanford et al., 1996; Gralak et al., 1997; Mustafa et al., 1997; Zebrowska et al., 1997). Nine studies observed greater RUP values (g/kg CP) for SBM than RSM, three studies reported the opposite and three studies noticed no differences between RUP values for SBM and RSM. Moreover, RUP values varied largely in all studies, more precisely results for SBM ranged between 200 to 500 g/kg CP and from 120 to 560 g/kg CP for RSM. Thus, data reported by Südekum et al. (2003) appears acceptable and may more closely mimic recent and current SBM and RSM qualities than tabular values. In conclusion it can be stated that it is currently recommended to state a mean RUP concentration of 300 g/kg CP for RSM and SBM (Südekum and Spiekers, 2002).

Other recent experiments tested the hypothesis that SBM can be fully replaced by RSM in dairy cow diets when fed on an approximate isonitrogenous and isocaloric basis (without considering differences in ruminal degradation and/or amino acid pattern. Table 3.14 summarizes the data and indicates that milk yield and milk component concentrations were similar for diets containing SBM or RSM, and thus the hypothesis can still be sustained. The energy concentration of the whole diet seems to be a key factor for the successful replacement

of RSM for SBM as lower energy concentrations generally mean insufficient DM intakes and this may be further aggravated if RSM (moderate energy density) is included at the expense of SBM (high energy density).

Steingass et al. (2010) tested in which concentrations rapeseed cake could replace SBM. A feeding trial, including 60 dairy cows and 7 time periods (4 control + 3 periods with rapeseed cake or rapeseed cake plus RSM) revealed higher DM intake and milk yield as well as lower milk fat and protein values when rapeseed cake was fed. The authors suggested that even though rapeseed cake and RSM differ widely in their protein values, both feedstuffs can be regarded as suitable full protein supplements in diets for dairy cows.

Moreover it should also be pointed out that the overall quality of RSM and rapeseed cake depends also on the concentration of glucosinolates and, in case of rapeseed cake, the content and quality of the lipid proportion. Generally, average glucosinolate concentrations of RSM are low while glucosinolate concentrations of rapeseed cake are considerably higher. However, a great variation of this item applies to both feedstuffs. In addition, crude fat in rapeseed cake fluctuates, making ration formulation a difficult task. Increasing crude fat content lowers CP concentrations and vice versa. Hence, grouping of rapeseed cakes according to crude fat concentration (g/kg) appears necessary. Additionally, storage stability should also be considered, since the fat is in a non-protected form after the mechanical extraction of the seed. It has also been reported by farmers and consultants that physical characteristics resulting from plaque forming during oil extraction may handicap rapeseed cake handling, e.g. a homogenous distribution in complete diets or silage mixtures is difficult to achieve.

Table 3.14. Comparative evaluation of rapeseed (RSM) and soybean (SBM) meals in diets for high-producing dairy cows - summary of German trials (Spiekers and Südekum, 2004; Steingass et al., 2010).

Location, duration of trials and diets	Protein supplement kg/(day x cow)	Milk kg/day	Fat g/kg milk	Protein g/kg milk
LWZ Haus Riswick[5]: lactation weeks 5 - 35				
Basal diet	SBM 2.3 kg	31.1	39	31
1/3 MS[1] + 2/3 GS[2]	RSM 3.1 kg	31.3	39	32
LWZ Haus Riswick: lactation weeks 2 – 44				
TMR[3] with	SBM 1.6 kg	25.2	42	34
50% MS + 25% GS	RSM 2.2 kg	25.8	41	34
LLFG Iden[6]: until lactation week 17				
TMR[3] with 40% (MS +	SBM 4.0 kg	40.0	38	33
EMS[4]) + 25% GS	RSM 4.3 kg	40.5	39	33
LVA Köllitsch[7]: 17 weeks				
Basal diet	SBM 1.6 kg	31.2	39	34
50% MS + 50% GS	RSM 2.0 kg	32.7	40	34
Universität Hohenheim[8]:				
TMR[3] with	SBM 1.2 kg	30.9	45	35
22% MS + 21% GS	RSM 1.8 kg	32.4	43	35

[1]MS, maize silage
[2]GS, grass silage
[3]TMR, totally mixed ration
[4]EMS, ear-maize silage
[5]Chamber of Agriculture of North Rhine-Westphalia, Landwirtschaftszentrum (LWZ) Haus Riswick, Kleve, Germany
[6]Centre for Livestock Husbandry and Equipment, Regional Institute for Agriculture, Forestry and Horticulture Saxony-Anhalt (LLFG), Iden, Germany
[7]State Office for Environment, Agriculture and Geology, Lehr- und Versuchsgut (LVA) Köllitsch, Germany
[8]Institute of Animal Nutrition, University of Hohenheim, Stuttgart, Germany

3.3.7. Rapeseed cake and meal – pigs and poultry

Other than ruminants, pigs and poultry react more sensitive to the glucosinolate content in rapeseed meal and cake. Even though the amino acid composition in rapeseed products is well balanced and favourable to monogastric animals, there are two limiting factors: the concentration and structural type of glucosinolates and the dietary fibre. There are two different types of glucosinolate including aliphatic glucosinolate derived from methionine and indole glucosinolate derived from tryptophan. Aliphatic glucosinolate, which causes the most negative antrinutritive effect, may be reduced by plant breeding to levels close to zero while indole glucosinolate contributes with 2-4 µmoles/g seed (Sørensen, 1990). The high content of fibre and fibre-associated CP, contributes to a relatively low digestibility of CP and energy in RSM. This is mainly due to the high lignin content of the hull, which may vary largely (47 - 517 g/kg) depending on genotype and processing of the seed (Jensen et al., 1990). Table 3.15 presents average amino acid contents of SBM, RSM and wheat. Lysine content of RSM is slightly lower than that of SBM, however threonine and sulfur amino acids (methionine, cysteine) are higher in RSM.

Table 3.15. Amino acid profiles (g/100 g crude protein) of rapeseed meal, soybean meal and wheat (Degussa Feed Additives, 1996)

	Rapeseed meal	Soybean meal	Wheat
Lysine	5.6	6.3	2.8
Methionine+Cysteine	4.6	3.0	3.8
Threonine	4.4	4.0	2.9
Tryptophan	1.3	1.3	1.2

The acceptance of using RSM in pig diets increased highly in the last years. This is mainly due to the beneficial price as well as declined concentration of glucosinolates and an improved quality monitoring. Moreover, RSM reveals similar values for protein quality when compared with SBM, however lysine concentration and digestibilities are lower in RSM. For the practical use this means that other protein supplements or free amino acids should compensate the loss. In contrast, RSM includes higher concentrations of sulphur amino acids than SBM.

Several trials throughout Germany were performed in order to ascertain the tolerance towards the maximum supplementation of RSM in pig diets. In early trials, amounts of 50 g/kg for

growing and 100 g/kg RSM for finishing pigs replaced SBM as a protein supplement in the diet. As a result no differences were observed between groups receiving RSM or SBM. The proximate trial increased the amount of RSM to 100, respectively 150 g/kg in the diets. Similarly, no differences in performance and carcass quality were observed when compared with pigs that were fed SBM. Concluded it can be stated that diets may contain 100 g/kg RSM in the grower diet (40 – 70 kg live weight) and 150 g/kg RSM in the finishing diet (70 -120 kg live weight). It is recommended that piglets, which are more sensitive to glucosinolate and high fibre concentrations can receive up to 50 g/kg RSM in diets and may also tolerate levels of up to 100 g/kg RSM (12 -15 kg live weight). However, levels of glucosinolates should not exceed 10 mmol/kg RSM (Weiß and Schöne, 2008; Weber, 2010; Weber et al., 2011).

Other than RSM, rapeseed cake is only produced at smaller oilmills and represents around one tenth of the total rapeseed feed consumption. The major difference to RSM is that rapeseed cake comprises a much higher and varying concentration of crude fat (20 vs. 100- 160 g/kg) as well as twice as high glucosinolate concentrations (6.2 – 9.4 vs. 11.6 – 17.1 mmol/kg cake). Recommendations for the practical use of rapeseed cake depend mainly on glucosinolate levels. If the compliant amount is exceeded animals react with a decrease of feed intake and performance and in the worst case an enhancement of the thyroid. Weiß and Schöne (2010) summarized 5 different trials that were carried out in order to estimate the maximum supplementation of rapeseed cake. It was concluded that fattening pigs may receive between 70 to 100 g/kg rapeseed cake, while sows and piglets may be fed between 50 up to 100 g/kg rapeseed cake. The exact amount depends on the glucosinolate level which should not exceed 1.5 mmol/kg diet. Moreover, crude fat content should be more standardized to be able to use commodities easier and more reliable.

The smallest application of rapeseed products can be found in poultry nutrition. For this reason not much research has been conducted, results vary to great extents and unfortunately, no declaration on glucosinolate levels of the used RSM can be found in most of the literature. Richter et al. (1996) noticed a decrease in performance when adding 50 g/kg RSM while Faghani and Kheiri (2007) observed no differences when RSM was added at levels of 100 g/kg. Few studies with rapeseed cake revealed that it is possible to use approximately 150 g/kg diet without any losses in performance (Peter and Dänicke, 2003). Jeroch et al. (2008) reviewed several trials and concluded that broiler, when fed rapeseed cake, tolerate between 3 and 5 mmol/kg glucosinolate. Moreover, it is highly important to add iodine, since glucosinolates act as antagonists. It is suggested that iodine supplementation should be twice

general recommendations (GfE, 1999). However, if glucosinolates are present in high concentrations, the negative effects may not be compensated, even if iodine is supplemented in high amounts.

Concluding, it becomes evident from these data that a more widespread use of RSM and rapeseed cake in diets for pigs and poultry requires further reduction of glucosinolate levels.

3.4. Energy utilisation efficiency and sustainability

The biofuel yield per tonne rapeseed varies between 250 and 350 kg rapeseed oil and per tonne maize or wheat grain between 300 and 350 kg bioethanol (Pinkney, 2009). Some losses are caused as CO_2 during alcohol fermentation. All other products may be considered as by-products and may be used in various ways as feedstuff in animal nutrition in wet and dry form or as fertilizer. Biofuel by-products can be considered as valuable protein sources for farm animals. Their CP concentration varies between 300 and 400 g/kg DM. Land use scenarios using wheat for biofuel or using wheat and soybean meal to match animal feed value of DDGS have been evaluated by Pinkney (2009). The most effective way to utilize the DGS resulting from biofuel production in large plants is feeding of this low DM material (80 g DM/kg) to farm animals. As it is unrealistic to distribute large amounts of DGS in nearby areas of the biofuel plant and due to the short shelf-life of DGS, it becomes necessary to dry the material in other to preserve the by-product. Therefore, additional energy expenditures and GHG emissions must be considered in any assessment of ecobalances (CF, life-cycle assessment) of the by-products or the whole biofuel production chain.

Up to this day, no definite regulations exist in order to classify emissions of the main product and the by-product (Bockisch et al., 2000; Flachowsky et al., 2011). When considering the causation principle, the producer or the responsible party should be accountable for all emissions. However, drying of DGS is only of interest if the products will be utilized as feedstuffs for animals and thus emissions associated with processing of by-products are not of interest or necessity for biofuel producing companies.

3.5. Knowledge gaps and future research

Even though, much research has already been conducted in the utilisation of bioethanol and biodiesel by-products for animal nutrition there are important aspects which need further consideration. Dose-response studies are required for all by-products covered in this chapter, in order to evaluate the exact mode of action as well as the appropriate inclusion level in diets of farm animals. More precisely this means that methanol must be removed from glycerine as far as technically possible since separation or purification of glycerine can be fluctuating depending on the plant and the applied process. Rapeseed products which are fed to pigs and poultry should contain as few glucosinolates as possible. This might be achieved through the breeding process, while the antinutritive impact of the remaining glucosinolates may be compensated by iodine addition.

Further attention should also be paid to the influence of processing conditions on composition and nutritive value of by-products in dependence on raw materials. Especially, rapeseed cake need further consideration and more reliable data because variations in the processing conditions result in varying chemical composition, particularly regarding the crude fat and CP content. These circumstances currently lead to difficulties in prediction of the feeding value of rapeseed cake for all categories of farm animals and could also affect storage stability. Therefore, the value of rapeseed cake would benefit from a standardization of composition. Similarly, a standardisation of processing and moreover using constant proportions of raw materials for the production of distillers grains would be desirable.

Future research should also focus on measuring additional expenditures of the processing of by-products in order to be able to evaluate CF and identify GHG reduction potentials. Factors like harvesting, pressing, drying, conservation and transportation should be accounted for in the same way as animal emissions and manure management since focussing on single factors, does not provide an assessment that reflects the complexity of this subject.

3.6. Conclusion

The results of a number of experiments with lactating dairy cows and fattening bulls suggest that distillers grains as the main protein source may support a high productive performance. Trials with grower-finisher pigs suggested that DDGS up to 200g/kg diet did not influence the growth performance and fattening and slaughtering variables. Similarly, laying intensity of

hens as well as egg quality and health were not affected by inclusion levels ranging from 150 g/kg to 300 g/kg diet. Trials with broilers suggest that diets that contain more than 100 g/kg DDGS may lower performance. Hence, it is recommended to add non-starch polysaccharide (NSP)-degrading enzymes (e.g., xylanase or xylanase mixed with other enzymes) to poultry diets rich in DDGS.

Table 3.16 summarizes current German recommendations for rapeseed products in diets for cattle and pigs. Pigs would particularly benefit from breeding or production progress in further reduction of glucosinolate levels, whereas in cattle, a safer quality assessment of the rapeseed cake is needed.

Table 3.16. Practical recommendations for daily amounts or dietary concentrations (as fed basis for dry diets) of rapeseed products for cattle, pigs and poultry (Weiß, 2007; Jeroch et al., 2008).

Animal category	Rapeseed meal, solvent-extracted	Rapeseed cake, mechanically extracted
Dairy cow	Maximum 4 kg	1.5 - 2.0 kg
Beef cattle	Maximum 1.2 kg	1 kg
Fattening pigs	Maximum 100 g/kg	70 – 100 g/kg
Sows	50 – 100 g/kg	50 – 100 g/kg
Piglets	Maximum 50 g/kg	50 – 100 g/kg
Broiler	50 -150 g/kg	50 -100 g/kg
Laying hens	0 -100 g/kg	0 – 50 g/kg

The current chapter reviewed, upon other the use of glycerine as a by-product from biodiesel production, as well as rapeseed products such as rapeseed meal and cake for farm animals. For the benefit of a fail-safe usage of glycerine in diets for all farm animals, methanol should be removed as far as technically possible. Glycerine at different purities may help to stabilise the hygienic quality of pelleted compound feeds without compromising physical quality of pellets. Furthermore, glycerine is no direct competitor of propylene glycol, since data on ruminal turnover suggest that glycerine, other than propylene glycol, should replace rapidly

fermentable carbohydrates. Mature cattle may consume up to 1 kg glycerine per day, while it may still be necessary to investigate if the sweet taste of glycerine may improve feed intake of diets with inferior palatability.

In conclusion, glycerine can be used a versatile feedstuff, in particular for ruminants, however, further research is thus required to explore the full potential of glycerine in dairy cows.

Other rapeseed products for ruminants, such as rapeseed meal, compare well with soybean meal for dairy cows. Recent research on rapeseed meal has shown that it can fully replace soybean meal within dairy cow diets when fed on an approximate isonitrogenous and isocaloric basis, i.e. without considering differences in ruminal degradation and (or) amino acid pattern. Moreover, milk and milk component yields were similar for diets containing soybean meal or rapeseed meal.

Nevertheless, rapeseed cake needs further consideration and more reliable data because variations in the processing conditions result in varying chemical composition, particularly regarding the crude fat and protein content. These circumstances currently lead to difficulties in prediction of the feeding value of rapeseed cake for all categories of farm animals and could also affect storage stability. Therefore, the value of rapeseed cake would benefit from a standardization of composition

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4. COMPARATIVE EVALUATION OF EQUATIONS PREDICTING METHANE PRODUCTION OF DAIRY CATTLE FROM FEED CHARACTERISTICS

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ABSTRACT

Techniques that allow direct measurements on animals to quantify methane (CH_4) emissions are costly and difficult to transfer to herd level. Mathematical approaches have been developed to predict methane emissions of cattle based on diet and intake characteristics which were calibrated against largely varying calorimetry data. In this study nine CH_4 prediction equations were applied to five typical Central European dairy cow diets in order to compare their applicability. The five diets differed in respect of forage proportion and type. In a first attempt regression equations were selected containing easily accessible data such as dry matter intake (DMI, kg/d) forage proportion (forage DMI/DMI), as well as neutral and acid detergent fibre, both expressed exclusive residual ash (NDFom, ADFom) that can also be extracted from on-farm datasets. Smallest differences to mean values were observed with the application of equations using NDF, while standard deviations were highest, and therefore showed the best capability to differentiate between diets, when using equations that operated with forage proportion and DMI. Nevertheless, the role of CH_4 prediction equations should not be overestimated. The differences in levels of CH_4 estimates show that frequently used equations are still inaccurate and may only serve as implications to locate trends. It should be taken into consideration to expand datasets, involving future CH_4 measurements, on animal and herd level, feeding typical up to date regional diets in order to get more precise equations, suitable for a greater range of estimations. To ease and simplify the future applications, the prediction equations could be classified into groups, clearly stating by which data they were derived, for example regional origin and diet composition.

Keywords: dairy cattle; greenhouse gas; methane; prediction

4.1. Introduction

Methane (CH_4) is one of the major greenhouse gases which may contribute greatly to global warming. The main sources of CH_4 output into the atmosphere include lowland marshes and wetlands, the burning of forests and grassland, the strongly increasing numbers of termites in the harvested tropical forest, rice fields, coal mines, the oceans and around 1.3 billion cattle (Jentsch et al., 2009). Globally, ruminants produce approximately 80 million tonnes of CH_4 a year, accounting for around one third of anthropogenic emissions of CH_4 (Beauchemin et al., 2008). Cattle lose approximately 2-10% of their ingested energy as eructated CH_4, depending on diet quality (Johnson and Johnson, 1995). Due to these facts agriculture has a responsibility to help decrease CH_4 emissions, which in the case of cattle can be achieved through optimized feeding strategies. There are several approaches in evaluating the contribution of different feedstuffs to CH_4 output. Techniques that allow for direct measurements on animals to quantify CH_4 emissions are costly and difficult to transfer to herd level. As an alternative to that, mathematical approaches have been developed to predict CH_4 emissions of cattle based on diet and intake characteristics which have been calibrated against largely varying calorimetry data. The challenge is that some of these models were developed solely on the basis of their regional data sets, whilst other models were developed with an insufficient amount of data. Likewise datasets for typical Central European diets are rare and partly overage. The practical use of certain regression equations is questionable since available data for different feedstuff is often not complemented (Wilkerson et al., 1995). Even though this subject is of great interest and a current issue, there are only few studies dealing with the evaluation of prediction equations, especially for Central European data. Even in a recently published issue on greenhouse gases in animal agriculture (McAllister et al., 2011), no evaluation or proceeding study on this subject was reported. In this study, several CH_4 prediction equations were applied to five typical Central European dairy cow diets in order to compare their applicability in regard to dietary measures to mitigate CH_4 production in dairy cattle and to evaluate their overall performance.

4.2. Materials and Methods

4.2.1. Diets

Five different typical Central European dairy cow diets were chosen to compare performance of the different regression equations. All diets have been or are in practical use (Research

Farm “Frankenforst”, University of Bonn; Research Farm “Haus Riswick”, Agricultural Chamber North Rhine Westphalia) and differ mostly in their maize and grass silage fraction (Table 4.1).

Diet 1 which was composed for dairy cows with a body weight of 630 kg and 32 kg milk yield had a relatively high proportion of grass silage (332 g/kg DM) combined with 357 g/kg DM of compound feed in the total mixed ration (TMR). Available data from this diet included a complete proximate constituents analysis as well as NDFom (neutral detergent fibre, expressed exclusive residual ash) and ADFom (acid detergent fibre, expressed exclusive residual ash) analysis. Diet 2 consisted mainly of maize silage (653 g/kg DM) and was composed for dairy cows with a milk yield of 39 kg combined with a body weight of 630 kg. Diet 3 had a major proportion of grass silage (553 g/kg DM), likewise designed for dairy cows with a milk yield of 39 kg combined with a body weight of 630 kg. Diet 4 and 5 did not differ in their forage proportion, both consisting of 461 g/kg DM grass silage and 326 g/kg DM maize silage. Only their actual amount differed as Diet 4 was developed for cows yielding 36 kg milk and Diet 5 for cows with a 25 kg milk yield. The diets also differed in DM intake (DMI). Cows which were fed Diet 5 consumed an average of 18.2 kg DM, while cows who were fed Diet 2 consumed an average of 22.8 kg DM. Cows fed Diet 1, 3 and 4 consumed 19.3, 22.3 and 22.4 kg DM respectively. Because of differences in performance and DMI all variable inputs have been transformed to a comparable daily DMI of 22 kg DM/(cow x d). This transformation allows to rule out the major effect of DMI on daily methane output but allows at the same time to determine the effect of diet composition more accurately.

There were differences in the available data concerning the chemical composition of the different diets. While Diet 1 covered the required information sufficiently, Diet 2 provided only few variables. Diet 3, Diet 4 and Diet 5 included basic information but lacked details on fibre fractions. Missing information was calculated using data and recommendations from literature (DLG, 2001).

Table 4.1. Ingredient and chemical composition of selected diets.

	[g/kg DM]¤				
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredients					
Grass silage	332	117	553	461	461
Maize silage	153	653	194	326	326
Beet pulp silage				134	134
Wheat grain	118		58		
Compound feed	357	209	120	58	54
Straw	28		15		
Mineral mix	4	11	5	3	5
Other	8	10	55	18	20
Chemical composition					
DM	443	532	446	359	359
CP†	165	177	173	175	175
Ash	70	62	65	72	70
NDFom‡	434	383	413	442	446
ADFomα	215	196	189	267	275
NEL# [MJ/kg DM]	6.8	7.1	7.0	7.0	6.6

Notes: ¤DM, Dry matter; †CP, Crude protein; ‡NDFom, Neutral detergent fibre, expressed exclusive residual ash; αADFom, Acid detergent fibre, expressed exclusive residual ash; #NEL = Net energy for lactation.

4.2.2. Equations

Regression equations were selected based on the available nutritional analysis of the diets, as well as their appearance in scientific literature. In addition, it was important that equations were to apply simply and practicable for regular use. The most available data was dry mater intake (DMI), forage proportion and in some cases NDFom and ADFom. Table 4.2 presents 9 suitable equations that were found in the literature. Equations (1) - (4), as well as equations (6) and (8) are simple regression equations, whereas the rest are multiple regression equations. The equations differed in their input factors - equations (1) – (3) used DMI as a sole factor, Equations (4) and (5) used forage proportion as an input factor, while Equations (6) – (9) relied on information of fibre fractions. Equations by Ellis et al. (2007) were developed from a database of 83 beef and 89 dairy cattle from North America. It was important to the author that the research to conduct the database was done either in the northern United States or in Canada, to ensure similarity of feedstuff. What should be noticed in this case is that Equations (1), (7) and (9) were developed using both, the dairy and the beef databases, while Equations (4), (6) and (8) were developed using only the dairy database. The data derived from the dairy

database included diets with forage proportion ranging from 28 to 100%. The combined database included diets with forage proportions from 9 to 100%. Equation (3), which was developed by Jentsch et al. (2007) used a Central European database of 337 cattle, including oxen, young bulls, cows and heifers. The diets fed in this database were composed of dry feedstuff and compounds ranged from 100% dried roughages (without concentrates) to 30% dried roughages plus 70% mixed concentrates. Equations (2) and (5) by Mills et al. (2003) were developed using a dataset of 159 dairy cattle from the U.K. and North America. The U.K. data included diets with forage proportions ranging from 48 to 100% while forage proportion of the Northern American diets ranged between 54 to 69%. The milk yield of the used animals ranged between 8.9 up to 30.8 kg/d. All of the estimated results of CH_4 were calculated in grams in order to have better comparability.

Table 4.2. List of statistical models used to predict CH4 production.

Source	Equation no.	Equation	r^2
Ellis et al. (2007)	(1)	CH_4 [MJ/d] = 3.272 + 0.736 · DMI[¤] [kg/d]	0.68
Mills et al. (2003)	(2)	CH_4 [MJ/d] = 5.93 + 0.92 · DMI[kg/d]	0.60
Jentsch et al. (2007)	(3)	CH_4 [kJ] = 1802 - 21.1 · DMI [g/kg BW]	0.22
Ellis et al. (2007)	(4)	CH_4 [MJ/d] = 8.56 + 0.139 · forage [%]	0.56
Mills et al. (2003)	(5)	CH_4 [MJ/d] = 1.06 + 10.27 · dietary forage proportion + 0.87 · DMI [kg/d]	0.61
Ellis et al. (2007)	(6)	CH_4 [MJ/d] = 3.14 + 2.11 · NDF[†] [kg/d]	0.46
Ellis et al. (2007)	(7)	CH_4 [MJ/d] = 3.44 + 0.502 · DMI [kg/d] + 0.506 · NDF [kg/d]	0.67
Ellis et al. (2007)	(8)	CH_4 [MJ/d] = 5.87 + 2.43 · ADF[‡] [kg/d]	0.56
Ellis et al. (2007)	(9)	CH_4 [MJ/d] = 3.41 + 0.520 · DMI [kg/d] - 0.996 · ADF[3] [kg/d] + 1.15 · NDF [kg/d]	0.67

Notes: [¤] DMI, Dry matter intake; [†]NDFom, Neutral detergent fibre, expressed exclusive residual ash, [‡]ADFom, Acid detergent fibre, expressed exclusive residual ash.

4.2.3. Analysis and calculations

The evaluation and descriptive statistics of the results was performed using the means procedure of SAS Version 9.2 (SAS 2002). As the true CH_4 output of all diets is unknown, the mean of all estimated values within diets was calculated. These means can be interpreted as the most likely values and serve as reference values in the analysis. In the next step, the estimated CH_4 emissions were expressed as a deviation between reference values and calculated value. Over- or underestimation of the different equations can be seen by the algebraic sign and dimension of these deviations. The capability of the equations to differentiate the possible CH_4 emission of the different diets can be explained by the standard deviations across diets. Determination coefficients of equations, which were also used in

analysis of data were taken from respective literature (Mills et al., 2003; Ellis et al., 2007; Jentsch et al., 2007).

4.3. Results

Table 4.3 presents the results of calculated CH_4 emissions with different diets and equations. Overall the results ranged from 298.4 g (Diet 5, Equation (1)) up to 612.3 g (Diet 2, Equation (5)). Mean values ranged from 367.2 g up to 471.8 g. Respective mean values [g/(cow x d)] and their standard deviations are presented in Table 4.4. Smallest differences to mean values were observed with the application of Equation (6), (9) and (3). Equations (6) and (3) tend to overestimate CH_4 emissions, while equation (9) rather tends to underestimate. Standard deviations were highest when using Equation (4), (5) and (8) and therefore have the best capability to differentiate various diets. In this case, two equations seem to underestimate results (equation (4) and (8)) and one equation overestimates the CH_4 emissions (equation (5)). Overall, equations which use forage proportion or ADF seem to be more practicable when different types of diets are applied. Nevertheless, no clear position can be concluded on which variables to use when differences to mean values are compared.

Table 4.3. Methane production from different diets [g/(cow x d)] estimated by several simple and multiple regression equations

Equation no.	Diet¤				
	1	2	3	4	5
[1]	326.7	431.8	413.8	418.2	298.4
[2]	445.3	576.6	553.9	559.5	409.9
[3]	428.6	482.1	477.8	490.7	408.0
[4]	306.1	385.3	378.9	389.9	389.9
[5]	428.8	612.3	585.0	598.6	457.0
[6]	388.2	465.4	475.0	510.2	360.8
[7]	325.0	415.4	405.3	416.8	298.7
[8]	302.6	355.2	344.8	429.0	323.0
[9]	354.2	449.1	445.8	433.6	358.9
Mean	367.3	463.7	453.4	471.8	367.2

Notes: ¤ Following conditions were assumed (see Materials and Methods): Because of differences in performance and DMI all variable inputs have been calculated to a comparable DMI of 22 kg DM/(cow x d) with: Diet1: cow of 630 kg body weight, 32 kg milk yield ; Diet 2: cow of 630 kg body weight, 39 kg milk yield; Diet 3: cow of 630 kg body weight, 39 kg milk yield; Diet 4: cow of 650 kg body weight, 36 kg milk yield; Diet 5: cow of 650 kg body weight, 25 kg milk yield

Table 4.4. Differences from CH4 estimates to their reference values, respective mean values and standard deviations [g/(cow x d)]

Equation no.	Diet¤ 1	2	3	4	5	Mean	sd
[1]	40.6	31.9	39.6	53.6	68.8	46.9	13.0
[2]	-78.0	-112.9	-100.6	-87.7	-42.7	-84.4	23.9
[3]	-61.4	-18.4	-24.4	-18.9	-40.8	-32.8	16.4
[4]	61.2	78.4	74.5	81.9	-22.7	54.7	39.3
[5]	-61.5	-148.6	-131.6	-126.7	-89.8	-111.7	31.6
[6]	-20.9	-1.7	-21.7	-38.4	6.4	-15.3	15.9
[7]	42.3	48.3	48.1	55.0	68.5	52.4	9.0
[8]	64.7	108.5	108.6	42.9	44.1	73.8	29.4
[9]	13.0	14.6	7.6	38.2	8.3	16.3	11.3

Notes: ¤ Following conditions were assumed (see Materials and Methods): Because of differences in performance and DMI all variable inputs have been calculated to a comparable DMI of 22 kg DM/(cow x d) with: Diet1: cow of 630 kg body weight, 32 kg milk yield ; Diet 2: cow of 630 kg body weight, 39 kg milk yield; Diet 3: cow of 630 kg body weight, 39 kg milk yield; Diet 4: cow of 650 kg body weight, 36 kg milk yield; Diet 5: cow of 650 kg body weight, 25 kg milk yield.

4.4. Discussion

4.4.1. Dry matter intake

It is widely recognized that DMI is one of the dominant factors determining CH_4 production in cattle. Therefore various studies have been performed to examine this effect in order to use it for developing equations for predicting CH_4 emissions in ruminants. In this study six equations included DMI as a variable, while three of these equations used DMI as a sole factor. Equation (3) developed by Jentsch et al. (2007), was a well performing equation when comparing its result to the respective mean value, however the standard deviations were average. This could be due to the fact that in this case, DMI was related to body weight which seems to make results more accurate. Also, Equation (3) was developed using a bigger dataset (n = 337) and Central European data, which may contribute to its preciseness.

Generally it can be stated that DMI is a factor which is easy to obtain and even under practical circumstances to apply simply in equations. Even if farms do not have access to their herds' exact DMI, there are several approaches to estimate these values reliably. For example Gruber et al. (2004) developed several estimation equations to quantify DMI depending on variables such as animal data, feed and management data. These estimates were based on an extensive dataset from 10 research institutions and are now widely used to calculate on-farm DMI of dairy cows. The findings of this study are in agreement with previous studies, where DMI was present on average as the best predictor (Axelsson 1949; Johnson and Johnson, 1995; Mills et

al., 2003; Ellis et al., 2007). Nevertheless, it has to be kept in mind that even if CH_4 production increases almost linearly with a higher feed intake (Kirchgeßner et al., 1995), the fraction of consumed gross energy lost as CH_4 decreases. This was also shown in a theoretical study performed by Mills et al. (2001) who found that there is an inverse relationship between feed intake and gross energy loss as CH_4. In their study, they increased feed intake from 10 to 24 kg/d with a diet containing a 1:1 ratio of grass silage and concentrate. They assumed that the decrease of CH_4 emissions was due to a reduction in rumen digestibility, shifts in the rumen fermentation and higher passage rates. The degree of the fermentation process in the fore stomachs is known to rely on rumen retention time which is reduced with rising levels of feed intake (Kirchgeßner et al. 1995; Benchaar et al. 2001). This leads to the conclusion that CH_4 production does not solely depend on feed intake but also depends on the quality, quantity and composition of the diet (Johnson and Johnson, 1995; Moss et al., 2000; Benchaar et al., 2001; Jentsch et al., 2007). Thus, DMI is a fair prediction factor which is easy to apply on farm level, but if results need to be more exact, other variables should be included.

4.4.2. Forage proportion

Two of the evaluated equations included forage proportion as a parameter. Equation (4) used forage proportion as a sole parameter, while Equation (5) also included DMI in its prediction. Surprisingly, results differed to a great extent. While Equation (4) resulted in average CH_4 values, Equation (5) showed relatively high CH_4 values when compared to the mean. The effect of forage proportion on CH_4 production has been the subject of many studies. Generally, CH_4 production rises when forage proportion in a diet is increased (Shibata et al., 1992; Johnson and Johnson, 1995). This is due to methanogenic Archaea which use CO_2 and H_2 to form CH_4 (McAllister and Newbold, 2008). High proportions of cell wall carbohydrates promote methanogenesis and favour acetic acid production which leads to a higher CH_4 production (Shibata et al., 1992; Johnson and Johnson, 1995; Beauchemin and McGinn, 2005). Consequently this means that if lower forage proportions are fed and replaced by concentrates containing more non-structural, rapidly fermentable carbohydrates, CH_4 production decreases. This effect is due to a shift in ruminal fermentation toward propionate production and a decrease of ruminal pH (Fahey and Berger, 1988). Propionate promotes competitive pathways for H_2 use in the rumen and thereby decreases overall CH_4 production (Moss et al., 2000; Monteny et al., 2006). This finding was confirmed in this study, revealing highest CH_4 production with the largest proportion of forage (Diet 4).

Forage proportion as a parameter to estimate CH4 production is practicable if no other data is

available. Nevertheless this factor implies no statement on forage quality. Therefore, more accurate results may be obtained using parameters which give more information on forage composition, such as cell wall substances and their ruminal degradability, starch and sugar to predict CH_4 production.

4.4.3. Fibre fraction

Equations (6) – (9) included ADFom, NDFom, DMI or all three as parameters. Results of these applications gave satisfactory results. Equation (7) and (9) had the lowest deviations of the whole dataset. Again, these equations were developed by Ellis et al. (2007) and were designed specifically for Northern American data, which confirms their low capability to differentiate between diets. Nevertheless, it has been shown that ADFom, NDFom and their components cellulose, hemicellulose and lignin are valuable factors in estimating CH_4 production (Moe and Tyrell, 1979; Ellis et al. ,2007). Generally, cellulose promotes CH_4 production three times more than hemicellulose (Moe and Tyrell, 1979). Moreover, cellulose and hemicellulose ferment at slower rates than non-structural carbohydrates (McAllister et al. 1996). Decreased passage rates out of the rumen favour a high acetate:propionate ratio and therefore lead to increased CH_4 production (Hegarty and Gerdes, 1998). This was also reported by Benchaar et al. (2001) who performed a rumen simulation with increasing forage proportion from 30 to 80% of DMI. While observing an increase in CH_4 production up to 80%, the authors noticed a decline when simulating proportions of more than 80%. It was suggested that this is due to higher passage rate, decreased ruminal digestion of starch, increased digestion of NDF and increased microbial efficiency. Similarly, Popova et al. (2011) found that bulls which were fed a fibrous diet produced 21% more CH_4 than those receiving a starch-rich diet. The authors concluded that this was attributed to methanogen activity and furthermore suggested that it is essential to use a holistic approach in studying the rumen ecosystem in order to better understand the effect of dietary CH_4 mitigation in ruminants.

4.4.4. Estimation vs. Measurement

There are several approaches to estimate or measure CH_4 emissions. This study used nine common estimation equations to compare their applicability for typical Central European diets. The range of the levels of the estimated results showed that there is still uncertainty and results depend strongly on the dataset which was used to develop the respective equation. Storm et al. (2012) reviewed the most common methods for measuring and estimating CH_4

emissions from ruminants. When comparing ten of the most common CH_4 prediction equations the authors derived that the application of the respective models leads to large differences. They concluded that no method is flawless and knowledge of advantages and disadvantages of the experimental methods is essential and should be taken into account when planning, interpreting and publishing results.

Klevenhusen et al. (2010) tested the accuracy of the Intergovernmental Panel on Climate Change (IPCC) default values, which is the standard model usually used for calculating cattle CH_4 emissions. The authors indicated that the CH_4 conversion rate is slightly underestimated by the IPCC (2006) for several diet types, in particular good quality forage-dominated diets, typical for Central Europe.

Likewise, in a recently published review, Flachowsky et al. (2011) stated that several frequently used prediction equations are imprecise and resulting methane emissions vary to great extents. Most equations do not account for different feeding strategies. For example, equations which were derived from cows that were fed a forage and high fibre diet estimated much higher methane emissions than equations that were developed with data from cows which were fed a diet with a very high concentrate fraction.

Nevertheless, policy makers depend on mathematical approaches to estimate regional, national and global GHG emissions and as long as datasets for CH_4 emissions for individual animals and whole barn systems are lacking, frequently applied equations may still serve as indications. To ease and simplify future applications, the prediction equations could be classified into groups, clearly stating by which data they were derived, for example regional origin and diet composition.

4.5. Conclusions

An evaluation of dietary measures at farm level requires a close look at animal level, following an evaluation at herd level in terms of productivity and nutrient utilisation (Monteny et al., 2006). Subsequently measures to reduce CH_4 production should imply increasing the level of rapidly fermentable carbohydrates to enhance propionate production and altering the diet concerning feed intake and feed composition to allow a better performance (Monteny et al. 2006; Beauchemin et al., 2008).

Overall it can be stated that all equations are suitable for practical use to some extent.. DMI, ADFom, NDFom and forage proportion seem to be helpful dietary factors which can be easily

extracted, even from on-farm data sets, although fibre fractions might not necessarily be needed for rough estimations of CH_4 emissions. Mitigation strategies should be considered for future research, while prediction equations can be of help for developing optimised feeding strategies. Nevertheless, the role of modelling and CH_4 prediction equations should not be overestimated. The range in levels of CH_4 estimates show that frequently used equations are still imprecise and may only serve as implications to locate trends (Walter 2009). It should be taken into consideration to expand and classify datasets, involving future CH_4 measurements, on animal and herd level, feeding typical up to date regional diets in order to get more precise equations, suitable for a greater range of estimations.

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5. ESTIMATION OF INTESTINAL PROTEIN DIGESTIBILITY OF PROTEIN SUPPLEMENTS USING A THREE-STEP ENZYMATIC IN VITRO PROCEDURE

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ABSTRACT

This study included 33 samples with main focus on unprotected or rumen protected rapeseed and soybean feedstuffs, which were analysed using an enzymatic *in vitro* procedure (EIVP) in order to determine intestinal crude protein (CP) digestibility (IPD) of ruminally undegraded CP (RUP). The EIVP involved the sequential digestion of samples with a protease from *Streptomyces griseus*, pepsin-HCl and pancreatin. The activity of *S. griseus* protease was related to the true protein content of the feed sample. Briefly, the EIVP started with determination of true protein. Feeds were incubated for 18 h in a buffer solution at a constant ratio (14 U/g) of *S. griseus* protease activity to feed true protein. The dried residues were incubated in pepsin-HCl solution for 1 h and residues from this step were incubated in pancreatin solution for 24 h. Results appeared to have lower IPD dimensions than literature-data of previous studies. In addition, correlation analysis of IPD in relation to different nutrient values revealed a negative correlation between acid detergent fibre and IPD, as well as a positive correlation between crude protein, true protein and IPD. To sum up, the EIVP seems to be a reliable, simple laboratory method to estimate IPD of RUP in concentrate feeds. However, future studies may be constricted since sufficient reference values, e.g. *in vivo* data is missing.

Keywords: Rumen, protein, rapeseed meal, soybean meal, enzymatic *in vitro* procedure

5.1. Introduction

Crude protein (CP) values of feeds do not supply precise information about the protein that may actually be digested in the small intestine by ruminants. The CP reaching the small intestine consists of both, the ruminally synthesized microbial CP as well as the feed CP that escaped ruminal degradation. Several techniques are available to determine ruminal degradation and whole-tract digestibility of CP. These techniques include *in vivo* and *in vitro* methods, and differ highly in complexity, cost and effort. Calsamiglia and Stern (1995) developed a three-step in *situ-in vitro* procedure (ISIVP) to estimate intestinal CP digestibility (IPD) by simulating physiological conditions in the ruminants` digestive tract. The method is supposed to be rapid, reliable and inexpensive, can be applied to a wide variety of protein supplements and accurately reflects differences in protein digestion (Calsamiglia and Stern, 1995). However, the procedure includes ruminal incubation of samples which may be regarded as an additional error source and still might be unable to compete with simple laboratory methods. Subsequently, Irshaid (2007) refined the procedure and developed an enzymatic *in vitro* procedure (EIVP) by replacing the rumen incubation step of Calsamiglia and Stern (1995) with an enzymatic treatment using a protease from *Streptomyces griseus* to mimic ruminal degradation of CP. Although values for IPD of ruminally undegraded dietary protein (RUP) are existing for a number of feeds, there are remarkable gaps in regard to reliable data, in particular for protein supplements like solvent-extracted oilseed meals, especially rapeseed and soybean commodities, which are considered to be an important source for high-quality protein to all farm animal species. For this reason, the main objective of this study was to evaluate IPD of RUP of several protein supplements which were predominantly characterised as protected from ruminal degradation through specific technical treatments, via the EIVP (Irshaid, 2007). The second aim of this study was to evaluate relationships and interactions between calculated IPD values and analysed chemical variables of feedstuffs.

5.2. Materials and Methods

5.2.1. Feedstuffs

This study included 33 commodities that are commonly used as protein supplementation (Table 5.1), with a main focus on rapeseed meal (RSM) and soybean meal (SBM). Twenty-

three samples were protected from rumen degradation either by a physical, namely thermal treatment (13 samples) or by chemical treatment (10 samples). Chemical treatments included formaldehyde (4 samples), xylose (5 samples) or polyurea-formaldehyde (1 sample) additions, in order to decrease ruminal CP degradation. Further, five samples were specifically assembled for experimental purposes. Three of these assembled RSM were extracted with hexane as a solvent, either directly (1 sample) or after squeezing (2 samples). The remaining two RSM samples were treated with supercritical CO_2 (300 bar, 40°C), with or without squeezing. The supercritical CO_2 treatment is supposed to be more gentle than other procedures and therefore does only little damage to the native protein of the rapeseed. The remaining samples were commercially purchased. Moreover, this study included samples of rapeseed hulls, rapeseed cake, protected wheat grain, unprotected lupine and solvent-extracted sunflower meal (protected and unprotected). Unfortunately information about specific treatments of samples was not provided for the commericially purchased samples.

5.2.2. General analytical procedures

The DM was estimated by oven-drying at 105 °C overnight. The N content was determined using the standard Kjeldahl procedure (4.1.1.) using a Vapodest 50s carousel (Gerhardt, Königswinter, Germany) for automated distillation and titration. The CP was calculated by multiplying N by 6.25. Acid detergent fibre (ADF) was analyzed according to AOAC (1990) and is expressed inclusicve residual ash.

Table 5.1. Feedstuff description including analyzed results for dry matter (DM), Ash, ADFom (Acid detergent fibre, expressed inclusive residual ash), crude protein (CP) and true protein (TP) expressed as g/kg DM unlessed stated differently.

No.	Feedstuff	DM (g/kg)	Ash	ADF	CP	TP
1	Wheat grain, protected[a]	865.9	2.6	25.7	143.5	111.3
2	Lupine	886.3	2.8	201.6	296.5	224.7
3	Lupine, protected[b]	892.5	6.9	201	309.1	287.2
4	Sunflowerseed meal	918.6	5.6	297.7	278.9	244.9
5	Sunflowerseed meal, protected[b]	907.0	5.9	312.1	280.2	242.4
6	Rapeseed hulls[c]	892.2	4.8	628.8	135.3	113.8
7	Rapeseed cake, protected 1[b]	911.5	6.3	208.9	336.8	290.1
8	Rapeseed cake, protected 2[a]	895.0	5.6	244.1	291.4	275
9	Rapeseed meal 1	896.8	6.6	226.5	354.6	325.4
10	Rapeseed meal 2[c]	929.9	6.4	110.5	375.4	291
11	Rapeseed meal 3[c]	942.3	5.4	199.2	290.7	193.4
12	Rapeseed meal 4[c]	921.5	6.7	161.4	387.6	286.2
13	Rapeseed meal 5[c]	931.1	6.2	148.4	371.4	253.7
14	Rapeseed meal 6[c]	917.7	6.8	228.2	353.7	242.6
15	Rapeseed meal, protected 1[b]	907.7	6.1	213.4	353.0	294
16	Rapeseed meal, protected 2[b]	908.9	6.2	217.2	341.3	322.9
17	Rapeseed meal, protected 3[b]	898.8	5.8	223	341.1	314.7
18	Rapeseed meal, protected 4[b]	910.3	6.4	208.5	340.3	331.4
19	Rapeseed meal, protected 5[b]	888.6	6.1	235.8	338.3	293.8
20	Rapeseed meal, protected 6[b]	899.7	5.8	216.3	335.5	303.1
21	Rapeseed meal, protected 7[b]	894.9	6.2	226	346.8	316.7
22	Rapeseed meal, protected 8[b]	914.9	6.4	203.2	355.4	282.4
23	Rapeseed meal, protected 9[d]	902.6	6.7	216.3	341.8	333.2
24	Rapeseed meal, protected 10[d]	924.3	5.9	274.8	362.4	323.5
25	Rapeseed meal, protected 11[a]	921.6	6.1	225.9	323.3	303.3
26	Rapeseed-/Soybean meal, protected 1[b]	883.2	6.4	229.3	337.9	318.4
27	Rapeseed-/Soybean meal, protected 2[b]	889.8	6	155.4	400.6	382.7
28	Soybean meal	891.5	6.3	64.8	513.3	460.6
29	Soybean meal, protected 1[d]	884.6	5.8	103.1	431.8	414.7
30	Soybean meal, protected 2[d]	922.7	6.6	179.6	486.7	446.8
31	Soybean meal, protected 3[a]	875.1	5.1	98.5	456.7	424.5
32	Soybean meal, protected 4[a]	921.0	6.3	51.6	447.8	436.9
33	Soybean meal, protected 5[e]	895.3	5.6	126.3	501.3	454.9

[a] treated with xylose in lignosulphonate solution to increase ruminally undegraded CP fraction
[b] treated with a physical- thermal method to increase ruminally undegraded CP fraction
[c] specifically assembled for experimental purposes
[d] treated with formaldehyde to increase ruminally undegraded CP fraction
[e] treated with polyurea formaldehyde to increase ruminally undegraded

5.2.3. Enzymatic in vitro procedure

Before enzymatic treatment, samples were ground through a 1-mm screen (Model M 20; IKA, Staufen, Germany). The three-step enzymatic procedure (ISIVP) followed Calsamiglia and Stern (1995) except for the first step that stimulates rumen incubation, which was done according to Irshaid (2007) and Irshaid and Südekum (2007), who replaced the original *in situ* rumen degradation step with a standardized *Streptomyces griseus* protease incubation. The true protein (TP) contents of all samples were determined using trichloroacetic acid (1000 g/l) as precipitating agent (Licitra et al., 1996). Based on the TP concentration of the samples, addition of a *S. griseus* protease solution was adjusted to the ratio of 41 U/g TP (Licitra et al., 1998; 1999) for ruminal protein degradation. Samples (2.5 g) were accurately weighed into 500 ml Erlenmeyer flasks and 200 ml of borate-phosphate buffer (pH 6.7-6.8) were added. Flasks were then kept in a shaking water bath at 39 °C for 1 h. The required amount of fresh protease solution was added to the flask. The flasks were removed after 18 h.. Following, the content was filtered with the aid of a mild vacuum through a filter bag (38 µm pore size). Mild vacuum was used to facilitate the filtration. Residues were washed with 1.25 l deionized water and dried in a forced-air oven at 55 °C for 48 h.

Four replicates of each feed sample residue were weighed into 50-ml centrifugation tubes in an amount corresponding to 15 mg N for intestinal protein digestion. Subsequently, 10 mL of a 0.1 N HCl solution (pH 1.9) containing 1 g/l of pepsin were added to each tube and tubes were incubated at 38 °C for 1 h in a shaking water bath. After incubation, pH was neutralized with 0.5 ml of 1 N NaOH; then 13.5 ml of a phosphate buffer (pH 7.8) containing 37.5 mg of pancreatin were added to each tube which was then vortexed and incubated at 38° C in a shaking water bath. Immediately after 24 hours incubation, 3 ml of trichloroacetic acid solution (1000 g/l) were added to each tube to stop enzymatic action and precipitate undigested protein. After about 15 minutes , the samples were centrifuged at 10,000 x g for another 15 minutes at 5 °C. The precipitate was then filtrated through filter paper (no. 589, Schleicher und Schuell, Dassel, Germany) and the residue on the filter paper was analysed for insoluble N by Kjeldahl procedure.

5.2.4. Statistical analysis and calculations

Intestinal protein digestibility (IPD; g/kg CP) was estimated as:

IPD = (RUP - RCP)/ RUP x 1000;

where RUP, the rumen undegradable CP content (g) of the feed sample which was weighed into a 50-ml centrifugation tube and RCP, residual CP content (g) of the precipitate.

PROC CORR of SAS 9.2 (SAS® 2009) tested potential relations between calculated IPD values in relation to DM, ash, acid detergent fibre (ADF, expressed inclusive residual ash) and CP. Pearson's correlation coefficient was reported from PROC CORR as an indicator of the strength and the direction of these relationships. Relations between these variables and IPD were considered significant at $P < 0.05$.

Data for IPD was analyzed as a completely randomized design using the GLM procedure of SAS (SAS® 2009) separately for all 33 samples. The following orthogonal contrasts were used to compare treatment means (for explanation of feedstuff and treatment see Table5.1): 'protected vs. unprotected'(Feedstuff No. 2, 4, 9-14, 28 vs. Feedstuff No. 3, 5, 15-25, 29-33); 'RSM protected vs. SBM protected' (Feedstuff No.15-25 vs. Feedstuff No. 29-33); 'other protein supplements vs. RSM' (Feedstuff No. 1, 3, 5 vs. Feedstuff No. 15-25); 'other protein supplemts vs. SBM' (Feedstuff No. 1, 3, 5 vs. Feedstuff No. 29-33).

5.3. Results

Table 5.2 shows analysed IPD values, as well as effects of treatment and feedstuff combinations. In general, rapeseed products mean IPD values for RUP gathered at 648 g/kg CP and soybean feedstuffs exhibited an average value of 755 g/kg CP. The highest IPD value for RUP was shown by a formaldehyde-treated SBM with 880 g/kg CP, followed by xylose treated wheat grain with a IPD value of 840 g/kg CP. While the SBM also resulted in average to high CP values (446 g/kg DM), the analysed wheat grain exhibited CP value of (143 g/kg DM). The highest IPD value for RUP for a RSM was shown by a meal which was extracted with supercritical CO_2 (820 g/kg CP). Likewise this RSM is characterised by a relatively average CP value (290 g/kg TM). The lowest IPD value for RUP for a RSM was displayed by a meal which was extracted with hexane (498 g/kg CP). The SBM generally showed high IPD values and ranged between 722 g/kg CP up to 880 g/kg CP. Lowest IPD values were found in

hexane extracted rapeseed hulls (182 g/kg CP). Untreated SBM and RSM did not reveal numerically lower IPD values (SBM: 821 g/kg CP; RSM: 533 g/kg CP) although it has to be stated that the tested untreated SBM showed a relatively high CP value (513 g/kg DM).

The IPD values were affected by treatment (Table 5.3. protected vs. unprotected; $P<0.001$) and moreover, indicated that the type of protein supplement has an influence on the IPD (RSM protected vs. SBM protected; $P<0.001$; other protein supplements vs. RSM; $P<0.001$; other protein supplemts vs. SBM; $P<0.001$).

Analysis of correlation coefficients revealed a strong negative correlation between ADF values and IPD (r = -0.718, P < 0.001) as well as positive correlations between CP and IPD (r= 0.4535, P = 0.008) as well as TP and IPD (r = 0.46111, P = 0.0069).

Table 5.2. Intestinal protein digestibility (IPD) of ruminally undegraded protein (RUP), respective standard deviations (sd), expressed in g/kg crude protein (CP).

No.	Feedstuff	IPD	sd
1	Wheat grain, protected[a]	840	7,1
2	Lupine	625	47,7
3	Lupine, protected[b]	617	9,3
4	Sunflowerseed meal	685	31,6
5	Sunflowerseed meal, protected[b]	625	13,5
6	Rapeseed hulls[c]	182	17,4
7	Rapeseed cake, protected 1[b]	645	13,2
8	Rapeseed cake, protected 2[a]	680	17,4
9	Rapeseed meal 1	533	21,0
10	Rapeseed meal 2[c]	816	14,4
11	Rapeseed meal 3[c]	820	11,5
12	Rapeseed meal 4[c]	541	18,6
13	Rapeseed meal 5[c]	498	10,7
14	Rapeseed meal 6[c]	596	7,1
15	Rapeseed meal, protected 1[b]	544	17,0
16	Rapeseed meal, protected 2[b]	599	12,0
17	Rapeseed meal, protected 3[b]	592	18,8
18	Rapeseed meal, protected 4[b]	666	17,7
19	Rapeseed meal, protected 5[b]	639	27,1
20	Rapeseed meal, protected 6[b]	663	12,3
21	Rapeseed meal, protected 7[b]	660	14,9
22	Rapeseed meal, protected 8[b]	580	11,1
23	Rapeseed meal, protected 9[d]	648	5,4
24	Rapeseed meal, protected 10[d]	789	11,3
25	Rapeseed meal, protected 11[a]	749	10,6
26	Rapeseed-/Soybean meal, protected 1[b]	619	7,2
27	Rapeseed-/Soybean meal, protected 2[b]	723	19,4
28	Soybean meal	821	7,2
29	Soybean meal, protected 1[d]	757	21,0
30	Soybean meal, protected 2[d]	880	11,4
31	Soybean meal, protected 3[a]	722	5,4
32	Soybean meal, protected 4[a]	750	7,1
33	Soybean meal, protected 5[e]	770	14,9

[a] treated with xylose in lignosulphonate solution to increase ruminally undegraded CP fraction
[b] treated with a physical- thermal method to increase ruminally undegraded CP fraction
[c] specifically assembled for experimental purposes
[d] treated with formaldehyde to increase ruminally undegraded CP fraction
[e] treated with polyurea formaldehyde to increase ruminally undegraded
[e] specifically assembled for experimental purposes

Table 5.3. Contrasts, effects of treatment combinations of analysed feedstuff

'protected vs. unprotected' (Feedstuff No. 2, 4, 9-14, 28 vs. Feedstuff No. 3, 5, 15-25, 29-33)	0.001
'RSM[a] protected vs. SBM[b] protected' (Feedstuff No.15-25 vs. Feedstuff No. 29-33)	0.001
'other protein supplements vs. RSM' (Feedstuff No. 1, 3, 5 vs. Feedstuff No. 15-25)	0.001
'other protein supplements vs. SBM' (Feedstuff No. 1, 3, 5 vs. Feedstuff No. 29-33)	0.001

[a] RSM, Rapeseed meal
[b] SBM, Soybean meal

5.4. Discussion

There are several attempts to standardize the method to evaluate IPD. One big aim in the last years was to avoid the rumen incubation step which is mandatory in the most common procedures (Hvelplund,1985; Hvelplund et al., 1992). Efforts included the incubation of a feed sample with mixed rumen microorganisms or proteolytic enzymes extracted from rumen contents (Kohn and Allen, 1995; Luchini et al., 1996). Results of these studies were promising but fistulated animals were still needed. Other studies found that feed samples can also be incubated in a solution containing a protease from *S. griseus* to replace rumen fluid or ruminal proteolytic enzymes (Kopency et al., 1989; Roe et al., 1991; Aufrere et al., 1991; Assoumani et al., 1992, Cone et al., 1996; Coblentz et al., 1998). Finer aspects in this context were added by Licitra et al. (1998, 1999) who determined the activity of protease to estimate the ruminal in situ CP degradability values more precisely. Moreover, the used variable ratio of enzyme units for each tested feed sample depending on TP content instead of using the same constant ratio for all samples.

With this knowledge, Irshaid (2007) complemented the well established three-step ISIVP by Calsamiglia and Stern (1995) and found evidence that IPD values estimated from the new procedure (EIVP) compared well with data derived from ISIVP (r^2 = 0.98, P < 0.0001) and data obtained by the mobile bag technique (MBT; r^2 = 0.66, P < 0.0001) by Hvelplund (1985) and Hvelplund et al. (1992). Similarly, present results are in agreement with previous studies which have been performed to evaluate IPD in concentrate feedstuffs with either the MBT or the ISIVP (Table 5.4). In this study wheat grain exhibited the highest IPD value- this is in agreement with work by Frydrych (1992) who observed an IPD value of 886 g/kg CP using the MBT, and Irshaid (2007) who found a IPD value of 802 g/kg CP for wheat grain using the EIVP. A slightly higher IPD value was observed by Tomankova and Homolka (2002), who observed a relatively high IPD value of 946 g/kg CP for wheat grain using the MBT.

Similarly, sunflowerseed meal analysed with the MBT was expressed with an IPD value of 850 – 980 g/kg CP in a study by Alcaide et al. (2003) and hence was higher then the sunflowerseed meal in this study. Likewise to our study, IPD values found in literature for RSM and SBM differ to great extents. Studies which used the MBT found IPD values similar to this study for RSM (Frydrych et al., 1992; Tomankova and Homolka, 2002) and higher IPD values for SBM (Frydrych, 1992; Tomankova and Homolka, 2002). Similar findings can be observed by studies which used the ISIVP to analyse the IPD. Intestinal protein digestibility found in literature for RSM ranged comparable to this study (Kopecny et al., 1998; Woods et al., 2003), while IPD values for SBM were found higher in other studies (Kopecny et al., 1998;, Woods et al., 2003; Samadi and Yu, 2011). However, Can et al. (2011) who also used the EIVP to estimate IPD from SBM found alike results to this study.

It appears that findings of ISIVP and MBT result in higher IPD values than the ones determined in the present study by EIVP. Since both procedures include ruminal incubation of the samples, higher variations of results are possible, mainly due to factors like animal characteristics, bag or temporal properties as well as other procedural aspects (Kopecny et al., 1998; Vanzant et al., 1998). Consequently, the EIVP could be more repeatable than established *in situ* methods since laboratory standardization may be easier to accomplish than diminishing individual animal effects.

Table 5.4. Literature values of intestinal protein digestibility (g/kg CP) of feedstuff estimated by mobile bag technique (MBT), in situ in vitro procedure (ISIVP) or by the three-step enzymatic in vitro procedure (EIVP).

Feedstuff	Frydrych, 1992	Tomankova and Homolka, 2002	Gargallo et al., 2006	Kopecny et al., 1998	Alcaide et al., 2003	Woods et al., 2003	Samadi and Yu, 2011	Irshaid, 2007	Can et al., 2011
Wheat grain[a]	886	946	-	-	-	-	-	802	-
Lupine	-	-	882	-	-	-	-	-	-
Sunflowerseed meal	-	-	-	-	850-980	605	-	-	-
Rapeseed meal	571	745	-	720		620	-	297-710	-
Rapeseed meal[b]	-	-	-	-	-	-	-	-	-
Rapeseed meal[b]	-	-	-	-	-	-	-	705	-
Rapessed meal[b]	-	-	-	-	-	-	-	-	-
Soybean meal	990	973	-	965-972	-	840	824	-	779
Soybean meal[b]	-	-	963	-	-	-	799-898	-	690-778
Soybean meal[c]	-	-	-	-	-	-	-	793-801	-
Soybean meal[a]	-	-	-	-	-	-	-	822	-
Method	MBT	MBT	ISIVP	ISIVP	ISIVP	ISIVP	ISIVP	EIVP	EIVP

[a]treated with xylose in lignosulphonate solution to increase ruminally undegraded CP fraction
[b]treated with a physical- thermal method to increase ruminally undegraded CP fraction
[c]treated with formaldehyde to increase ruminally undegraded CP fraction

5.5. Conclusions

The comparison between experimentally determined results and literature data resulted in agreements as well as variations. In order to evaluate if absolute results are plausible and applicable, further research regarding IPD of RUP is required. However, it is possible that the EIVP is more standardized than the ISIVP and the MBT due to replacing the incubation of feed samples in the rumen with a more repeatable enzymatic procedure. This study showed that the EIVP seems to be an adequately working method to estimate IPD of RUP in concentrate feeds. The EIVP in its current, strictly standardized form can be applied to develop a database that can be used for protein evaluation systems for establishing tabular values of IPD. Nevertheless, future studies may be hindered since sufficient reference values, e.g. *in vivo* data is completely missing.

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6. GENERAL DISCUSSION AND CONCLUSION

Three different topics in animal nutrition have been discussed in the previous parts of this thesis – methane emissions, co-products of biofuel production and a new method to estimate intestinal protein digestibilities of the ruminally undegraded crude protein. At first sight these topics seem to be very different and the approach to look at them from one perspective is difficult. Nevertheless, they have certain things in common, which can be detected at a closer view. The background of all three topics is the better and more efficient use of limited resources, their impacts on the environment and a better understanding of agroecosystems. The aim of the general discussion and conclusion is to draw links between these topics and establish new approaches to see connections from a greater perspective.

Two of the studies of this work deal with topics that are seemingly unrelated but inherently connected. Biofuel production is considered to be one of the “hot topics” these days. Not only is there a fast development in the different methods and techniques, there is also a huge public debate about the ecological and economical sense of its use. Apart from the question of the general use there is also a recurring discussion about the use of respective co-products as feedstuff for farm animals. As the world moves forward to a population of nine to ten billion people by 2050 (Godfray et al., 2010), land availability becomes a bigger issue. There is competition for land providing food, water, timber, energy, settlements, infrastructure and biodiversity. Two of the greatest challenges facing humanity are the need to feed this growing population and trying to avoid climate change and adapting to the impact that cannot be avoided. There is also need to improve the resilience of food production to environmental change (Easterling et al., 2007), protect biodiversity (FAO, 2010), protect the freshwater resource (Frenken and Kiersch, 2011), move to healthier diets (WHO, 2004) and reduce the adverse impacts of food production on the whole ecosystem (Firbank et al., 2011).

Society faces important decisions regarding climate change and about the potential implications of the build-up in atmospheric concentrations of greenhouse gases (GHG). This build-up will affect the global climate, most likely stimulate global warming and moreover, will take a long time to reverse (IPCC 2000). Agriculture can play a role in an effort to reduce net emissions of GHG. It has the potential to absorb emissions, particularly CO_2, through changes in land use including conversion of cropland to grassland or forest. Agriculture can also offset GHG emissions by increasing the production of biomass commodities, which can serve either as feedstock for electricity generating power plants or as a substitute for fossil

fuel based petrol. The biofuel product ethanol has desirable environmental/health attributes relative to petroleum- based fuels. The belief that biofuels reduce GHG emissions is promoting a great interest in them throughout the world. This belief rests on Life Cycle Analysis models, which include calculations of GHG emissions from the manufacture of the fertilizers and pesticides used in crop production; from fossil fuel used to transport the fertilizer to the farm, farming operations and transport of the crop to the biofuel refinery (Schneider and McCarl, 2003).

In virtually all lifecycle analyses, the GHG emissions from producing, transporting and refining cereals and vegetable oil into ethanol and biodiesel substantially exceed the emissions from mining and refining crude oil into petrol or diesel. Reductions of GHG are concluded as a result of ignoring the carbon emitted as CO_2 from the exhaust pipes of vehicles that use biofuels, as well as the CO_2 emitted by fermentation. In a world that needs to produce more food while reducing emissions, it would be surprising to discover benefits from biofuels that use much of the world's best cropland. A more relevant focus of biofuel policy should be on the generation of additional biomass from waste feedstock, or high-yielding bioenergy crops with low nitrogen demand on land that is capable of generating these yields (Smith and Searchinger, 2012). However, biofuels can be produced in many different ways and in many different locations in the world with widely varying conditions. It is not possible to generalise the debate about whether production of biofuels is a threat or an opportunity. To stimulate a more varied discussion, as well as providing better decision data for various organisations, more knowledge needs to be developed and disseminated so the arguments for and against biofuels can be reviewed critically (Börjesson, 2009).

One of the major contributions to atmospheric pollution is caused by Nitrogen (N) derived from cattle, especially dairy cows. On the one hand dairy cows are able to make efficient use of low levels of dietary N because microbes in the rumen can synthesize a large proportion of the animals required N (Broderick, 2009). However, on the other hand, there is a limited potential of cows to convert feed N into milk. Subsequently, excessive N intake, mainly through high protein supplements, leads to large losses of N through animal excretion. One approach to define and border the feed N use efficiency (NUE) is calculated as the percentage mass of N output per mass of N input. Chase et al. (2003) specified the feed NUE of less than 20% as very low; 20 - 25% indicates substantial improvements can be made; 25 - 30% is the normal, average value while 30 - 35% is seen as above average and greater than 35% is considered as excellent. Table 6.1 gives an overview about ranges of NUE found in literature.

Powell et al. (2010) found that feed NUE is generally greater on confinement- than on grazing-based dairy farms due to several factors. Confinement farms have more detailed information on the nutritive values of the fed diets, so they can more efficiently control N levels in dairy cow rations. This allows more precisely balanced diets and better strategic use of concentrates and other protein supplements. On grazing farms it is more difficult to control the feed protein because pastures, particularly during early growth, are higher in (crude) protein than the requirement of dairy cows (NRC, 2001). Therefore, recommendations for improving feed NUE are farm specific and may also vary by region. The main aim is to narrow the gap between the actual feed NUE and the potential feed NUE, which leads to benefits, such as reductions in the need to import feeds and fertilizers (Kohn et al., 1997), increasing whole-farm NUE, reducing costs and the decrease of N excretion through manure.

Table 6.1. Indicative range of N inputs, N outputs and feed N use efficiency (NUE) on dairy farms (Powell et al., 2010).

Input to output parameters	N input range (g x cow/day)	NUE range (%)	Source
Feed to milk (feed-NUE)	26-33	26-33	Powell et al. (2006a)
	22-29	22-29	Kebreab et al. (2001)
	21-32	21-32	Castillo et al. (2000)
	21-36	21-36	Chase (2004)
	16-24	16-24	Aarts et al. (2000)

One of the big aims in animal nutrition is an efficient N feeding strategy. In the ideal case, the animal is neither undersupplied nor oversupplied with protein. Nitrogen losses through faeces and urine contribute to environmental pollution, either as ammonia, nitrous oxide, N oxides in air, or as nitrate in soil and ground water (Tamminga, 1992). Nevertheless, cattle and other ruminants are able to convert vast renewable resources from rangeland, pasture, crop residues and other by-products into food. With ruminants, land that is too poor to cultivate becomes productive. Moreover, nutrients in co-products are utilized and do not become a waste-disposal problem. In an ideal case, the dairy cow nutrient requirements should be met by their natural feed to forage ratio. High production demands and limited space make protein supplements an essential part of the diet. The challenge is to establish the minimal amount of protein required by high yielding dairy cows to achieve optimal milk production while minimizing environmental emissions.

To secure efficient feeding strategies there have to be methods estimating precisely how much

nitrogen the animal needs, or how much for the animal utilisable protein is available in the feedstuff. The current feed evaluation systems recognise the need to estimate the protein value as the amount of protein truly absorbed in the small intestine (NRC, 1985; NKJ, 1985; AFRC, 1992; Volden, 2011). The application of these systems requires data on the digestibilitiy of rumen- undegradable protein in the small intestine. Currently, methods to estimate intestinal digestibilites rely heavily on fistulated animals. *In situ* and *in vitro* methods are high in cost, labour, time and results may vary to great extents, due to the lack of standardization. In general *in vitro* methods are preferable but must first be validated with *in vivo* or *in situ* data.

In conclusion it can be stated that there is still research needed to improve existing systems in order to optimise feeding strategies to meet the animals' nutrient requirement as well as minimising GHG emissions and energy loss in agricultural production systems. This reseach should include the improvement of GHG estimation systems towards a more differentiated view to regional conditions and resources (e.g.biofuel co-products) as well as an improvement of the protein evaluation system and standardised, easy to apply laboratory methods to estimate nutrient requirements in order to achieve a more effienct usage of local resources. Growing agricultural production, high demand for food, food security, the emerging biofuel development and climate change are all linked to each other and in the future will all have a significant impact on the world food system

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CONFERENCES and PRESENTATIONS

2013	67th Symposium for the Society of Nutrition Physiology (GfE), Göttingen, Germany **(attended only)**
2012	VDLUFA (German Association for Agricultural Research) congress, Passau, Germany **(poster: Quantifizierung der Methanemissionen in einem freibelüfteten Milchviehstall bei Gras- oder Maissilage betonten Mischrationen)**
2011	VDLUFA (German Association for Agricultural Research) congress, Speyer, Germany **(presentation: Schätzung der Gehalte an praecaecal verdaulichem Rohprotein von Mischfuttermitteln für Pferde)**
2011	65th Symposium for the Society of Nutrition Physiology (GfE), Göttingen, Germany **(attended only)**
2011	8th International Symposium on the Nutrition of Herbivores, Aberystwyth, Wales. **(poster: Application of neutral detergent soluble crude protein to estimate protein digestibility of concentrate feedstuffs for horses)**
2011	10th International GCIRC Rapeseed Congress, Prague, Czech Republic. **(presentation: Estimation of intestinal protein digestibility of rapeseed and soybean products using a three-step enzymatic in vitro procedure)**
2010	4th Greenhouse Gases and Animal Agriculture Conference, Banff, Canada **(poster: Prediction of methane production from dairy cows)**
2010	64th Symposium for the Society of Nutrition Physiology (GfE), Göttingen, Germany **(attended only)**
2008	10th Tagung “Schweine und Geflügelernährung”, Halle (Saale), Germany **(attended only)**

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