Institut für Tierernährung

Universität Hohenheim

Prof. Dr. Markus Rodehutscord

VARIABILITY OF THE PROTEIN AND ENERGY VALUES OF EUROPEAN DRIED DISTILLERS´ GRAINS WITH SOLUBLES FOR RUMINANTS

DISSERTATION

Submitted in fulfilment of the requirements for the degree

„Doktor der Agrarwissenschaften"

(Dr. sc. agr./Ph.D. in Agricultural Sciences)

to the

Faculty of Agricultural Sciences

presented by

M. Sc. Edwin Westreicher Kristen

Born in Lince, Peru

2013

This work was funded through a doctoral scholarship by the “Ministerium für Wissenschaft, Forschung und Kunst Baden Württemberg”, Germany, which is gratefully acknowledged.

Die vorligende Arbeit wurde am 17. Juli 2013 von der Fakultät Agrarwissenschaften der Universität Hohenehim als „Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften“ angenommen.

Tag der mündlichen Prüfung:	29. Juli 2013
In Vertretung der Dekanin:	Prof. Dr.-Ing. Stefan Böttinger
Berichterstatter, 1. Prüfer:	Prof. Dr. Markus Rodehutscord
Mitberichterstatter, 2. Prüfer:	Prof. Dr. Andreas Susenbeth
3. Prüfer:	Prof. Dr. Hans Schenkel

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

3D	dry-degerm-fibre
AA	amino acids
ADF	acid detergent fibre
ADF	acid detergent fibre
ADiN	acid-detergent insoluble nitrogen
ADL	acid detergent lignin
AFRC	Agricultural and Food Research Council
NDF	neutral detergent fibre
BMELV	Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz
BW	body weight
C	corn
CA	crude ash
CDS	condensed distillers soluble
CF	crude fibre
CNCPS	Cornell Net Carbohydrates and Protein System
CM	canola meal
CP	crude protein
CV	coefficient of variation
DAPA	diaminopimelic acid
dCF	digestible crude fibre
dEE	digestible ether extract
d-DDGS	dietary dried distillers´ grains with solubles
DDG	dried distillers´ grains
DDGHP	high protein distillers´ grains
DDGS	dried distillers´ grains with soluble
DDGS-r	residue of dried distillers´ grains with solubles

DG	distillers´ grains
DGS	distillers´ grains with solubles
DLG	Deutsche Landwirtschafts-Gesellschaft
DM	dry matter
DMI	dry matter intake
dOM	digestible organic matter
EAAs	essential amino acids
ECM	energy corrected milk
ED	effective degradability
EDCP	effective degradability of crude protein
EDDM	effective degradability of dry matter
EE	ether extract
E-Mill	enzymatic milling
ES	elutriation and sieving (“elusive”)
EU	Europe Union
FM	fish meal
GE	gross energy
GfE	Gesellschaft für Ernährungsphysiologie
IADP	intestinally absorbable dietary protein
ID	intestinal digestibility
IVDOM	*in vitro* digestibility of organic matter
IWR	Institut der Regenerativen Energiwirtschaft
LSD	least significance difference
MCP	microbial crude protein
ME	metabolizable energy
MEEE	metabolizable energy without ether extract
MJ	Mega Joule

moHGT	modified Hohenheim gas test
MPS	milk protein score
n	number of samples/replicates
NDF	neutral detergent fibre
NDiCP	neutral-detergent insoluble crude protein
NDiN	neutral-detergent insoluble nitrogen
NEAAs	non-essential amino acids
NE_L	net energy for lactation
NFE	nitrogen-free extract
NPN	non-protein nitrogen
NRC	National Research Council
NSP	non-starch polysacharide
OM	organic matter
peNDF	physically effective neutral detergent fibre
pNDF	neutral detergent fibre measured using filter papier
PPS	pepsin-pancreatin solubility
QG	quick germ
QGQF	quick germ and quick fibre
r	correlation coefficient
RDP	rumen degradable protein
RFA	Renewable Fuels Association
RMSE	root mean square error
RNB	ruminal nitrogen balance
RSC	rapeseed cake
RSM	rapeseed meal
SARA	sub-acute rumen acidosis
SD	standard deviation

SBM	soybean meal
TMR	total mixed ration
uCP	utilizable crude protein
UDP	undegraded crude protein
UK	United Kingdom
USA	Unites States of America
UTP	utilizable crude protein
VDLUFA	Verband Landwitschaftlicher Untersuchungs- und Forschungsanstalten
vs.	versus
WDG	wet distillers´ grains
WDGS	wet distillers´ grains with solubles

CHAPTER 1

GENERAL INTRODUCTION

Ethanol production incremented in the European Union (EU) from 2007 to 2011 at a rate of more than 100% reaching more than 4 million tonnes (Renewable Fuels Association (RFA), 2012). Ethanol production yields dried distillers´ grains with solubles (DDGS) as the main by-product. After the removal of starch from the grains during ethanol production, content of fibre fractions, crude protein and crude fat are enriched. This makes DDGS very attractive as a feedstuff for ruminants. Moreover, DDGS is worldwide the third most used protein source for animal nutrition after soybean meal (SBM) and rapeseed meal (RSM) (Bundesministerium für Ernährung, Landwirtschaft und Verbraucherschutz (BMELV), 2012).

One important requisite to consider when formulating diets is that nutrients supply matches as much as possible the requirements of the animal. To precisely formulate diets and improve the biological and economical production efficiency under environmental considerations, knowledge of the nutritional requirements and of the nutritive value of feedstuffs is needed. To calculate or predict the nutritional requirement of animals, different models or feed evaluation systems have been developed around the world based on standardized and controlled research conditions. Some of the most important were developed e. g. in Germany, UK, The Netherlands, France and USA.

Variation of nutrient contents and feed values of grains should be considered and monitored. Moreover, even higher variation in nutrient contents should be expected from by-products due to the influence of different processing methods (e.g., techniques, drying, addition of supplements) and variation of the quality of raw materials.

Chemical composition is a relative rapid and cost effective method to evaluate and monitoring the feed value. However, this does only give limited information of a feedstuff for diet formulation. For ruminants, besides chemical composition, rates of degradation of nutrients in the rumen, microbial production, nutrient supply at duodenal level and intestinal digestibility of nutrients are of great importance. However, such evaluations require complex, costly and labour intensive research methods. This should be normally run under controlled research conditions with sophisticated methods that are not accessible in field condition.

The expected further increase of DDGS production and the limited information about the feed value of DDGS from the EU demands for investigation of this by-product. However a single study may limit complete and specific interpretations. Therefore, a study in systematic sequence and interrelated considering several possible questions is required to draw a complete overview and better conclusions. In addition, emphasis in estimation or prediction of feed values of DDGS through alternative simple methods should be considered, since this could rapidly and cost-effectively deliver information for practical feeding.

This doctoral thesis will review research results from European DDGS for ruminants and aims to evaluate and characterize the variation of the protein and energy values to provide updated data for livestock producers, researchers and the feed industry for the improvement of cattle nutrition. Moreover, the thesis will describe the advantages, limiting factors and challenges of feeding dairy cows with DDGS. Finally it will provide recommendations to consider in further studies concerning the use of DDGS.

REFERENCES

Bundesministerium für Ernährung, Landwirtschaft und Verbrauscherschutz (BMELV) 2012. Eiweißpflanzstrategie des BMELV. 16 pp

Renewable Fuels Association (RFA) 2012. Annual World Ethanol Production by Country. In: http://www.ethanolrfa.org/pages/statistics. Accessed March 2013

CHAPTER 2

LITERATURE REVIEW

2.1 ETHANOL PRODUCTION PROCESS AND RESULTING BY-PRODUCTS

The raw materials used for ethanol production are classified into three categories of agricultural raw materials: sugars, starch and lignocellulose (Balat and Balat, 2009). During the process, di- and oligosaccharide sugars, starch or cellulose is converted to monosaccharides such as glucose and fructose through the addition of enzymes and finally fermented into ethanol. The principal sources of starch are grains. In the USA, corn is predominantly used for ethanol production, but other grains such as barley, wheat and sorghum can also be used (Schingoethe, 2006). In western Canada, wheat is the principal grain used for ethanol production and barley can also be used alone or in combination with wheat to reduce cost of ethanol production (Mustafa *et al*., 2000a). In France, one of the largest ethanol producers of Europe, ethanol is mostly produced from beet molasses (Sánchez and Cardona, 2008), but from this raw material DDGS is not produced. And around 80% of the commercialized DDGS in France is originated from wheat and the rest from corn (Vilariño *et al*., 2007). In Europe, the main grain generally used is wheat (Rodehutscord, 2008), resulting in DDGS normally with higher CP and lower EE content compared to the North American corn-DDGS.

When grains are used for ethanol production, processing of grains is mainly classified into two types namely wet milling and dry grinding processes (Bothast and Schlicher, 2005). Figure 1 shows schematically the comparison between dry and wet grinding methods. In the USA, most of the ethanol is produced via dry grinding (Rausch and Belyea, 2006). The main difference is the focus of resourcing and the target product. In wet milling, the grain kernel is fractionated into primary components (germ, fibre and starch) (Rausch and Belyea, 2006) resulting in different valuable by-products (e. g. crude oil, corn gluten feed and corn gluten meal and germ meal). Target product of wet milling is starch for industrial and chemical use that principally can be also fermented to ethanol (Bothast and Schlicher, 2005). Wet milling requires extensive equipment and high capital investment (Belyea *et al*., 2004). Since wet milling does not yield DDGS, the by-product from this process will not be further discussed.

In the dry grinding process, the grain kernel is not fractionated (Rausch and Belyea, 2006) and the focus is maximizing the capital return per litre of ethanol yielding DDGS as only by-product (Bothast and Schlicher, 2005). Dry grinding plants are smaller, require less equipment, have lower capital investment (Belyea *et al*., 2004) and ethanol is the target product. Quantitatively, dry grinding process produces from 100 kg grain around 40 l of ethanol, 32 kg of carbon dioxide (CO_2) and 32 kg of DDGS (Schingoethe, 2006). Thus, for

easy calculations, when ethanol is produced from grains, it is assumed that the production process will yield around 1/3 of ethanol, CO_2 and DDGS each.

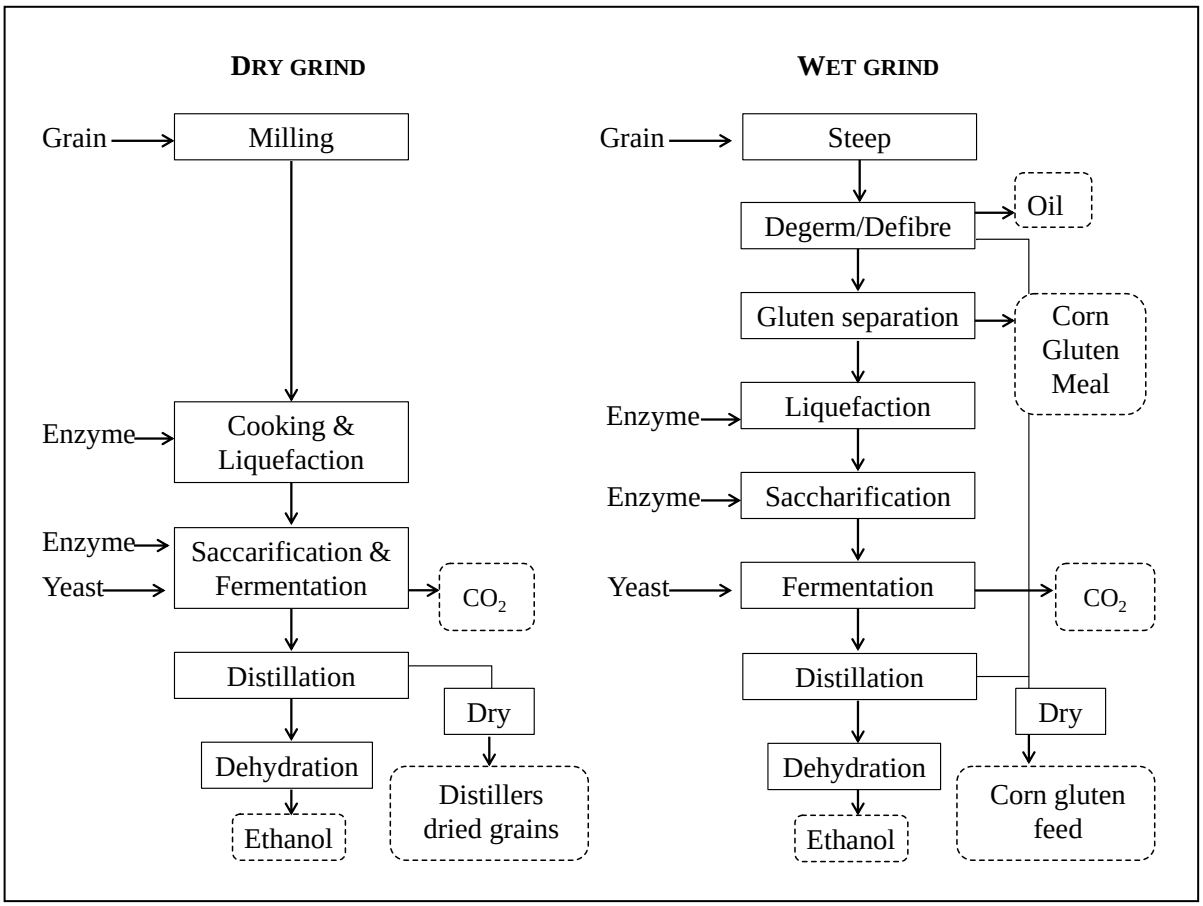

Figure 1 Schematic comparison of dry and wet grinding processes for the ethanol production from grains (Bothast and Schlicher, 2005)

Dry grinding process is designed to ferment as much of the grain kernel as possible and is based in the following basic steps: grinding, cooking, liquefaction, saccharification, fermentation, distillation and by-product recovery. These steps are briefly described according to Bothast and Schlicher (2005) as follows: (1) the entire grain is ground into coarse flour through a hammer mill and mixed with water to form a mash. (2) The pH of the mash is adjusted to pH 6.0 and thermo stable alpha-amylase enzyme is added to begin breaking down the starch polymer. The mash is heated above 100°C using a jet cooker, this provides the temperature and mechanical shear necessary to cleave and rupture starch of high molecular weight. (3) After temperature is allowed to fall to 80-90 °C, additional alpha-amylase is added and the mash is liquefied for at least 30 min, necessary to reduce the size of the starch polymer. (4) The mash is cooled, adjusted to pH 4.5 and gluco-amylase enzyme is added to convert liquefied starch into glucose through saccharification. (5) When temperature reaches 32°C, mash is transferred to fermenters where yeast is added. Additionally, ammonium sulphate or urea is added as a nitrogen source for the growth of yeast. Fermentation requires

48-72 h. (6) Ethanol is separated from the solids and the water in the mash through distillation and concentrated by dehydration. (7) After distillation and obtaining ethanol, a solid together with a liquid fraction referred as whole stillage remains.

The whole stillage obtained after distillation is further processed to recover several optional by-products of the whole ethanol production process. Which by-product will be finally produced in a factory may depend on many factors like price and demand in the market, costs and technology available for by-products production, transport and warehouse access. All the by-product alternatives can be used as feed, but normally DDGS is the preferred final by-product. Figure 2 shows the process of recovering these by-products beginning with the step of distillation. The whole stillage containing the non-fermentable portions of grains is centrifuged (Kingsly *et al*., 2010) or pressed/extruded (Bothast and Schlicher, 2005) to separate the insoluble solids from the liquid portion (thin stillage). The thin stillage (5-10% solids; Belyea *et al.* 1998) is further condensed by removing water to syrup referred as condensed distillers' solubles (CDS) (Cao *et al*., 2009) which has about 30-50% solid content (Kingsly *et al*., 2010; Belyea *et al*. 1998). Even when it is not common, CDS may be fed to animals (Schingoethe, 2006; Belyea *et al*., 1998).

The insoluble solid portion is known as wet distillers grains (WDG) and has about 65-70% moisture (Kingsly *et al*., 2010). When WDG are dried, the resulting by-product is named dried distillers grains (DDG). When the CDS are added back to the WDG, the resulting product is referred as wet distillers´ grains with solubles (WDGS) and can be used directly as feed product having only a shelf-life of about 1-2 weeks (Bothast and Schlicher, 2005) because of its high moisture content of about 70% (Birkelo *et al*., 2004; Schingoethe *et al*., 1999). WDGS can be dried to obtain DDGS. From the drying process results a product with moisture ranging between 10-13% (Kingsly *et al*., 2010; Berger and Singh, 2010). Drying WDGS to obtain DDGS is a energy-intensive process that increments the cost, but results a uniform, stable and high quality feed by-product, increase shelf-life and reduce transportation cost, which is essential for the profitability of plants (Bothast and Schlicher, 2005).

Nowadays, ethanol producers seek to optimize the efficiency of ethanol production and the value of the by-products they produce (Berger and Singh, 2010). Therefore, conventional dry grinding process has been modified and new technologies are been implemented. Innovative technologies have been developed to fractionate corn or DDGS, or both, for recovering additional by-products and improving nutritional composition of DDGS (Berger and Singh, 2010) resulting in a variety of distillers grains of different chemical

composition (Mjoun *et al*., 2010). Thus, resulting in modified processes referred as fractionation process (Li *et al*., 2012) which can be divided into wet and dry fractionation (Berger and Singh, 2010).

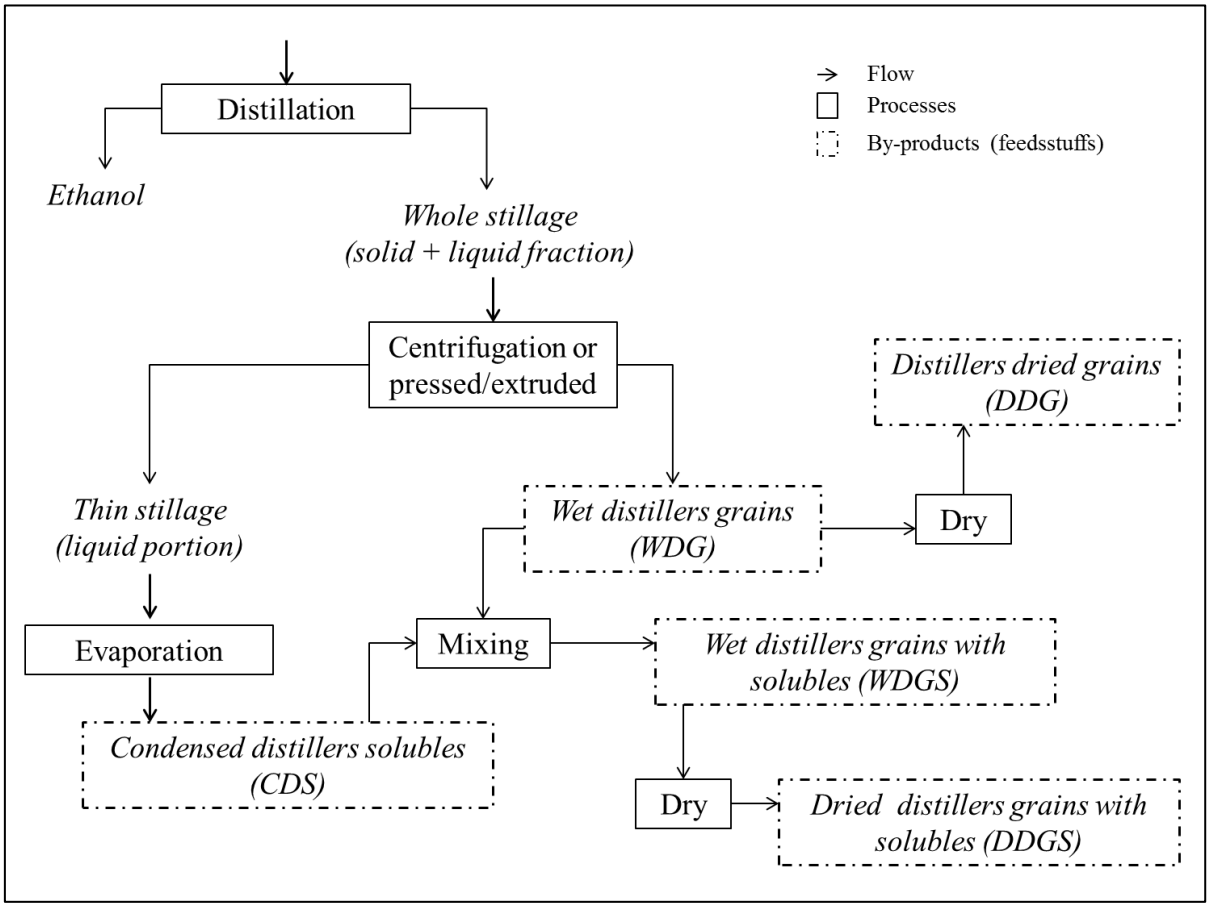

Figure 2 Steps and recovery of optional by-products for animal feeding after distillation and ethanol production in the dry grinding process.

The wet fractionation- that is a combination of the wet and dry grinding processes- involves grain fractionation in an aqueous medium to recover germ, pericarp fibre, and endosperm fibre as valuable by-products. Three processes have been developed such as quick germ (QG) (Singh and Eckhoff, 1996), quick germ and quick fibre (QGQF) (Singh *et al*., 1999) and the enzymatic milling (E-Mill), incrementing the ethanol production by 8-27%, reducing the fibre content, and increasing the CP content compared to conventional dry grinding process (Singh *et al*., 2005). These three process modifications are schematically shown in Figure 3 and compared with the conventional dry grinding process.

The QG process has the finality of recovering the high valued grain germ before fermentation for production of germ oil. Grain is soaked in water for 12 h at an optimal temperature of 59°C and germ is recovered by flotation and skimming (Singh and Eckhoff, 1996). The QGQF process is an improvement of the QG and allows the additional separation

of hull fractions prior to fermentation, resulting DDGS with low fat and fibre and increased CP content (Singh *et al*., 1999). These methods improve overall fermentation and energy efficiency in the ethanol production process (Singh *et al*., 2005).

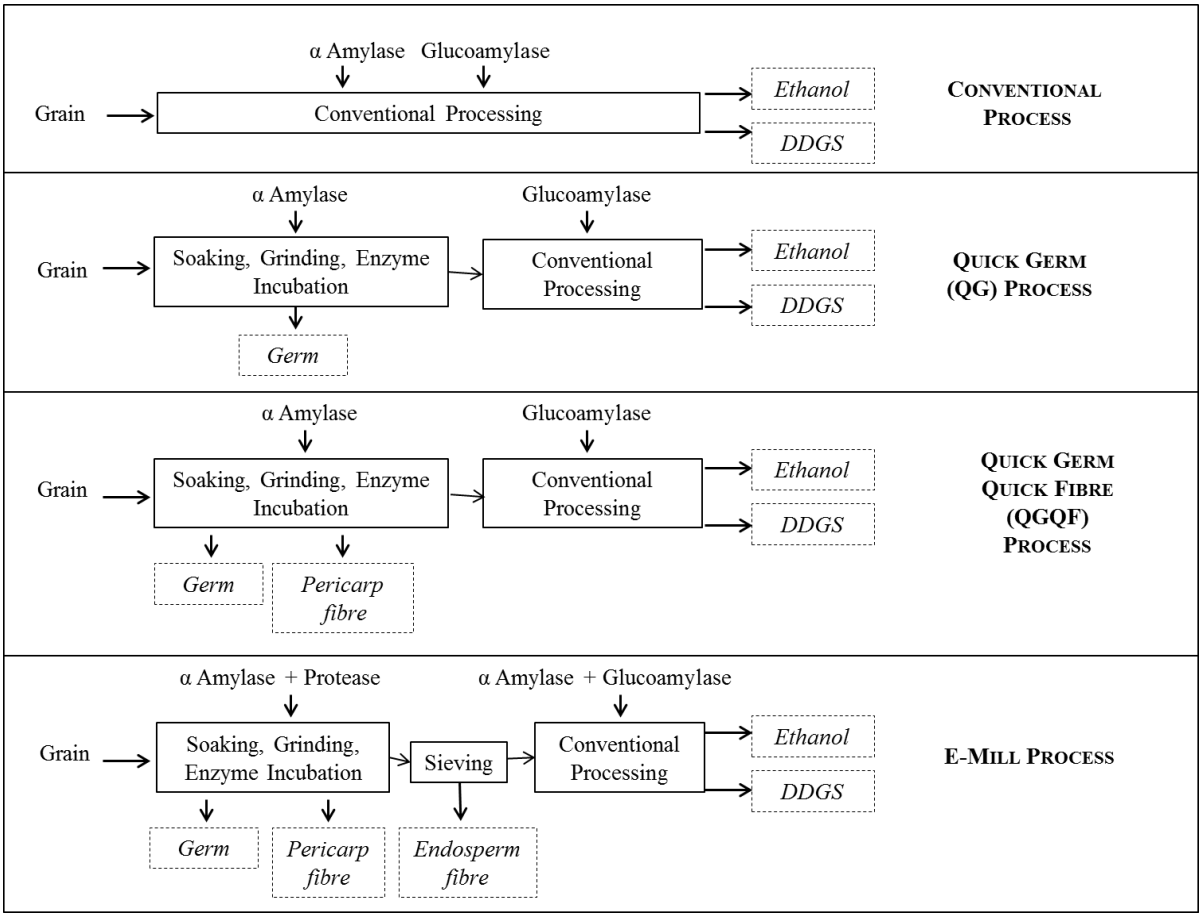

Figure 3 Schematic of conventional dry grinding process and quick germ (QG), quick germ quick fibre (QGQF), and enzymatic milling (E-Mill) wet fractionation processes (Singh *et al*., 2005)

In the E-Mill process, grains are soaked in water for 6 to 12 h, then are coarse ground and incubated with protease and starch degrading enzymes for 2 to 4 h. This process increase specific gravity of the slurry and aid separation of individual corn components (germ, pericarp fibre and endosperm fibre) before or after fermentation. This process increments CP and reduces fat and ADF (Berger and Sigh, 2010). E-Mill is an improvement of the QGQF process and allows the additional recovery of endosperm fibre as a valuable product (Singh *et al*., 2005).

In the dry fractionation process, grain kernels are physically separated into high fibre bran, germ and endosperm prior to mashing and fermentation process (Li *et al*., 2012). The non-fermentable portion (bran and germ) are not subjected to fermentation and can be processed into different products like a high protein meal, high fat corn germ and corn bran

(Baker and Babcock, 2008). This process is called dry-degerm-defibre (3D) (Murthy *et al*., 2006). In this process, corn is tempered with hot water or steam for 5 to 10 min, ground to break corn endosperm into smaller pieces called grits, germ is separated through gravity tables, and fibre is separated by aspiration. Grits are further ground and processed using conventional dry grinding method (Berger and Singh, 2010). Compared to wet fractionation, the 3D process does not recover the endosperm fibre.

DDGS from fractionation processes generally contains higher protein, lower fat, and lower fibre content than conventional (Li *et al*., 2012) as well as lower phosphorus than traditional DDGS (Depenbusch *et al*., 2008). From the dry fractionation process results also high protein distillers grains (DDGHP) (Robinson *et al*., 2008) which does not contain CDS (Mjoun *et al*., 2010).

Other dry fractionation process is the so called elusieve (ES), which uses sieving and elutriation to separate fibre from DDGS in a dry grinding process (Berger and Singh, 2010). Elutriation is an air classification process aimed to separate particles into two or more groups. The lighter or fibre fraction is carried to the top of the elutriation column and can be used for recovery of other value-added by-products like corn fibre oil and corn fibre gum. Whereas the heavier or enhanced DDGS with higher CP and fat and lower fibre content is settled to the bottom of the column (Srinivasan *et al*., 2005).

Other fractionation technologies include oil extraction from CDS or from DDGS. A disk stack centrifuge is used to recover oil from CDS resulting in DDGS with lower fat and more protein (Berger and Singh, 2010). Oil may be also removed from DDGS by solvent extraction methods. Another example of improvement of by-products of ethanol production is the modified WDGS (Berger and Singh, 2010), that is the same as conventional except this feed goes through only one dryer and soluble are added back to achieve 50% DM (Mjoun *et al*., 2010).

Development of new technologies or modifications of the dry grinding process will improve and diversify nutritional characteristics of DDGS both for ruminants and non-ruminants and will increase profitability of ethanol factories. However, this diversification will also promote a higher variability in chemical composition and feed value of DDGS.

2.2 SOURCES OF VARIATION OF DDGS

In general, DDGS has a higher concentration of chemical components such as protein, fat, minerals and fibre fractions than its original grain. These nutrients are concentrated due to the removal of most of the cereal starch that is fermented into ethanol

(Widyaratne and Zijlstra, 2007). Although corn and wheat are the major grain used for ethanol production, barley, triticale, rye, barley malt, sorghum as well as sugar beet syrup or different combination of them may be used. Moreover, difference in chemical constituents between grains species is expected. For example, corn, wheat and barley have 10.6, 13.8 and 12.4% CP, 4.5, 2.0 and 2.7% EE, and 2.6, 2.9 and 5.7 CF, respectively (Deutsche Landwirtschafts-Gesellschaft (DLG), 1997). Therefore, variation of nutrient concentration and quality of DDGS is expected to be related to the type of grain. In addition, it is inappropriate to assume fixed nutritive values for DDGS without considering factors such as DDGS type (Nuez-Ortín and Yu, 2010a). Li *et al.* (2012) found that differences in nutrient composition and amino acids (AA) profiles of DDGS varied with grain source (wheat vs. corn). Similarly, Azarfar *et al*. (2012) observed differences in protein and carbohydrates fractions, ruminal degradation characteristics of OM, CP, NDF and starch among three DDGS types. Mustafa *et al*. (2000b) found lower ruminal degradability of nutrients of barley-DDGS compared to those from wheat, rye and triticale in terms of ruminal degradability of nutrients. Nuez-Ortín and Yu (2009, 2010b) studied also the magnitude of the differences in nutritive value among different types of DDGS. Among the DDGS types they found different chemical characterisation, mineral concentration (Ca, P, S), estimated energy values, protein and carbohydrate sub-fractions and *in situ* degradability.

Variation of nutrients within the same grain is also expected. This may be due to growing conditions, varieties, seasonal variation, environmental factor, fertilization and soil conditions. Therefore, even when the same grain is used, variations in nutrient composition of DDGS might still be expected. Moreover, Belyea *et al*. (2004) found no significant correlations between components of corn and components of DDGS attributing the variation to processing methods. Contradictory, Liu (2009) found some correlation for protein and non-starch carbohydrates between corn and DDGS. Differences could be attributed to the different approaches used in both studies. However, Liu (2009) concluded that even when raw material affected DDGS to some extent, other factors such as processing method and variable proportions of yeast were responsible for larger variation in chemical attributes of DDGS. The proteins in CDS are a mixture of residual corn and yeast proteins and the exact proportion of each probably varies from batch to batch and is difficult to determine (Belyea *et al*., 1998). Therefore a proportion of CP in DDGS is of yeast origin (Belyea *et al*., 2004). When producing ethanol from grains, starch usually is removed by fermentation. However, Cozannet *et al*. (2011) found high variability of starch content (2.5-10.1% of DM) in 19 European DDGS samples; therefore, variation of starch content due to differences in

fermentation efficiency among and within plants may influence the variation of chemical composition.

Nuez-Ortín and Yu (2009, 2010a) studied the magnitude of the differences in nutritive value among ethanol plants and suggested differences in protein and carbohydrates sub-fractions, *in situ* degradability and in true protein supply predicted according to the Dutch DVE/OEB system. However, these variables differed to a lesser extent compared to the effect of DDGS types. Azarfar *et al*. (2012) found different nutritional values of DDGS among different batches within DDGS type. In addition, Belyea *et al*. (2010) found that fermentation batches were the more important source of variation of chemical composition, than ethanol plants. They assumed the variation among batches to be a result of differences in composition (starch) or physical form and particle size distribution of ground corn, and differences in processing condition among fermentation batches. Moreover, they proposed that these sources of variation can be present as single factors or there could be interactions, making the identification and control of variation in processing steps and ultimately, composition of DDGS more difficult.

CDS is an important component of DDGS susceptible to variations in chemical composition. Belyea *et al*. (1998) found higher variation in nutrient composition of CDS in long periods (week to week) rather than day to day. They suggest as possible variation factors the amount and quality (light or heavy) of steep water added to fermenters, purity of starch, temperature of water and evaporator, and the addition of other carbohydrates process streams into the fermenter. Additionally, they found low digestibility of lysine and methionine and variable AA content in CDS possibly due to drying conditions of CDS and the type of grain used for the ethanol production, respectively.

WDG and CDS differ in chemical properties. WDG is greater in content of CP (Schingoethe, 2006) and CF than CDS. Since upon centrifugation, more minerals go to the liquid fraction (CDS) than the solid fraction (WDG), CDS has higher concentration of minerals (Liu and Han, 2011), especially in P (Schingoethe *et al*., 2009), and as well as in fat content (Schingoethe, 2006) than WDG. Kingsly *et al*. (2010) suggested that more AA are present in the WDG than in the CDS fraction. Since nutrient composition of CDS is variable (Belyea *et al*., 1998) and differs with WDG (Kingsly *et al*., 2010), variation of nutritional composition of both WDGS and DDGS depends also on the proportion of CDS added back to the WDG (Cao *et al*., 2009). Moreover, Cao *et al*. (2009) found increased ruminal DM and CP degradability when the inclusion of CDS to the WDG increased. Drying WDGS to obtain DDGS can reduce the ruminal digestibility incrementing the UDP. However, heating

exposure can damage the protein resulting in reduced AA availability, especially lysine (Kleinschmit *et al*., 2007). Nevertheless, Cao *et al*. (2009) found that intestinal and total digestible protein for dried and wet DG seems to be primarily affected by processing procedures rather than the rate of inclusion of CDS in the distillers´ grains.

Ethanol production consists of many different and separated steps, each factory applies its own know-how modifying one or many steps according to own conditions, experience and availability of resources and technology, resulting in no standardized processing methods with variability of technologies among factories. Additionally, each plant has its own raw material and by-product concept. According to the literature, it is possible to summarize that probably processing methods are the predominant factor that influences variability of chemical composition and feed value characteristics of DDGS between and even within ethanol production plants.

New process modifications to improve ethanol production efficiency and quality of by-products have been developed and are been applied. These new technologies (e. g. QG, QGQF, E-Mill, 3D and ES; see chapter 2.1) recover essential part of the grains before fermentation, after fermentation and before obtaining DDGS consecutively, or remove materials directly from DDGS. Therefore, more variation of chemical composition and feed value of DDGS are expected due to a larger variation of the processing methods and new technologies available. Singh *et al*. (2005) compared conventional dry grinding with dry fractionation processes (QG, QGQF and E-Mill) and found high variation of content of CP (28-58%), EE (3.8-12.7%) and ADF (2.0-10.8%) along methods. In addition, fractionation methods produced DDGS with higher CP and lower fibre content than conventional dry grinding. Srinivasan *et al*. (2005) similarly observed an increase in CP, EE and reduction of NDF when DDGS were submitted to the ES method.

The number and variation of nutrient composition of new ethanol by-products will expand as ethanol producers seek to optimize efficiency of ethanol production and the value of the by-products they produce. And the nutritional value of these products will vary based on the technique being used (Berger and Singh, 2010).

2.3 DDGS FOR ANIMAL FEEDING

DDGS have been fed for more than 100 year; however, it is only during recent years that large quantities have become available and at competitive prices (Schingoethe *et al*., 2009). Due to the good feed value of DDGS in terms of energy, CP, NDF and fat, it has been

largely used for animal feeding around the world. DDGS are used for feeding dairy cows, beef cattle, sheep, goats, horses, pigs and poultry.

2.3.1 FEEDING RUMINANTS WITH DDGS

Due to high content of fibre fractions, CP and rumen undegraded CP (UDP), DDGS are more widely used for ruminants, especially in feedlot cattle and dairy cows feeding. Most of the published research regarding the suitability of DDGS for dairy cows feeding comes from USA and Canada. Among published results, the objectives of the studies varied widely but commonly they evaluated the effect of substituting partially or completely protein sources of diets with DDGS. Some studied the effect of partial substitution by one corn-DDGS (Grings *et al*., 1992; Anderson *et al*., 2006; Janicek *et al*., 2008). Others studied the effect of partial substitution by three different corn-DDGS (Powers *et al*., 1995; Kleinschmit *et al*., 2006). Whereas others completely substituted protein sources of the diets with DDGS (Liu *et al*., 2000; Mulrooney *et al*., 2009). In Canada, Chibisa *et al*. (2012) replaced canola meal (CM) and SBM as main protein source with wheat-DDGS at incrementing rates until 20% of DM in the ration. In Europe, Dunkel (2010) and Urdl *et al*. (2006) studied the effect of wheat-DDGS and wheat-DDGS or corn-DDGS, respectively, as a sole protein source in dairy cows. For a better overview, the results of the mentioned studies are summarized in Table 1. Grings *et al*. (1992) replaced ground corn with DDGS and found improved milk production, milk protein content and production with increasing levels of DDGS; however, this may be as a result of increased energy and protein in the diets at incremented levels of DDGS. Powers *et al*. (1995) found no effects on performance when substituting SBM with different DDGS sources. Similar results were reported by Urdl *et al*. (2006), Liu *et al*. (2000) and Mulrooney et al. (2009) when replacing different protein sources with DDGS or DDG. However, Kleinschmit *et al*. (2006) found higher milk yield and fat and protein production when SBM was replaced with DDGS. In addition, they found no differences between DDGS sources. Similarly, Janicek *et al*. (2008) and Anderson *et al*. (2006) found improved milk, protein and fat production when incrementing levels of DDGS, probably due to higher UDP intake in conjunction with incrementing level of DDGS. Whereas Dunkel *et al.* (2010) found reduced milk production when feeding cows with DDGS but no effect in milk components. In addition, Chibisa *et al*. (2012) found mixed results among traits, higher milk production with DDGS, no effects on fat and protein content of milk but higher fat and protein production at an inclusion level of 15% of DDGS compared to control diet. Different results among the mentioned trials seem to be probably due to variation of feed value between the DDGS

studied, regarding the UDP content and availability of AA, especially lysine, and the choice of the protein supplement serving as control.

Table 1 Effect of feeding distillers grains on intake, milk yield and milk composition of cows according to different feeding trials.

Level of inclusion	DMI	Milk	ECM	Fat		Protein		
% of DM	kg/d	kg/d	kg/d	%	kg/d	%	kg/d	Source
34.2 C	25.3	37.8[a]				2.63[a]	0.99[a]	Grings *et al.*, 1992
10.1 DDGS, 21.9 C	26.3	40.2[b]				2.66[b]	1.07[b]	
20.8 DDGS, 10.5 C	26.4	41.9[c]				2.78[c]	1.16[c]	
31.6 DDGS,	26.5	42.0[c]				2.80[d]	1.18[d]	
8.8 SBM	24.2	26.5	25.5	3.25	0.86	3.13	0.83	Powers *et al.*, 1995
13 $DDGS_1$	24.1	27.4	27.2	3.61	0.99	3.05	0.84	
13 $DDGS_2$	23.6	27.5	27.7	3.60	0.99	3.12	0.86	
13 $DDGS_3$	23.6	26.4	25.5	3.39	0.89	2.95	0.79	
17.6 SBM	23.5	27.0	26.0	3.47	0.94	3.25	0.88	
26 $DDGS_1$	24.3	28.0	28.5	3.68	1.03	3.25	0.91	
26 $DDGS_2$	23.8	28.0	27.1	3.32	0.93	3.07	0.86	
26 $DDGS_3$	24.4	27.4	27.6	3.59	0.98	3.08	0.84	
5.5 DDG, 10 FM, SBM	27.8	32.8	33.9	3.67	1.20	3.25	1.06	Liu *et al.*, 2000
18.9 DDG	28.4	32.6	33.7	3.72	1.20	3.23	1.05	
12.5 SBM	23.4	39.8[a]	38.4[a]	3.23	1.28[a]	3.05	1.20[a]	Anderson *et al.*, 2006
10 DDGS, 7 SBM	22.8	40.9[b]	39.6[b]	3.16	1.32[b]	3.01	1.22[b]	
20 DDGS, 2 SBM	22.5	42.5[c]	41.3[c]	3.28	1.39[c]	3.02	1.29[c]	
13.6 SBM	21.7	31.2[a]	32.2[a]	3.69	1.14[a]	3.28	1.02[a]	Kleinschmit *et al.*, 2006
20 $DDGS_1$	21.2	35.0[b]	35.5[b]	3.60	1.26[b]	3.13	1.09[b]	
20 $DDGS_2$	21.5	34.3[b]	34.8[b]	3.53	1.22[b]	3.19	1.09[b]	
20 $DDGS_3$	21.1	34.6[b]	35.9[b]	3.67	1.29[b]	3.17	1.09[b]	
12 RSC, 7 SBM	20.9	26.2		4.43		3.39		Urdl *et al.*, 2006
16 w-DDGS	20.9	25.9		4.48		3.34		
17 c-DDGS	20.8	26.4		4.46		3.33		
10.3 SBM, 6.6 CSO	21.4[a]	27.4[a]		3.70	1.00[a]	3.18	0.86[a]	Janicek *et al.*, 2008
10 DDGS	22.4[b]	28.5[b]		3.64	1.03[b]	3.19	0.91[b]	
20 DDGS	23.0[c]	29.3[c]		3.73	1.09[c]	3.16	0.92[c]	
30 DDGS	24.0[d]	30.6[d]		3.55	1.10[d]	3.14	0.95[d]	
6.6 RSM	25.2	35.2	36.7	3.81	1.34	3.05	1.08	Mulrooney *et al.*, 2009
4.6 RSM, 2.3 DDGS	25.4	35.8	38.4	4.05	1.45	3.06	1.10	
2.3 RSM, 6.6 DDGS	25.9	34.5	36.6	3.97	1.37	3.06	1.05	
10.4 DDGS	25.1	34.3	35.7	3.87	1.32	3.01	1.03	
5.7 RSC, 8.2 SBM	24.6	38.3[a]	36.3[a]	3.56		3.42		Dunkel *et al.*, 2010
9.3 RSM, 7.5 DDGS	23.4	35.7[b]	34.4[b]	3.71		3.37		
8.8 CM, 6.8 SBM	29.7[c]	42.9[b]	45.0	3.60	1.48[b]	3.32	1.44[bc]	Chibisa *et al.*, 2012
10 DDGS, 6.4 SBM	30.7[b]	44.7[a]	45.0	3.57	1.56[ab]	3.29	1.46[ab]	
15 DDGS, 2.6 SBM	30.0[bc]	44.1[ab]	44.5	3.43	1.62[a]	3.30	1.49[a]	
20 DDGS	31.8[a]	44.5[a]	45.4	3.56	1.55[ab]	3.30	1.42[c]	

C = ground corn, c-DDGS = corn-DDGS, CM = canola meal, CSO = cottonseed oil, DDG = dried distillers grains, DDGS = dried distillers grains with solubles, DM = dry matter, DMI = dry matter intake, ECM = energy corrected milk, FM = fish meal, RSC = rapeseed cake, RSM = rapeseed meal, SBM = soybean meal; w-DDGS = wheat-DDGS,

[a, b, c, d] Different superscripts within a column and within the same experiment indicate significant differences ($P<0.05$)

In addition, Table 2 resumes the meta-analysis study conducted by Kalscheur (2005) on the basis of 24 researches conducted between 1982 and 2005 using either WDG or DDGS

in dairy cow diets. This analysis gives a better general overview of the effects of inclusion of corn distillers grains (DG) at different level. Dry matter intake (DMI) remained statistically unchanged until a level inclusion of 30% of DM of DG in the diets. However, intake was slightly reduced with 20% of inclusion among DG diets and was statistically reduced with an inclusion higher than 30% compared to the control. Milk production was the same at all level of DG inclusion and was only slightly reduced with inclusion level higher than 30%. Results showed also no difference in milk fat content when feeding DG even at the highest rate in the total diet (>30% DM). However, fat content of milk was numerically superior with DG than control. Protein content of the milk was similar except, when DG was fed at higher levels than 30% resulting in reduced protein content. The latter was probably due to the lysine limitation in DG (Schingoethe, 1996; Kleinschmit et al., 2007).

Table 2 Resume of the effect of dried distillers grains on dry matter intake (DMI), milk production and milk composition according to a meta-analysis (Kalscheur, 2005)

Level of inclusion % of DM	DMI kg/d	Milk kg/d	Fat %	Protein %
0	22.1[b]	33.0[ab]	3.39	2.95[a]
4 – 10	23.7[a]	33.4[a]	3.43	2.96[a]
>10 – 20	23.4[ab]	33.2[ab]	3.41	2.94[a]
>20 – 30	22.8[ab]	33.5[a]	3.33	2.97[a]
>30	20.9[c]	32.2[b]	3.47	2.82[b]
SEM	0.8	1.4	0.08	0.06

[a, b, c] Different superscripts within a column indicate significant differences ($P<0.05$)

DDGS is also an excellent protein and energy source for feedlot cattle (Klopfenstein *et al*. 2008). Gibb *et al*. (2008) found similar feeding value of wheat-DDGS as barley when included at 20% of diet DM of feedlot cattle. Ham *et al.* (1994) found better daily weight gain (DWG) and gain/feed ratio when feedlot cattle were fed WDGS or DDGS as energy and protein source compared to control diet containing dry rolled corn. Depenbusch *et al*. (2008) found that DMI, DWG and final body weight (BW) was maximized with inclusion of corn-DDGS in diets of yearling heifers at a level of 15% DM and decreased at each level of DDGS above 15%. Moreover, Buckner *et al*. (2007) found an optimal inclusion level of 20% of DDGS in diets of feedlot cattle. Klopfenstein *et al*. (2008) concluded in their meta-analysis that distiller grains with solubles (DGS) has a good feeding value for feedlot cattle and this is dependent upon level of inclusion, and that WDGS has greater feeding value than DDGS.

For dairy goats, Baumgärtel *et al.* (2012) achieved a complete replacement of SBM with DDGS (25% DM) without changes in milk yield and milk composition. In a similar experiment, comparable results were obtained by Ringdorfer *et al*. (2010) when substituting SBM with a wheat-DDGS (19% DM).

For sheep, it was shown that feeding DDGS between 20 and 60% of lamb finishing rations resulted in acceptable performance, carcass quality and metabolite concentrations in serum (Huls *et al*., 2006; van Emon *et al*., 2012; Schauer *et al*., 2008). Although feed intake increased linearly as level of DDGS inclusion increased up to 60%, an increase in DWG was not observed, reflecting a decreased feed utilization efficiency of the rations with DDGS. Whitney and Braden (2010) found enhanced juiciness, tenderness, and flavour intensity without affecting off-flavor of meat when including 20% of DDG in diets of finishing lambs. McEachern *et al*. (2009) found no altered wool production or quality characteristics when feeding growing lambs with 20% of DDG. Moreover, Charles *et al*. (2012) found that lambs preferred diets containing DDGS over the control when they had free access to all diets, confirming a good palatability of DDGS.

2.3.2 FEEDING NON-RUMINANTS WITH DDGS

The high content of CP and EE in conjunction with the higher availability of DDGS due to increased production in Europe makes the inclusion of this feedstuff attractive as an alternative component in diets for monogastric animals like poultry and pigs. However, for the inclusion of DDGS as protein sources in diets of these species, in addition to the AA composition of the CP and its low lysine content, it is very important to consider the precaecal digestibility of the AA (Rodehutscord, 2008). In addition, high fibre content of DDGS may limit the level of inclusion in monogastric diets.

For pig feeding, Cozannet *et al*. (2010a, 2010b) and Stein *et al*. (2006) found high variability of nutrient composition and standardise precaecal digestibility of essential AA (EAA) in pigs, with lysine content in CP and lysine digestibility having more variation than other EAA in DDGS. Cromwell *et al*. (1993) found previously also similar high variability of nutrients and lysine content. Lower lysine content and digestibility was suggested as a result of the lysine destruction caused by excessive exposure to heat of DDGS (Batal and Dale, 2006). Additionally, total dietary fibre in DDGS is relatively high and three times greater than in corn and its apparent total tract digestibility is less than 50%, which results in reduced digestibility values for DM and energy (Stein and Shurson, 2009). Another limiting factor of DDGS for use in monogastric feeding may be its high content of non-starch polysaccharide (NSP), which has an inverse relationship with nutrient digestibility (Widyaratne *et al*., 2009). Therefore, the inclusion of NSP enzymes was proposed to enhance nutrient digestibility or feed intake of DDGS. However, results of feeding DDGS was found to be contradictory, with positive or no effect of enzymes (Richter *et al*., 2006; Punz *et al*., 2010; Emiola *et al*., 2009;

Widyaratne *et al*., 2009). Exposure of grains to fermentation and drying during the production process of DDGS may change the nature of NSP, thereby preventing enzymes from being effective (Widyaratne *et al*., 2009). Digestibility of P of DDGS is relatively high (up to 60%) (Stein and Shurson, 2009) and is higher than in grains likely because of partial breakdown of phytate during fermentation (Widyaratne *et al*., 2009).This provides the potential of reducing P excretion due to lower need of supplemental inorganic P. According to a large review of Stein and Shurson (2009), inclusion of DDGS in diets of nursery pigs, growing and finishing pigs and lactating sows up to 30% and in diets of gestating sows up to 50% of DM was proved to have no negative effects on animal performance. Nevertheless, its optimal level of inclusion might vary depending on the quality of feeding value of DDGS for pigs.

DDGS have been used normally in commercial poultry diets at a level of 5% or less for many years (Lumpkins *et al*., 2004). Cozannet *et al*. (2011) found high variability of lysine content and digestibility of DDGS in force-fed caecectomised cockerels. Kluth *et al*. (2008) found an average precaecal digestibility of EAA of 76%, which is lower than for SBM and RSM. An inclusion up to 20% of DDGS in diets of chicks and laying hens resulted in no negative effects on performance (Richter *et al*., 2006); however, these authors found reduced performance in finishing broilers when including more than 5% of DDGS in the diets. Contradictory, Lumpkins *et al*. (2004) suggested safe inclusion levels of DDGS at 6% in starter period and 12 to 15% in the grower and finisher period of broilers. Similarly, Thacker and Widyaratne (2007) and Wang *et al*. (2007) concluded that DDGS can be satisfactory used in broilers at a level between 15 and 20% of DM. Whereas, Shim *et al*. (2011) found no negative performance of broilers when feeding up to 24% of DDGS. Inclusion level of DDGS from 10 to 20% DM was found to have no negative effects on performance of laying hens (Roberts *et al*., 2007; Lumpkins *et al*., 2005; Nasi, 1990). However, diets containing 15 to 20% of DDGS was found to affect performance of laying hens, and supplementation of diets with NSP enzymes and lysine and methionine still resulted in low performance (Swiatkiewicz and Koreleski, 2008). In addition, they suggested a safely rate of 10 and 15% level of inclusion of rye-DDGS and corn-DDGS in diets of laying hen, respectively. Differences of the mentioned results may be due to differences in DDGS type and quality regarding content and digestibility of nutrient fractions, especially of lysine as detailed in chapter 2.3.1. Thus, inclusion level of DDGS in poultry diets will dependent on feeding value.

To summarize, high variation of chemical composition, low precaecal digestibility of AA especially lysine, low content of lysine, relative high content of NSP fraction and high

total dietary fibre will be still factors of main concern for the inclusion of DDGS in monogastric diets.

2.4 OBJECTIVES AND HYPOTHESIS OF THE STUDY

2.4.1 GENERAL OBJECTIVES AND HYPOTHESIS

A great number of publications related to the evaluation of DDGS as a feedstuff for ruminants are available. However, these studies were carried out in its majority in the USA and in Canada. Moreover, these studies concern mainly corn-DDGS. In the EU, other and diverse technological conditions regarding processing and drying methods, different grains and their mixtures for ethanol production predominate. Furthermore, considering the European conditions, jet little investigation has been carried out to evaluate the feed value for ruminants of DDGS. For these reasons, a project was conceived with the general objective to characterize the chemical composition and evaluate the protein and energy value for ruminants of DDGS from a greater number of ethanol plants of different European countries. Our general hypothesis was to find a high variation of the nutritional composition and feeding value of DDGS for ruminants. The project was carried out through *in situ*, *in vitro*, chemical and feeding approaches. The whole project is described and divided in the present doctoral thesis into three different studies and chapters.

2.4.2 SPECIFIC OBJECTIVES AND HYPOTHESIS

In the first study (Chapter 3) the specific objectives were (1) to characterize the variability in chemical composition and protein and energy values for ruminants of different DDGS available in Europe, and (2) to estimate UDP values from chemical constituents or protein fractions. We hypothesized that (1) there is a large range in nutritive value and composition, and (2) that it is possible to predict the UDP value from chemical composition or protein fractions.

In the second study (Chapter 4) the specific objectives were (1) to determine and compare the *in situ* ruminal degradation of CP and AA of DDGS from European ethanol plants and (2) to characterize the *in vitro* pepsin-pancreatin solubility (PPS) of UDP of DDGS. We hypothesized (1) that it is possible to predict ruminal degradation of individual AA from CP degradation, (2) that a large variation of PPS of UDP of DDGS exists and (3) there is a relationship between PPS and *in situ* UDP.

In the third study (Chapter 5) the specific objectives were (1) to evaluate the effect of a complete replacement of RSM as the main protein source by three different sources of

DDGS on milk production and milk composition in dairy cows in mid lactation and (2) to compare and characterize the three DDGS from different ethanol production plants in terms of feed value based on *in vivo* digestibility and *in situ* CP degradability and to compare them with RSM. We hypothesized (1) to confirm the feasibility of the use of DDGS as primary protein source in dairy cow rations and (2) to find differences between DDGS from different origin in terms of CP degradation, digestibility of nutrients and performance when fed to dairy cows.

REFERENCES

Anderson JL, Schingoethe DJ, Kalscheur KF and Hippen AR 2006. Evaluation of dried and wet distillers grains included at two concentrations in the diets of lactating dairy cows. Journal of Dairy Science 89, 3133-3142

Azarfar A, Jonker A, Hettiarachchi-Gamage KI and Yu P 2012. Nutrient profile and availability of co-products from bioethanol processing. Animal Physiology and Animal Nutrition 96, 450-458

Baker ML and Babcock BA 2008. Value maximization from corn fractionation: Feed, greenhouse gas reductions, and cointegration of ethanol and livestock. Proceeding of the Integration of Agricultural and Energy Systems. Georgia, USA. 12-13 February 2008. pp 67-74

Balat M and Balat H 2009. Recent trends in global production and utilization of bio-ethanol fuel. Applied Energy 86, 2273-2282

Batal A and Dale NM 2006. True metabolizable energy and amino acid digestibility of distillers dried grains with solubles. Journal of Applied Poultry Research 15, 89-93

Baumgärtel T, Potthast C and Peter K 2012. Einsatz von Trockenschlempe (ProtiGrain®) in der Milchziegenfütterung. Forum angewandte Forschung in der Rinder- und Schweinefütterung. Verband der Landwirtschaftskammern-Verlag, Bonn. Tagungsband S. 57-60

Belyea R, Eckhoff S, Walling M and Tumbleson M 1998. Variability in the nutritional quality of distillers solubles. Bioresource Technology 66, 207-212

Belyea RL, Rausch KD, Clevenger TE, Singh V, Johston DB and Tumbleson ME 2010. Sources of variation in composition of DDGS. Animal Feed Science and Technology 159, 122-130

Belyea RL, Rausch KD and Tumbleson ME 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresource Technology 94, 293-298

Berger L and Singh V 2010. Changes and evolution of corn for feed cattle. Journal of Animal Science 88 (E. Suppl.) 143-150

Birkelo CP, Brouk MJ and Schingoethe DJ 2004. The energy content of wet corn distillers grains for lactating dairy cows. Journal of Dairy Science 87, 1815-1819

Bothast RJ and Schlicher MA 2005. Biotechnological processes for conversion of corn into ethanol. Applied Microbial Biotechnology 67, 19-25

Buckner CD, Mader TL, Erickson GE, Colgan SL, Karges KK and Gibson ML 2007. Optimum levels of dry distillers grains with solubles for finishing beef steers. Nebraska 2007 Beef Cattle Report. University of Nebraska, Nebraska, USA. pp 36-38

Cao ZJ, Anderson JL and Kalscheur KF 2009. Ruminal degradation and intestinal digestibiliy of dried or wet distillers grains with increasing concentrations of condensed distillers solubles. Journal of Animal Science 87, 3013-3019

Charles EKR, Jonas E and Chaves AV 2012. Diet preference of lambs offered a choice of concentrate diets containing proportions of wheat dried distiller´s grain with solubles. Small Ruminant Research 108, 67-72

Chibisa GE, Christensen DA and Mutsvangwa T 2012. Effects of replacing canola meal as the major protein source with wheat dried distillers grains with solubles on ruminal function, microbial protein synthesis, omasal flow, and milk production in cows. Journal of Dairy Science 95, 824-841

Cozannet P, Primot Y, Gady C, Métayer JP, Callu P, Lessire M, Skiba F and Noblet J 2010a. Composition and amino acids ileal digestibility of wheat distillers dried grains and solubles in pigs: Sources of variability. Livestock Science 134, 176-179

Cozannet P, Primot Y, Gady C, Métayer JP, Callu P, Lessire M, Skiba F and Noblet J 2010b. Ileal digestibility of amino acids in wheat distillers dried grains with soluble for pigs. Animal Feed Science and Technology 158, 177-186

Cozannet P, Primot Y, Gady C, Métayer JP, Lessire M, Skiba F and Noblet J 2011. Standardised amino acid digestibility of wheat distillers' dried grains with solubles in force-fed cockerels. British Poultry Science 52, 72-81

Cromwell GL, Herkelman KL and Stahly TS 1993. Physical, chemical and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. Journal of Animal Science 71, 679-686

Depenbusch BE, Loe ER, Quinn MJ, Corrigan ME, Gibson ML, Karges KK and Drouillard JS 2008. Corn distillers grains with solubles derived from traditional or partial fractionation process: Growth performance and carcass characteristics of finishing feedlot heifers. Journal of Animal Science 86, 2338-2343

Deutsche Landwirtschafts-Gesellschaft (DLG) 1997. DLG Futterwerttabellen-Wiederkäuer. 7., erweiterte und überarbeitete Auflage, Herausgeber. Universität Hohenheim-Dokumentationsstelle, DLG-Verlag, Frankfurt am Main.

Dunkel S, Potthast C, Eggers J, Trauboth K, and Früh G 2010. Trockenschlempe und Rapsextraktionsschrot als alleiniger Proteinergänzer in Futterrationen für Hochleistungskühe. 122 VDLUFA-Kongress. VDLUFA-Verlag, Darmstadt. S. 613-622.

Emiola IA, Opapeju FO, Slominski BA and Nyachoti CM 2009. Growth performance and nutrient digestibility in pigs fed wheat distillers dried grain with solubles-based diets supplemented with a multicarbohydrate enzyme. Journal of Animal Science 87, 2315-2322

Gibb DJ, Hao X and McAllister TA 2008. Effect of dried distillers grains from wheat on diet digestibility and performance of feedlot cattle. Canadian Journal of Animal Science 88, 659-665

Grings EE, Roffler RE and Deitelhoff DP 1992. Responses of dairy cows to additions of distillers dried grains with soluble in alfalfa-based diets. Journal of Dairy Science 75, 1946-1953

Janicek BN, Kononoff PJ, Gehman AM and Doane PH 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. Journal of Dairy Science 91, 3544-3553

Ham GA, Stock RA, Klopfenstein TJ, Larson EM, Shain DH and Huffman RP 1994. Wet corn distillers byproducts compared with dried corn distillers grains with solubles as a source of protein and energy for ruminants. Journal of Animal Science 72, 3246-3257

Huls TJ, Bartosh AJ, Daniel JA, Zelinsky RD, Held J and Wertz-Lutz E 2006. Efficacy of dried distiller´s grains with solubles as a replacement for soybean meal and portion of the corn in a finishing lamb diet. Sheep and Goat Research Journal 21, 30-34

Kalscheur KF 2005. Impact of feeding distillers grains on milk fat, protein, and yield. Proceedings of the Distillers Grains Technology Council, 9th Annual Symposium, Kentucky, USA. 18-19 May 2005

Kingsly ARP, Ileleji KE, Clementon CL, Garcia A, Maier DE, Stroshine RL and Radcliff 2010. The effect of process variables during drying on the physical and chemical characteristics of corn dried distillers grains with solubles (DDGS) – Plant scale experiments. Bioresource Technology 101, 193-199

Kleinschmit DH, Schingoethe DJ, Kalscheuer KF and Hippen AR 2006. Evaluation of various sources of corn dried distillers grains plus solubles for lactating dairy cattle. Journal of Dairy Science 89, 4784-4794

Kleinschmit DH, Anderson JL, Schingoethe DJ, Kalscheur KF and Hippen AR 2007. Ruminal and intestinal digestibility of distillers grains with solubles varies by source. Journal of Dairy Science 90, 2909-2918

Klopfenstein TJ, Erickson GE and Bremer VR 2008. Board-invited review: Use of distillers by-products in the beef cattle feeding industry. Journal of Animal Science 86, 1223-1231

Kluth H, Wolf E and Rodehutscord M 2008. Untersuchungen zum Gehalt an ME und praecaecal verdaulichen Aminosäuren von Getreidetrockenschlempe beim Broiler. 120. VDLUFA-Kongress. VDLUFA-Verlag, Darmstadt. S. 142-148.

Li C, Li JQ, Yang WZ and Beauchemin KA 2012. Ruminal and intestinal amino acid digestion of distiller´s grain vary with grain source and milling process. Animal Feed Science and Technology 175, 121-130

Liu KS 2009. Effects of particle size distribution, compositional and color properties of ground corn on quality of distillers dried grains with solubles (DDGS). Bioresource Technology 100, 4433-4440

Liu KS and Han J 2011. Changes in mineral concentrations and phosphorus profile during dry-grind processing of corn into ethanol. Bioresource Technology 102, 3110-3118

Liu C, Schingoethe DJ and Stegeman GA 2000. Corn distillers grains versus blend of protein supplements with or without ruminally protected amino acids for lactating cows. Journal of Dairy Science 83, 2075-2084

Lumpkins BS, Batal AB and Dale NM 2004. Evaluation of distillers dried grains with solubles as a feed ingredient for broilers. Poultry Science 83, 1891-1896

Lumpkins BS, Batal AB and Dale NM 2005. Use of distillers grains with solubles as a fed ingredient for broilers. Poultry Science 83, 1891-1896

McEachern JK, Whitney TR, Scott CB, Lupton CJ and Salisbury MW 2009. Substituting distillers dried grains for cottonseed meal in lamb-finishing diets: growth, wool characteristics, and serum NEFA, urea N, and IGF-1 concentrations. Sheep and Goat Research Journal 24, 32-40

Mjoun K, Kalscheur KF, Hippen AR and Schingoethe DJ 2010. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grain products. Journal of Dairy Science 93, 4144-4154

Mulrooney CN, Schingoethe DJ, Kalscheur KF and Hippen AR 2009. Canola meal replacing distillers grains with soluble for lactating dairy cows. Journal of Dairy Science 92, 5669-5676

Murthy GS, Singh V, Johston DB, Rausch KD and Tumbleso ME 2006. Evaluation and strategies to improve fermentation characteristics of modified dry-grinding corn processes. Cereal Chemistry 83, 455-459

Mustafa AF, McKinnon JJ and Christensen DA 2000a. Chemical characterization and in situ nutrient degradability of wet distillers´ grains derived from barley-based ethanol production. Animal Feed Science and Technology 83, 301-311.

Mustafa AF, McKinnon JJ, Ingledew MW and Christensen DA 2000b. The nutritive value for ruminants of thin stillage and distillers´ grains derived from wheat, rye, triticale and barley. Journal of the Science of Food and Agriculture 80, 607-613

Nasi M 1990. Distillers feeds and feed fractions on barley in the diets of laying hens. Journal of Agricultural Science in Finland 62, 423-433

Nuez-Ortín WG and Yu P 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. Journal of Science Food and Agriculture 89, 1754-1761

Nuez-Ortín WG and Yu P 2010a. Effects of bioethanol plant and coproduct type on the metabolic characteristics of the protein in dairy cattle. Journal of Dairy Science 93, 3775-3783

Nuez-Ortín WG and Yu P 2010b. Estimation of ruminal and intestinal digestion profiles, hourly effective degradation ratio and potential N to energy synchronization of co-products from bioethanol processing. Journal of the Science of Food and Agriculture 90, 2058-2067

Powers WJ, Van Horn HH, Harris Jr. B and Wilcox CJ 1995. Effects of variable sources of distillers dried grains plus soluble on milk yield and composition. Journal of Dairy Science 78, 388-396

Punz C, Windisch W and Schedle K 2010. Einfluss von Trockenschlempe (DDGS) in Kombination mit NSP-abbauenden Enzymen auf die Mast- und Schlachtleistung von

Mastschweinen. 9. BOKU-Symposium Tierernährung. BOKU-Universität für Bodenkultur-Verlag, Wien. S. 99-104

Rausch KD and Belyea RL 2006. The future coproducts from corn processing. Applied Biochemistry and Biotechnology 128, 47-85

Richter G, Hartung H, Herzog E and Otto F 2006. Effect of distilled dried grains with solubles from wheat from bioethanol production in poultry. 9. Tagung Schweine- und Geflügelernährung. Universität Halle-Wittenberg-Verlag, Halle (Saale). S. 265-267

Ringdorfer F, Huber R and Gruber L 2010.Optimierung der Proteinversorgung von Milchziegen durch heimische Eiweißfuttermittel aus der Bioenergieerzeugung und durch die Qualität des Grundfutters. Abschlussbericht „Actiprot-Ziege“, Lehr und Forschungszentrum für Landwirtschaft, Raumberg-Gumpenstein. 13S. In: http://www2.actiprot.at/download/Proteinversorgung_Milchziegen_Raumberg_Gumpenstein_Ringdorfer.pdf. Access date 15 Februar 2013

Roberts SA, Xin H, Kerr BJ, Russell JR and Bregendahl K 2007. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. Poultry Science 86, 1716-1725

Robinson PH, Karges K and Gibson ML 2008. Nutritional evaluation of four co-products feedstuffs from motor fuel ethanol distillation industry in Midwestern USA. Animal Feed Science and Technology 146, 345-352

Rodehutscord M 2008. Nebenprodukte aus der Bioenergiegewinnung-Perspektiven für die Tierernährung. 22. Hülsenberger Gespräche 2008: Perspektiven der landwirtschaftlichen Energieerzeugung. H.-Wilhelm Schaumann Stiftung-Verlag, Hamburg. S. 122-127

Sánchez OJ and Cardona CA 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresource Technology 99, 5270-5295

Schauer CS, Stamm MM, Maddock TD and Berg PB 2008. Feeding of DDGS in lamb rations. Sheep and Goat Research Journal 23, 15-19

Schingoethe DJ 1996. Balancing the amino acid needs of the dairy cow. Animal Feed Science and Technology 60, 153-160

Schingoethe DJ 2006.Utilization of DDGS by cattle. Proccedings of the 27th Western Nutrition Conference. Manitoba, Canada. 19-20 September 2006. pp 61-74

Schingoethe DJ, Brouk MJ and Birkelo CP 1999. Milk production and composition from cows fed wet corn distillers grains. Journal of Dairy Science 82, 574-580

Schingoethe DJ, Kalscheur KF, Hippen AR and Garcia AD 2009. Invited review: The use of distillers products in dairy cattle diets. Journal of Dairy Science 92, 5802-5813

Shim MY, Pesti GM, Bakalli RI, Tillamn PB and Payne RL 2011. Evaluation of corn distillers dried grains with solubles as an alternative ingredient for broilers. Poultry Science 90, 369-376

Singh V and Eckhoff SR 1996. Effect of soak time, soak temperature and lactic acid on germen recovery parameters. Cereal Chemistry 73, 716-720

Singh V, Johnston DB, Naidu K, Rausch KD, Beleyea RL and Tumbleson ME 2005. Comparison of modified dry-grind corn process for fermentation characteristics and DDGS composition. Cereal Chemistry 82, 187-190

Singh V, Koreau RA, Doner LW, Eckhoff SR and Hicks KB 1999. Recovery of fiber in the corn dry-grind ethanol process: A feedstock for valuable coproducts. Cereal Chemistry 76, 868-872

Srinivasa R, Moreau RA, Rausch KD, Belyea RL, Tumbleson ME and Singh V 2005. Separation of fiber from distillers dried grains with solubles (DDGS) using sieving and elutriation. Cereal Chemistry 82, 528-533

Stein HH, Gibson ML, Pedersen C and Boersma MG 2006. Amino acid digestibility in ten samples of distillers dried grain with solubles fed to growing pigs. Journal of Animal Science 84, 853-860

Stein HH and Shurson GC 2009. The use and application of distillers dried grains with solubles in swine diets. Journal of Animal Science 87, 1292-1303

Swiatkiewicz S and Koreleski J 2008. The use of distillers dried grains with solubles (DDGS) in poultry nutrition. World´s Poultry Science Journal 64, 257-265

Thacker PA and Widyaratne GP 2007. Nutritional value of diets containing graded levels of wheat distillers dried grains with solubles fed to broiler chicks. Journal of the Science of Food and Agriculture 87, 1386-1390

Urdl M, Gruber L, Häusler J, Maierhoffer G and Schauer A 2006. Influence of distillers dried grains with soluble (Starprot) in dairy cow feeding. Slovak Journal of Animal Science 39, 43-50

Van Emon ML, Gunn PJ, Neary MK, Lemenager RP, Schultz AF and Lake SL 2012. Effects of added protein and dietary fat on lamb performance and carcass characteristics when fed differing levels of dried distiller´s grains with solubles. Small Ruminant Research 103, 164-168

Vilariño M, Gaüzere JM, Métayer JP and Skibaa F 2007. Energy value of wheat-DDGS in adult cockelers and growth performances of broiler chickens. Proceedings of the 16th European Symposium on Poultry Nutrition. Strasbourg, France. 26-30 August 2007. pp 83-86

Wang Z, Cerrate S, Coto C, Yan F and Waldroup PW 2007. Utilization of distillers dried grains with solubles (DDGS) in broiler diets using a standardized nutrient matrix. International Journal of Poultry Science 6, 470-477

Whitney TR and Braden KW 2010. Substituting corn dried distillers grains for cottonseed meal in lamb finishing diets: carcass characteristics, meat fatty acid profiles, and sensory panel traits. Sheep and Goat Research Journal 25, 49-56

Widyaratne GP, Patience JF and Zijlstra RT 2009. Effect of xylanase supplementation of diets containing wheat distiller´s grains with solubles on energy, amino acid and phosphorus digestibility and growth performance of grower-finisher pigs. Canadian Journal of Animal Science 89, 91-95

Widyaratne GP and Zijlstra RT 2007. Nutritional value of wheat and corn distiller´s dried grains with solubles: Digestibility and digestible contents of energy, amino acids and

phosphorus, nutrient excretion and growth performance of grower-finisher pigs. Canadian Journal of Animal Science 87, 103-114

CHAPTER 3

VARIATION IN CHEMICAL COMPOSITION AND *IN VITRO* AND *IN SITU* RUMINAL DEGRADATION CHARACTERISTICS OF DRIED DISTILLERS´ GRAINS WITH SOLUBLES FROM EUROPEAN ETHANOL PLANTS[1, 2, 3]

Edwin Westreicher-Kristen, Herbert Steingass and M. Rodehutscord

Institut für Tierernährung, Universität Hohenheim, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany

[1]Published in:Westreicher-Kristen E, Steingaß H and Rodehutscord M. 2012. Variations in chemical composition and *in vitro* and *in situ* ruminal degradation characteristics of dried distillers´grains with soluble from European ethanol plants. Archieves of Animal Nutrition 66, 450-472

[2]Presented in part at the annual Congress of the Association of German Agricultural Analytic and Research Institutes (VDLUFA), Speyer, Germany, September 13-16, 2011: Westreicher E, Steingaß H and Rodehutscord M 2011. Untersuchungen *in vitro* und *in situ* zum Protein- und Energiewert von Trockenschlempen. 123. VDLUFA-Kongress. Kurzfassungen der Referate S. 86

[3]Presented in part at the annual International Scientific Symposium on Nutrition of Farm Animals, Radenci, Slovenia, November 8-9, 2012: Steingaß H, Westreicher-Kristen E and Rodehutscord M 2011. Untersuchungen *in vitro* und *in situ* zum Protein- und Energiewert von Trockenschlempen. International Scientific Symposium on Nutrition of Farm Animals 21, 11-15

3.1 ABSTRACT

The objective of this study was to characterize variations in the composition and nutritive value of dried distillers´ grains with solubles (DDGS) for ruminants, and to estimate the undegradable crude protein (UDP) in DDGS. Thirteen samples originating from wheat, corn, barley, and blends of different substrates were studied. The rumen degradation of crude protein (CP) was determined using the nylon bag technique. Samples were incubated for 0, 1, 2, 4, 8, 16, 32, and 72 h, and in situ degradation kinetics were determined. UDP was estimated using a passage rate of 8 %/h. *In vitro* gas production was measured to estimate the metabolizable energy (ME), net energy for lactation (NE_L) and in vitro digestibility of organic matter (IVDOM). Chemical profiles varied among samples [in g/kg dry matter (DM) ± standard deviation, the values were 310 ± 33 CP, 86 ± 37 ether extract, 89 ± 18 crude fibre, 408 ± 39 neutral detergent fibre, 151 ± 39 acid detergent fibre, and 62 ± 31 acid detergent lignin], as well as in protein fractions according to the Cornell Net Carbohydrate and Protein System [in g/kg CP, the values were 161 ± 82 for fraction A, 24 ± 11 for fraction B1, 404 ± 105 for fraction B2, 242 ± 61 for fraction B3, and 170 ± 87 for fraction C]. ME, NE_L [MJ/kg DM] and IVDOM [%], also varied among samples: 12.1 ± 0.59, 7.3 ± 0.39, and 72.5 ± 4.30, respectively. The *in situ* rapidly degradable CP fraction (a) varied from 10.2 to 30.6%, and the potentially degradable fraction (b) averaged to 66.8%. UDP varied from 8.6 to 62.6% of CP. The present study suggests significant variations in composition and nutritive value among different sources of DDGS. UDP could be predicted on the basis of analysed CP fractions, but the accuracy of UDP prediction improved upon the inclusion of neutral-detergent insoluble nitrogen, explaining 94% of the variation in the UDP values. We conclude that chemical protein fractions may be used to predict the UDP values of DDGS and that the variability in the protein fractions of DDGS should be considered when formulating diets for dairy cows.

Keywords: DDGS, ruminants, gas production, protein fractionation, UDP, prediction

3.2 INTRODUCTION

The increasing demand for energy, elevated prices for mineral oil, and the promotion of the use of energy from renewable sources in the European Union (EU) have led to an increase in ethanol production. Ethanol production yields dried distillers´ grains with solubles (DDGS) as the main by-product, a valuable feedstuff for ruminant diets in terms of energy and crude protein (CP). Many systematic studies in the USA and in Canada concerning mainly corn-DDGS have been carried out to compare different types of DDGS and products from different ethanol plants (Nuez-Ortín and Yu 2009, 2010), and to compare different

batches within a single type of DDGS (Azarfar *et al*. 2012; Belyea *et al*. 2010). Spiehs *et al*. (2002) studied nutrient variability among and within ethanol plants. Belyea *et al*. (2004) determined the relationship between the composition of corn and the composition of DDGS.

In the EU, price fluctuations in grains have forced ethanol plants to use a wide variety of grains like wheat, corn, barley, and triticale, mixtures of different grains, or even the inclusion of barley malt and sugar beet syrup. This can result in an augmented variation of the nutritional composition and feeding value. Few studies have investigated European DDGS in the diets of lactating cows (Urdl *et al*. 2006). Furthermore, Cozannet *et al*. (2010, 2011) studied the variability of the feeding value of a large number of European DDGS in pigs and roosters. To the best of our knowledge, there have been no data published in scientific journals addressing the range of differences in the nutritive value of different European DDGS sources for ruminants. A lack of knowledge of the variations in DDGS nutritive value could lead to imprecise diet formulation, resulting in nutrient deficiency, reduced animal productivity, or nutrient wastage.

One important key variable for the characterization of feed CP is the ruminally undegradable protein (UDP) value, which is used in many protein evaluation systems for ruminants and is commonly calculated on the basis of in situ procedures. The UDP content of DDGS can vary depending on the raw materials used in the production process, time and temperature of drying (Nuez-Ortín and Yu, 2010), proportion of condensed distillers solubles (Cao *et al*. 2009) and also the proportion of neutral-detergent insoluble nitrogen (NDiN) or neutral detergent fibre (NDF) in the DDGS (Kajikawa *et al*. 2012). The in situ procedure requires rumen-fistulated animals, making this method time-consuming and labour intensive. Estimation of UDP through simple methods could perhaps quickly and cost-effectively deliver information. One proposed method is on the basis of the protein fractionation according to the Cornell Net Carbohydrate and Protein System (CNCPS). Shannak *et al*. (2000) and Kirchhof (2007) developed reliable equations for the UDP prediction of concentrates and forage legumes based on this approach, but these have not been validated for DDGS. Therefore, the objectives of this study were (1) to characterize the variability in chemical composition and protein and energy values for ruminants of different DDGS available in Europe, and (2) to estimate UDP values from chemical constituents or protein fractions. We hypothesized that (1) there is a large range in nutritive value and composition, and (2) that it is possible to predict the UDP value from chemical composition or protein fractions.

3.3 MATERIALS AND METHODS

3.3.1 SAMPLES AND CHEMICAL ANALYSES

Thirteen samples of DDGS were obtained from ethanol facilities located in European countries including Austria, Belgium, the Czech Republic, France, Germany, Poland, Slovakia, and the United Kingdom. Ten samples were in pelleted form. According to the producers, 5 DDGS samples originated from wheat, 3 from corn, 1 from barley, and 4 were blends (wheat:corn:triticale = 68:25:7, corn:barley malt = 85:15, wheat:barley malt = 85:15, and wheat, barley, corn, sugar beet syrup in unknown proportions, with wheat as the main constituent). All samples were ground through a 1.0 mm sieve and analysed following the official analytical methods in Germany (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten [VDLUFA], 2006) for dry matter (DM), crude ash (CA), CP, ether extract (EE), and crude fibre (CF) (methods 3.1, 8.1, 4.1.1, 5.1.1, and 6.1.1, respectively). NDF assayed using a heat-stable amylase ($aNDF_{om}$), and acid detergent fibre (ADF_{om}) were analysed (methods 6.5.1 and 6.5.2) and expressed exclusive of residual ash. Acid detergent lignin (ADL), starch, and sugar were also determined (methods 6.5.3, 7.2.1, and 7.1.1, respectively). Samples were analysed for Ca, P, Mg, K, Na, and Cl content (methods 10.2.1, 10.6.1, 10.4.1, 10.2.1, 10.1.1, and 10.5.1, respectively). Non-protein nitrogen (NPN), NDiN and acid-detergent insoluble nitrogen (ADiN) were determined according to Licitra *et al*. (1996) for the calculation of chemical protein fractions according to CNCPS (Sniffen *et al*. 1992).

3.3.2 *IN SITU* PROCEDURE

Four non-lactating cows (two Jersey cows with an average body weight of 470 kg and two Holstein cows with an average body weight of 670 kg) fitted with a rumen cannula were used. In previous experiments (unpublished) it was shown, that variation of degradation characteristics was smaller between breeds than between replicates of the same sample within a single cow. The cows were fed a daily ration consisting of 2 kg of a mixed concentrate containing 30% wheat, 30% maize, 22% rapeseed cake, 15% field beans and 3% of a mineral- and vitamin-premix and hay. Hay and water were offered for ad libitum consumption and cows were adapted to the diet for 14 days before commencing the incubations. Around 1.5 g of each sample, in original condition, was placed into nylon bags (5 × 10 cm, ~50-µm pore size, Type R510; Ankom Technology, Macedon, NY, USA) to be incubated in duplicate in the rumen of each cow for 1, 2, 4, 8, 16, 32, and 72 h. Immediately before placement in the

rumen, the filled nylon bags were soaked in warm water (~39°C) for 15 min. The samples were introduced into the rumen immediately after the morning feeding and fixed in the ventral sac of the rumen using an anchor weight (1 kg). After each corresponding incubation time, the bags were removed from the rumen and immediately immersed in ice-cold water to minimize microbial activity. Afterwards, the bags were rinsed in cold tap water to remove excess ruminal contents and were stored frozen (-20°C) until the end of the experiment. Six additional nylon bags were filled with each of the samples for the determination of zero time disappearance (0 h). The bags representing the 0-h time point were also soaked as described before and washed together with the rumen-incubated bags with cold water in a washing machine (extraKLASSE E12.18; SIEMENS, Munich, Germany) for 15 min without centrifugation and were subsequently dried at 65°C for 24 h, and weighed. Finally, the residues were analysed for their CP content. The water-soluble fraction was determined as follows: Pelleted samples were broken using a mortar, weighed in quadruplicate (~1 g) into 100-ml beakers, and soaked with 50 ml of distilled water (~37°C) for 1 min. The soaked samples were washed with distilled water through N-free filter paper (MN 615, Macherey-Nagel GmbH & Co. KG, Germany). After filtration, the remaining residue together with the filter paper, as well as blank filter papers, were dried at 103°C overnight, weighed, and analysed for N content.

3.3.3 *IN VITRO* GAS PRODUCTION

In vitro gas production was performed using the Hohenheim gas test method following the official method (25.1, VDLUFA, 2006). DDGS samples were ground to pass through a 1.0-mm sieve. Rumen liquor for incubation was obtained from 2 rumen-cannulated non-lactating Holstein cows 1 h prior to the morning feeding, filtered, and transferred into pre-warmed thermos flasks, and the rumen liquor from both cows were mixed. Cows were fed a daily ration consisting of 2 kg concentrate and hay. Hay and water were offered for *ad libitum* consumption. The liquor was added to a buffer solution (1:2 v/v) and maintained in a water bath at ~39°C under constant stirring. All laboratory handling of the liquor was carried out under continuous CO_2 flow. Samples were accurately weighed (200 ± 1 mg) into 100-ml glass syringes in triplicate and 4 repetitions were carried out. Additionally, 4 syringes containing only the rumen liquor/buffer solution, termed as blanks, 3 syringes with a hay standard, and 3 with a concentrate standard were included for the corresponding corrections of gas production. In all syringes, 30 ml of the mixed rumen liquor/buffer solution were injected. The gas production was recorded at 0, 2, 4, 8, 12, 24, 36, 48, and 72 h of incubation.

The syringes were affixed to a rotary shaker platform and oven incubated at a constant temperature (39°C).

3.3.4 CALCULATIONS, MATHEMATICAL MODELS AND STATISTICAL ANALYSES

The disappearance of CP from the bags with time was fitted to the equation proposed by Ørskov and McDonald (1979): $P = a + b\ (1\text{-}e^{-ct})$, where P = degradation after t hours, a = rapidly degradable fraction, b = potentially degradable fraction, c = rate of degradation of b, and t = time [h]. UDP was calculated as 100 – ED, where ED is the effective degradability [%] calculated with a rate of passage (k) of 8 %/h, as ED = a + [(b x c)/(c + k)]. The values of CP disappearance were corrected for the loss of small particles from the bags according to Weisbjerg *et al*. (1990), assuming that these particles are degraded at the same rate as those remaining in the bag. The small particle fraction escaping from the bags were calculated by subtracting the water soluble fraction from zero disappearance values.

The gas production values at 24 h (G24) were corrected for the obtained values of hay and concentrate standards. The *in vitro* digestibility of organic matter (IVDOM), metabolizable energy (ME), and net energy for lactation (NE_L) were calculated based on the corrected G24 [ml/200 mg DM] and nutrient composition of the samples, according to Menke and Steingass (1988), as follows: IVDOM [%] = 9 + 0.9991 x G24 + 0.595 x CP + 0.0181 x CA; ME [MJ/kg DM] = 1.06 + 0.157 x G24 + 0.084 x CP + 0.22 x EE – 0.081 x CA; NE_L [MJ/kg DM] = -0.36 + 0.1149 x G24 + 0.054 x CP + 0.139 x EE – 0.054 x CA; where CP, CA, and EE are in % DM and G24 in ml/200 mg DM.

For the gas production kinetics, the cumulative gas values at the corresponding incubation times were corrected for the blanks. Gas production kinetics were fitted to an exponential monophasic model, $Y = A\ (1\text{-}e^{-ct})$, where A = potential gas production [ml], c = constant rate of gas production [ml/h], and t = time [h]. Model parameters for *in situ* and *in vitro* data were estimated using the software GraphPad Prism 5.00 for Windows (GraphPad Software Inc., San Diego, CA, USA). Statistical analysis were run using the software package SAS (version 9.2, SAS Institute Inc., Cary, NC, USA). Correlations were tested between UDP values and chemical constituents and protein fractions using the CORR procedure. Stepwise linear multiple regression was used to predict UDP values from chemical composition and protein fractions. Linear regressions with the highest coefficient of determination (R^2) were chosen. Relationships were described using the R^2, root mean square error (RMSE), and regression coefficients. All significant differences were declared at $p < 0.05$, with tendencies associated with p-values between 0.05 and 0.10.

3.4 RESULTS

3.4.1 CHEMICAL COMPOSITION AND PROTEIN FRACTIONS

The chemical composition and protein fractions of the DDGS are shown in Table 3. Chemical profiles varied substantially among samples. The CP content ranged from 247 to 358 g/kg DM and averaged 310 g/kg DM. EE varied considerably, from 49 to 147 g/kg. The fibre fractions, especially ADF_{om}, and ADL, also varied widely. Most of the starch values ranged from 21 to 58 g/kg DM, but one sample contained 185 g/kg DM of starch. The NDiN and ADiN content varied from 12 to 31 and 3 to 16 g/kg DM, respectively. The $aNDF_{om}$ and sugar varied moderately. The CP fractions varied considerably, particularly fractions A, B2, and C. The B1 fraction was the lowest and averaged 24 g/kg CP. The B2 fraction was the highest and averaged 404 g/kg CP, followed by B3 and C averaging 242 and 170 g/kg CP, respectively. The most variable minerals were Ca, Na, and K. When classified by raw material groups, the barley-DDGS had the lowest CP concentration (247 g/kg DM) and the wheat-DDGS had the highest (330 g/kg DM). The corn-DDGS averaged the highest concentration of EE (142 g/kg DM), followed by blend-DDGS and wheat-DDGS with 82 and 58 g/kg DM, respectively.

3.4.2 *IN VITRO* GAS PRODUCTION

Gas production kinetics, ME, NE_L, and IVDOM are summarised in Table 4. The G24 ranged from 39.6 to 52.4 ml/200 mg DM. Potential gas production (A) and the rate of gas production (c) varied moderately. The predicted ME and NE_L averaged 12.1 and 7.3 MJ/kg DM, respectively. IVDOM varied between 67 and 80%. One of the wheat-DDGS samples obtained simultaneously the highest values of G24, potential gas production and IVDOM. The barley-DDGS showed the lowest values of ME (10.9 MJ/kg DM), NE_L (6.6 MJ/kg DM), and IVDOM (67%). The corn-DDGS samples tended to have higher NEL values than blend-DDGS, followed by wheat-DDGS averaging 7.6, 7.5, and 7.2 MJ/kg DM, respectively.

3.4.3 *IN SITU* DEGRADATION OF CP

The *in situ* kinetic and estimated UDP values are given in Table 5. All the estimated parameters varied widely among samples. Estimated parameters for the rapidly degradable fraction (a), potentially degradable fraction (b), and the rate of degradation (c) varied from 10.2 to 30.6%, 57.3 to 82.7%, and 2.7 to 267%/h respectively. The UDP varied from 8.6% to 62.6% of CP.

Table 3 Chemical composition of distillers dried grains with solubles (DDGS), including the mean, standard deviation (s.d.), and range (Min., Max.)

	DDGS sources																
	Corn			Wheat					Barley	Blends							
	1	2	3[#]	4[#]	5[#]	6	7	8	9	10[§]	11[ψ]	12[†]	13[‡]	Mean	s.d.	Min.	Max.
Nutrient [g/kg DM]																	
Crude ash	52	50	42	47	39	55	45	43	47	58	46	52	60	49	6	39	60
Crude protein	281	274	311	339	320	302	335	358	247	312	331	273	349	310	33	247	358
Ether extract	138	143	147	66	56	49	57	64	70	65	61	120	83	86	37	49	147
Crude fiber	78	85	89	82	82	59	90	80	134	89	107	103	85	89	18	59	134
Sugar	19	33	11	31	43	49	40	44	16	40	25	21	25	31	12	11	49
Starch	37	34	53	21	34	185	34	58	22	23	24	19	25	44	44	19	185
$aNDF_{om}$	397	382	445	450	392	334	391	435	475	380	382	401	441	408	39	334	475
ADF_{om}	144	132	158	242	120	75	167	184	140	136	153	182	127	151	39	75	242
ADL	48	38	44	130	45	31	103	95	25	52	77	63	53	62	31	25	130
NDiN	20	19	22	31	15	14	22	28	12	17	23	22	23	21	5.4	12	31
ADiN	8	6	6	16	5	3	11	11	3	7	12	15	5	8	4	3	16
Ca	0.4	0.4	0.2	0.7	0.9	1.3	1.2	1.0	1.3	0.8	0.9	1.2	0.9	0.9	0.4	0.2	1.3
P	7.8	8.3	7.0	8.6	7.5	8.3	8.5	8.2	7.2	7.9	8.0	7.9	8.9	8.0	0.5	7.0	8.9
Na	4.7	3.5	2.6	1.0	0.9	1.1	0.8	1.3	0.5	3.3	0.1	3.2	6.5	2.3	1.9	0.1	6.5
Cl	1.3	1.5	0.9	1.5	1.2	1.5	1.6	1.7	2.0	1.4	1.9	2.3	3.0	1.6	0.4	0.9	3.0
Mg	3.2	3.3	2.6	2.7	2.1	2.3	2.6	2.6	2.5	2.7	2.5	3.2	3.0	2.7	0.4	2.1	3.3
K	10.2	10.5	8.7	10.2	9.1	16.5	11.3	9.9	7.9	14.2	12.0	10.5	10.6	10.9	2.3	7.9	16.5
CP fractions[∞] [g/kg CP]																	
A	71	90	107	116	164	124	140	125	251	152	386	193	178	161	82	71	386
B1	31	6	10	22	26	29	24	21	34	28	6	33	40	24	11	6	40
B2	450	460	438	292	517	558	435	371	420	485	182	278	362	404	105	182	558
B3	274	302	328	275	188	222	188	287	218	190	195	141	332	242	61	141	332
C	174	142	117	295	105	67	213	196	77	145	231	355	88	170	87	67	355

DM = dry matter; $aNDF_{om}$ = neutral detergent fibre, amylase pretreated, ash free; ADF_{om} = acid detergent fiber, ash free; ADL = acid detergent lignin; NDiN = neutral detergent insoluble nitrogen; ADiN = acid detergent insoluble nitrogen.

[#] Not pelleted; [§] wheat:barley:corn:sugar beet syrup in unknown proportions; [ψ] wheat:barley malt = 85:15; [†] corn:barley malt = 85:15; [‡] wheat:corn:triticale = 68:25:7; [∞] According to Cornell Net Carbohydrate and Protein System (CNCPS).

Table 4 *In vitro* gas production constants, predicted metabolizable energy (ME), net energy for lactation (NE_L), and in vitro digestibility of organic matter (IVDOM) with standard deviation (s.d.)

	DDGS sources												
	Corn			Wheat					Barley	Blends			
Item[∞]	1	2	3[#]	4[#]	5[#]	6	7	8	9	10[§]	11[ψ]	12[†]	13[‡]
G24 [ml/200 mg DM]	40.9	42.3	39.6	40.2	44.3	52.4	43.1	45.3	42.2	46.4	47.6	45.1	45.5
s.d.	2.01	1.13	1.00	0.98	2.60	1.86	2.19	1.83	2.63	1.54	1.92	1.57	1.59
A [ml]	43.9	45.5	44.2	44.7	50.9	57.4	47.7	50.3	46.7	50.7	50.9	46.0	50.3
s.d.	1.76	1.77	0.96	1.64	2.57	1.00	1.77	1.22	1.77	0.64	0.96	1.07	1.05
c [ml/h]	12.7	12.4	10.6	12.7	12.1	12.9	14.4	12.7	12.4	13.3	13.7	14.1	12.1
s.d.	2.25	2.44	1.43	1.52	0.96	0.43	1.33	1.06	0.83	1.40	1.24	1.54	1.40
ME [MJ/kg DM]	12.5	12.7	12.8	11.3	11.6	12.4	11.5	12.2	10.9	11.9	12.3	12.6	12.5
s.d.	0.32	0.18	0.16	0.15	0.41	0.29	0.34	0.29	0.41	0.24	0.30	0.25	0.25
NE_L [MJ/kg DM]	7.5	7.7	7.7	6.8	7.0	7.7	7.0	7.4	6.6	7.2	7.5	7.7	7.6
s.d.	0.23	0.13	0.12	0.11	0.30	0.21	0.25	0.21	0.30	0.18	0.22	0.18	0.18
IVDOM [%]	67.5	68.4	67.8	70.2	73.0	80.3	72.8	76.4	66.7	75.0	77.1	71.2	76.3
s.d.	2.00	1.13	1.00	0.98	2.60	1.86	2.19	1.84	2.63	1.54	1.91	1.57	1.59

[#] Not pelleted; [§] wheat:barley:corn:sugar beet syrup in unknown proportions; [ψ] wheat:barley malt = 85:15; [†] corn:barley malt = 85:15; [‡] wheat:corn:triticale = 68:25:7; [∞] G24 = gas production after 24 h incubation; gas production constants calculated from $Y = A (1\text{-}e^{-ct})$, where A = potential gas production, c = constant rate of gas production, and t = time [h].

Table 5 Estimated parameters of in situ ruminal kinetic and UDP fractions with standard deviation (s.d)

	DDGS sources												
	Corn			Wheat					Barley	Blends			
Item[∞]	1	2	3[#]	4[#]	5[#]	6	7	8	9	10[§]	11[Ψ]	12[†]	13[‡]
a [%]	17.2	19.0	17.1	10.2	18.8	19.3	21.1	19.7	30.6	20.4	28.5	16.4	21.3
s.d.	2.63	0.98	1.36	1.12	1.44	1.09	3.43	1.39	1.64	1.46	0.01	0.22	0.32
b [%]	66.3	69.2	82.7	72.2	67.5	71.5	60.9	64.8	57.3	61.3	64.8	60.8	68.6
s.d.	12.7	6.82	8.07	2.85	2.09	1.64	1.66	2.43	1.30	0.95	0.55	1.55	1.11
c [%/h]	4.5	3.5	2.7	5.2	37.8	13.6	15.7	11.3	48.4	26.0	267	130	15
s.d.	2.61	0.79	0.56	0.46	6.05	2.93	6.72	2.45	9.22	4.51	10.6	18.2	1.27
UDP [% of CP]	61.2	60.2	62.6	61.4	25.6	36.1	40.1	42.8	20.4	32.9	8.6	26.4	34.1
s.d.	1.78	1.14	1.93	1.34	1.40	2.34	2.87	1.63	0.88	1.12	0.48	1.13	0.76

[#] Not pelleted; [§] wheat:barley:corn:sugar beet syrup in unknown proportions; [Ψ] wheat:barley malt = 85:15; [†] corn:barley malt = 85:15; [‡] wheat:corn:triticale = 68:25:7; [∞] Calculated from the fitted equation $P = a + b\ (1\text{-}e^{-ct})$, where P = degradation after t hours, a = rapidly degradable fraction, b = potentially degradable fraction, c = rate of degradation of b fraction [%/h] and t = time [h].UDP = undegradable CP, calculated as 100 – ED; ED was calculated from the equation $a + [(b \times c)/(c + k)]$ with rate of passage of 8%/h (k).

Among the blend-DDGS samples, those originating from a proportion of barley malt obtained the highest estimates for rates of degradation (130 and 267 %/h). The UDP [% CP] tended to be higher for corn-DDGS (61.3), followed by wheat-DDGS (41.2) and blend-DDGS (25.5), and was associated with their respective average rates of degradation of 3.6, 16.7, and 109.5 %/h.

3.4.4 CORRELATIONS AND ESTIMATION OF UDP

Correlation coefficients between UDP, and chemical constituents and protein fractions, are shown in Table 6. EE was not correlated with UDP (r = 0.44), but CF was (r = -0.55). The NDiN tended to correlate with UDP (r = 0.54, P = 0.05), whereas ADiN had no relationship with UDP. UDP was negatively correlated with protein fraction A (r = -0.83), and positively with fraction B3 (r = 0.70). Other chemical constituents and protein fractions neither had significant relations, nor tended to correlate, with UDP.

Table 6 Correlation coefficient (r) between UDP and chemical fractions

Item	UDP
Nutrient [g/kg DM]	
Crude protein	0.24
Ether extract	0.44
Crude fibre	-0.55*
$aNDF_{om}$	-0.39
ADF_{om}	-0.09
ADL	-0.06
NDiN	$0.54^{(*)}$
ADiN	0.12
Protein fractions[#] [g/kg CP]	
A	-0.83***
B1	-0.20
B2	0.22
B3	0.70**
C	0.06

UDP = undegradable CP [g/kg DM] with a rate of passage of 8 %/h; $aNDF_{om}$ = neutral detergent fiber, amylase pretreated, ash free; ADF_{om} = acid detergent fiber, ash free; ADL = acid detergent lignin; NDiN = neutral detergent insoluble N; ADiN = acid detergent insoluble nitrogen. [#] According to the Cornell Net Carbohydrate and Protein System (CNCPS). ***p < 0.001; **p < 0.01, *p < 0.05; $^{(*)}$0.10 < p> 0.05; otherwise p > 0.10.

Equations for the prediction of UDP values from nutritional composition and protein fractions are shown in Table 7. The equation based on the proximate constituents (EE and CF) was significant. The use of protein fractions A, B2, and B3 improved the accuracy of the prediction, explaining 91% of the variation of the UDP value, with a lower RMSE. When fraction A was replaced by fraction C, the equation was even better, explaining 93% of the variation in UDP with a reduced RMSE. In terms of the coefficient of determination, RMSE and deviation of the slope from zero, when considering parameters B3 + C and NDiN x

6.25/(A + B1), the equation was even more suitable for UDP prediction (R^2 = 0.94, RMSE = 14.6). With this equation, the difference between the reference and predicted values of UDP varied from -18 to 23 g/kg DM (Table 8). The equations of Shannak *et al*. (2000) and Kirchhof (2007) overestimated substantially the UDP values of DDGS from 496 to 5241 and 291 to 639 g/kg DM, respectively.

Table 7 Equations calculated to predict UDP from nutrient fractions and protein fractions

	Regression			
Item	1	2	3	4
Intercept	221.7**	235.6**	-383.6***	48.01*
Ether extract	6.82$^{(*)}$			
Crude fibre	-17.67*			
A		-0.62***		
B2		-0.20*	0.45***	
B3		0.28*	0.89***	
C			0.65***	
(B3+C) x A				-0.0003*
NDiN x 6.25/(A + B1)				120.31***
p_{global}	*	***	***	***
R^2	0.51	0.91	0.93	0.94
RMSE	42.4	19.5	16.3	14.6

UDP = undegradable CP [g/kg DM] with a rate of passage of 8 %/h; A, B2, B3, and C are protein fractions according to CNCPS [g/kg CP]; RMSE = root mean square error; ***p < 0.001; **p < 0.01; *p < 0.05; $^{(*)}$0.10 < p > 0.05.

3.5 DISCUSSION

3.5.1 ESTIMATION OF UDP

In this study, we attempted to predict UDP values from the chemical constituents of DDGS. The moderate coefficient of determination and high RMSE between EE and CF, and the *in situ* UDP values, indicate that these fractions alone are not reliable for UDP prediction. Although significant, this relationship probably is an artefact, since corn-DDGS samples have the highest UDP and EE values and the other types of DDGS have lower UDP and EE content. Given that the 5 protein fractions according to the CNCPS (A, B1, B2, B3 and C) have different rates and extents of ruminal degradation, it was hypothesized that they are good predictors for UDP values of DDGS. Kirchhof (2007) and Shannak *et al*. (2000) proved that UDP in concentrates and forages can be predicted from protein fractions and NDF. DDGS were not considered in their studies. Therefore, we attempted to predict UDP values from protein fractions, using *in situ* UDP values as the reference. Equations developed by Kirchhof (2007) and Shannak *et al*. (2000) considering a passage rate of 8%/h proved to be unsuitable for the DDGS studied. Overestimations seem to be due to a higher proportion of fraction C in DDGS (67-355 g/kg CP) compared to feedstuffs studied by Shannak *et al*. (2000) and

Kirchhof (2007). They reported that protein fraction C made up 5 to 95 g/kg CP in feeds of plant origin and 20 to 78 g/kg CP in forages. Therefore, we calculated new equations for the prediction of UDP in DDGS. The NDiN fraction was additionally considered, because NDiN was proposed as an appropriate indicator for UDP and tended to correlate with UDP. Based on the coefficient of determination and the RMSE, the inclusion of NDiN into the equation proved more efficient than only protein fractions or other chemical fractions. Dividing the CP into different fractions provides more detailed information than CP alone. Since the CP fractions are divided based on the different rates of ruminal degradation, this could indirectly account for the dynamics of ruminal degradation of CP. This present calculations show that CP fractions have good potential as predictors of UDP in DDGS, and a similar approach should be followed for other types of protein feedstuff. However, specific studies and adaptations should be carefully considered for each type of feedstuff. In our study, the resulting equations for UDP prediction in DDGS differed from those of Shannak *et al.* (2000) and Kirchhof (2007). The differences were basically due to different protein fraction profiles of DDGS compared to those of the feedstuff samples used in the two prior studies. Additionally, a higher number of samples of DDGS should be further investigated to independently validate the equations predicted in the present study before applying them to routine UDP prediction.

Table 8 UDP of DDGS and differences between predicted UDP values based on a new regression equation as well as Shannak *et al*. (2000) and Kirchhof (2007)

		Difference of UDP		
	In situ UDP	New equation[#]	Shannak[§]	Kirchhof[ψ]
Sample		[g/kg DM]		
1	172	15	1292	392
2	165	23	941	357
3	195	-18	751	313
4	208	-11	4161	465
5	82	11	726	377
6	109	-3	519	291
7	134	-4	2172	445
8	153	19	1873	412
9	50	6	496	359
10	103	0	1031	393
11	28	14	2345	461
12	72	19	5241	639
13	119	-13	522	297

UDP = undegradable CP with a rate of passage of 8 %/h;

[#] UDP = 48.01 - 0.0003 x ((B3+C) x A) + 120.31 x (NDiN x 6.25/(A+B1)).

[§] UDP = - 98.663 - 275.125 x (CP/pNDF) + 0.0028 x (CP x B2) - 0.0220 x (CP x C) + 0.0032 x (CP x (A+B1)) + 0.0002 x (CP x C^2) - 0.0020 x (pNDF x B1) + 0.0035 x ((B3 + C) x B2).

[ψ] UDP = 285.5459 + 1.2143 x C + 0.0005 x (pNDF x B2) - 110.1740 x ((A+B1)/pNDF); pNDF denotes that the measurement of NDF was accomplished using filter paper.

3.5.2 *IN SITU* DEGRADABILITY OF CP

UDP values for wheat-DDGS and barley-DDGS are lower than for corn-DDGS, reflecting the degradability of the grain substrate (Schingoethe 2006). Nuez-Ortín and Yu (2009) found a relationship between *in situ* CP degradability of the original grain and the type of DDGS. In the present study, corn-DDGS averaged the highest UDP value (61.3% of CP), associated with the lowest rate of degradation 3.6%/h, followed by wheat-DDGS (41.2% of CP). The high variation in UDP observed among the wheat-DDGS samples in the present study (25.6-61.4%), could be due to differences in processing methods, the nutritional composition of raw materials, and drying temperatures and processes applied after ethanol distillation. The lower UDP values of the 2 blend-DDGS samples originated from the proportion of barley malt (8.6 and 26.4%), and were accompanied by the highest rates of degradation (267 and 130 %/h) and with the highest concentration of CP fraction A (386 and 193 g/kg CP), the fraction that contains NPN and is rapidly degraded in the rumen. The variations in the estimated value of the rapidly degradable fraction (a) of DDGS (*in situ*) could be due to differences in raw materials, time and temperature of drying, and the amount of solubles blended back (Nuez-Ortín and Yu 2010). A similar hypothesis can be raised for the potentially degradable fraction (b). The heating process could incorporate part of fraction "a" into "b" (Kajikawa *et al*. 2012), influencing their variation. Since NDiN and ADiN are considered slowly degradable and undegradable, respectively, in the rumen, they were proposed to be appropriate indicators for rumen UDP. However, NDiN only tended to correlate, and ADiN did not correlate, with the UDP values of DDGS in the present study. Kajikawa *et al*. (2012) reported relatively higher *in situ* degradation of NDiN in DDGS and suggested partial degradation of ADiN. They proposed that the low levels of lysine and xylose in DDGS, which are involved in the Maillard reaction, may be one cause of the partial degradation of NDiN and ADiN. Interpretations about corn-DDGS, wheat-DDGS, barley-DDGS, and blend-DDGS should be carefully considered, since different processing methods could influence the *in situ* degradation of CP and consequently UDP values. Specific information about the technical process was not provided by the processing plants, and this represents an important limiting factor in the present survey and in further data interpretations. However, this lack of information is also a reality in the feed market. Therefore, it is important to try to predict feed values of DDGS from chemical fractions.

3.5.3 CHEMICAL COMPOSITION AND PROTEIN FRACTIONS

The nutrient concentrations of DDGS usually reflect proportionately increased concentrations of those components relative to the original substrate after the extensive removal of starch during ethanol production (Schingoethe 2006). In general, the CA, EE, and CF concentrations of DDGS were duplicated and the CP content was almost triplicated compared to the published values of original corn, wheat, and barley (Deutsche Landwirtschafts-Gesellschaft, 1997). However, when the same grain is used, variability is likely caused by differences in the effectiveness of fermentation, drying temperatures (Stein *et al*., 2006), and duration of fermentation (Belyea *et al*. 2004). Ammonium sulphate or urea, and recently, proteases are added to provide nitrogen directly or indirectly by breaking down grain protein to free amino acids for the growth of yeast (Bothast and Schlicher 2005). Thus, the CP concentration and CP fraction A are probably influenced, simultaneously affecting *in situ* CP degradability and UDP values. The yeast used during ethanol production should also be considered as a protein source in the DDGS, but information about the proportion of yeast protein is lacking and difficult to determine. Han and Liu (2010) found that yeast contributed about 20% to DDGS protein. Condensed distillers solubles added back to the DDGS influence the CP and EE content (Kingsly *et al*. 2010; Cao *et al*. 2009). Variations in the NDiN and ADiN content are related to the heating processes used in ethanol plants (Kajikawa *et al*. 2012), since heat damage incorporates CP into the NDF and ADF fractions. Variations in A and B2 fractions could be explained by differences in plant processing methods and drying temperatures, as well as variability in the original grains used for the ethanol production (Nuez-Ortín and Yu 2009). The higher fraction A of both blend-DDGS samples originating partly from barley malt were perhaps due to free amino acids generated from protein hydrolysis during the malting process.

The high variation of Ca and Na content in DDGS is probably related to the addition of exogenous sources to sanitize process lines, to adjust pH, or to optimize enzyme and yeast performance during ethanol production (Liu and Han 2011). Variations in K concentration could be attributed to the addition of pH regulators, molasses, and sugar beet syrup or, according to Cao *et al*. (2009), due to condensed distillers solubles added back. However, when higher proportions of condensed distillers solubles are added, variations in P content can be expected (Cao *et al*. 2009; Schingoethe 2006), which seems not to be reflected in our results. The low variation in P and Mg concentrations among samples could reflect the reduced influence or lack thereof of processing techniques and conditions on the DDGS studied. In general, large variations in nutrients and protein fractions were due to large

variations in raw materials and their combinations in different proportions, and due to different processing methods between ethanol production plants.

3.5.4 *IN VITRO* GAS PRODUCTION

The variation in potential gas production, G24, and IVDOM can also be explained by differences in nutrient composition among the DDGS samples. Potential gas production was significantly affected by EE (r = -0.71), starch (r = 0.67), sugar (r = 0.70), $aNDF_{om}$ (r = -0.56), and ADF_{om} (r = -0.62). Consequently, IVDOM was correlated to EE (r = -0.67), CP (r = 0.59), and sugar (r = 0.67). This explains the highest values for G24, potential gas production, and IVDOM observed in one of the wheat-DDGS, related to its higher starch content (185 g/kg DM) and lower fibre fractions content. In contrast, the lowest values for the predicted ME, NE_L, and IVDOM seen in the barley-DDGS reflect its lower nutritional value and could be explained by lower concentrations of CP, sugar, and starch, and higher concentration of fibre fractions due to the enriched proportion of low digestible hulls. The energy values of DDGS are expected to be lower than of the corresponding original grains due to an increase in fibre fractions and reduction in starch after ethanol production. However, Nuez-Ortín and Yu (2009) found that different types of DDGS were similar or superior to their respective raw materials in terms of energy values for ruminants. The ratio between G24 and potential gas production "A" showed that 90.2-91.3% of the fermentation occurred during the first 24 h of incubation, confirming the good quality of the DDGS, since at high feed intake and high passage rates it is desirable that the major part of nutrient fermentation occurs in a relative short period of time. Moreover, half of the potential gas production was produced in 5.5 h, with the time taken ranging from 4.8 to 6.6 h. The ratio between gas production at 72 h and the potential gas production (93.7%) proves that the incubation time was long enough to express the potential fermentation of the DDGS.

3.6 CONCLUSIONS

The chemical composition and energy values differed among the DDGS samples studied. DDGS have good potential as a source of UDP, but the variability of UDP content of DDGS was also very high. These variations should be of major concern for the ethanol industry and reveal the challenging importance of more standardized products to be used in practice. Ethanol plants should deliver specific information about production processes in further studies and consider frequent analysis for product specification. The prediction of UDP from proximate nutrient analyses was not reliable. Equations to predict UDP from

protein fractions gave better results. However, additional research to estimate UDP by simple methods and independent validation is needed. Further studies on the amino acid profiles of DDGS and the intestinal digestibility of UDP are also recommended.

REFERENCES

Azarfar A, Jonker A, Hettiarachchi-Gamage IK, Yu P. 2012. Nutrient profile and availability of co-products from bioethanol processing. J Anim Physiol Anim Nutr. 96, 450-458.

Belyea RL, Rausch KD, Tumbleson ME. 2004. Composition of corn and distillers dried grains with solubles from dry grind ethanol processing. Bioresource Technol. 94:293-298.

Belyea RL, Rausch KD, Clevenger TE, Singh V, Johnston DB, Tumbleson ME. 2010. Sources of variation in composition of DDGS. Anim Feed Sci Tech. 159:122-130.

Bothast RJ, Schlicher MA. 2005. Biotechnological processes for conversion of corn into ethanol. Appl Microbiol Biot. 67:19-25.

Cao ZJ, Anderson JL, Kalscheur KF. 2009. Ruminal degradation and intestinal digestibility of dried or wet distillers grains with increasing concentrations of condensed distillers solubles. J Anim Sci. 87:3013-3019.

Cozannet P, Primot Y, Gady C, Métayer JP, Callu P, Lessire M, Skiba F, Noblet J. 2010. Composition and amino acids ileal digestibility of wheat distillers dried grains and solubles in pigs: Sources of variability. Livest Sci. 134:76-179.

Cozannet P, Primot Y, Gady C, Métayer JP, Lessire M, Skiba F, Noblet J. 2011. Standardised amino acid digestibility of wheat distillers´ dried grains with soluble in force-fed cockerels. Brit Poultry Sci. 52:72-81.

Deutsche Landwirtschafts-Gesellschaft (DLG) 1997. DLG Futterwerttabellen-Wiederkäuer. 7., erweiterte und überarbeitete Auflage, Herausgeber. Universität Hohenheim-Dokumentationsstelle, DLG-Verlag, Frankfurt am Main.

Han J, Liu K. 2010. Changes in composition and amino acids profile during dry grind ethanol processing from corn and estimation of yeast contribution toward DDGS proteins. J Agr Food Chem. 58:3430-3437.

Kajikawa H, Miyazawa K, Yanase A, Tanabe Y, Tsuchida Y, Mitsumoto Y, Kozato Y, Mitsumori M. 2012. Variation in chemical composition of corn dried distillers grains with solubles in relation to *in situ* protein degradation profiles in the rumen. Anim Sci J. 83:299-304.

Kingsly ARP, Ileleji KE, Clementson CL, Garcia A, Maier DE, Stroshine RL, Radcliff S. 2010. The effect of process variables during drying on the physical and chemical characteristics of corn dried distillers grains with solubles (DDGS)-Plant scale experiments. Bioresource Technol. 101:193-199.

Kirchhof S. 2007. Untersuchungen zur Kinetik des ruminalen in situ-Nährstoffabbaus von Grünlandaufwüchsen des Alpenraumes unterschiedlicher Vegetationsstadien sowie von Maissilagen und Heu- ein Beitrag zur Weiterentwicklung der Rationsgestaltung für Milchkühe. Doctoral thesis, University of Kiel, Germany.

Licitra G, Hernandez TM, van Soest PJ. 1996. Standarization of procedures for nitrogen fractionation of ruminant feeds. Anim Feed Sci Tech. 57:347-358.

Liu K, Han J. 2011. Changes in mineral concentrations and phosphorus profile during dry-grind processing of corn into ethanol. Bioresource Technol. 102:3110-3118.

Menke KH, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim Res Dev. 28:7-55.

Nuez-Ortín WG, Yu P. 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. J Sci Food Agr. 89:1754-1761.

Nuez-Ortín WG, Yu P. 2010. Estimation of ruminal and intestinal digestion profiles, hourly effective degradation ratio and potential N to energy synchronization of co-products from bioethanol processing. J Sci Food Agr. 90:058-2067.

Ørskov ER, McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. J Agr Sci. 92:449-503.

Shannak S, Südekum K-H, Susenbeth A. 2000. Estimating ruminal crude protein degradation with *in situ* and chemical fractionation procedures. Anim Feed Sci Tech. 85:195-214.

Shingoethe DJ. 2006. Utilization of DDGS by cattle. Proc. 27th Western Nutrition Conference, Manitoba, Canada, 19-20 September, 2006. pp 61-74.

Sniffen CJ, O´Connor JD, van Soest PJ, Fox DG, Russell JB. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J Anim Sci. 70:3562-3577.

Spiehs MJ, Whitney MH, Shurson GC. 2002. Nutrient database for distiller´s dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. J Anim Sci. 80:639-2645.

Stein HH, Gibson ML, Pedersen C, Boersma MG. 2006. Amino acid and energy digestibility in ten samples of distillers dried grains with solubles fed to growing pigs. J Anim Sci. 84:853-860.

Urdl M, Gruber L, Häusler J, Maierhofer G, Schauer A. 2006. Influence of distillers dried grains with soluble (Starprot) in dairy cow feeding. Slovak J Anim Sci. 39:43-50.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) (ed.) 2006. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungmethodik (VDLUFA-Methodenbuch), Bd. III Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Weisbjerg MR, Bhargava PK, Hvelplund T, Madsen J. 1990. Anveldelse af Nedbrydningsprofiler I Fordemiddelvurderingen (Use of Degradation Curves in Feed Evaluation), Report 679, National Institute of Animal Science, Denmark, pp. 33

CHAPTER 4

IN SITU RUMINAL DEGRADATION OF AMINO ACIDS AND *IN VITRO* PROTEIN DIGESTIBILITY OF UNDEGRADED CRUDE PROTEIN OF DRIED DISTILLERS´ GRAINS WITH SOLUBLES FROM EUROPEAN ETHANOL PLANTS[1, 2]

Edwin Westreicher-Kristen, Herbert Steingass and M. Rodehutscord

Institut für Tierernährung, Universität Hohenheim, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany

[1] Submitted to Animal on March 9th 2013

[2] Presented in part at the annual meeting of the Society of Nutrition Physiology, Göttingen, Germany, March 20-22, 2012: Westreicher E, Steingaß H and Rodehutscord M 2012. *In situ* ruminal degradation of crude protein and amino acids and *in vitro* protein digestibility of undegraded protein in DDGS. Proceedings of the Society of Nutrition Physiology 21, 99

4.1 ABSTRACT

The objectives were to determine and compare the *in situ* ruminal degradation of crude protein (CP) and amino acids (AAs) of dried distillers' grains with solubles (DDGS) and to characterize the *in vitro* pepsin-pancreatin solubility of CP (PPS) from dietary DDGS (d-DDGS) and DDGS residue (DDGS-r) obtained after 16-h ruminal incubation. Thirteen samples originating from wheat, corn, barley and blends were studied. The rumen degradation of AAs and CP was determined using nylon bag incubations in the rumen of cows. Lysine and methionine content of d-DDGS varied from 1.36 to 4.00 and 1.34 to 1.99 g/16 g N, respectively. The milk protein score (MPS) of d-DDGS was low and ranged from 0.36 to 0.51, and lysine and isoleucine were estimated to be the most limiting AAs in d-DDGS and DDGS-r. DDGS-r contained slightly more essential AAs than did the d-DDGS. Rumen degradation of CP after 16 h varied from 44% to 94% between DDGS samples. Rumen degradation of lysine and methionine ranged from 39% to 90% and from 35% to 92%, respectively. Linear regressions showed that ruminal degradation of individual AAs can be predicted from CP degradation. The PPS of d-DDGS was higher than that of DDGS-r and it varied from 70% to 89% and from 47% to 81%, respectively. There was no significant correlation between the PPS of d-DDGS and PPS of DDGS-r (R^2 = 0.31). The estimated intestinally absorbable dietary protein (IADP) averaged 21%. Moderate correlation was found between the crude fibre content and PPS of DDGS-r (R^2 = 0.43). This study suggests an overestimation of the contribution of undegradable crude protein (UDP) of DDGS to digestible protein supply in the duodenum in currently used protein evaluation systems. More research is required and recommended to assess the intestinal digestibility of AAs from DDGS.

Keywords: Undegraded protein, pepsin-pancreatin solubility, intestinal digestibility, DDGS, amino acids

4.2 IMPLICATIONS

Differences in the raw materials used for ethanol production in the European Union and in processing details result in a high variability of the chemical composition of dried distillers' grains with solubles (DDGS), which is an important by-product largely used in the feeding of dairy cows. For an efficient protein and amino acid utilisation of the cow, it is important to consider that not only the content of rumen undegradable protein but also the concentration of amino acids such as lysine and methionine and protein digestibility vary greatly between different batches of DDGS.

4.3 INTRODUCTION

Ethanol production has increased considerably in the European Union. The increasing demand of energy, increasing raw oil prices and the implementation of the European Programme for the use of energy from renewable sources favours further growth of ethanol production. Subsequently, the production of dried distillers' grains with solubles (DDGS) as the main by-product of ethanol production might be expected to increase. DDGS is well recognized as a good energy and protein source for feeding of livestock, especially cattle. However, European DDGS varies substantially in nutritional composition, energy value or content of rumen undegradable crude protein (UDP) (Cozannet *et al*., 2010, 2011; Westreicher-Kristen *et al*., 2012). The variability in the feeding value in conjunction with the expected increase of DDGS production demand for further and more specific characterization of this by-product.

In high-yielding dairy cows, UDP substantially contributes to the supply of amino acids (AAs) at the duodenum. Hence, the AA composition of UDP and its intestinal digestibility (ID) are further important characteristics of the protein value of a feedstuff (Weisbjerg *et al*., 1996). Unlike the well-balanced AA profile supplied by the microbial protein, AAs coming from the UDP largely reflect the AA pattern of the original feed (Kleinschmit *et al*., 2007; Li *et al*., 2012; Mjoun *et al*., 2010). However, AAs of the UDP sometimes do not match the AA needs of the cow in terms of concentration and digestibility. This may become especially relevant if the UDP content of a feedstuff is high and one AA is particularly low, as is the case for lysine in DDGS. Knowledge about whether or not the AA composition of DDGS and UDP fraction of DDGS are similar is important for ration formulation and has been reported so far (Kleinschmit *et al*., 2007; Li *et al*., 2012; Mjoun *et al*., 2010). However, these experiments only considered a maximum of five samples of DDGS mainly originating from corn and did not consider blend-DDGS or DDGS originating from a wide variety of grains or mixtures like those produced under European conditions.

The determination of ID of UDP is a prerequisite for assessing AA supply to the cow. It has often been assumed that ID of UDP is constant. However, ID of UDP from corn-DDGS was found to be reduced in batches that had a high UDP content (Kleinschmit *et al*., 2007). As the UDP content in European DDGS samples was largely variable (Westreicher-Kristen *et al*., 2012), the question is whether any variation of ID of UDP is related to the UDP content of DDGS. *In vivo* determination of ID of protein fractions in ruminants is expensive and time consuming. Additionally, endogenous losses of protein at the intestinal level might have a great influence on *in vivo* trial results. Boisen and Fernández (1995) proposed an *in vitro*

method based on the pepsin-pancreatin solubility of CP (PPS) to predict the ID of feedstuffs for pigs. Although *in vitro* methods cannot precisely simulate an *in vivo* situation, the proposed method is found to be reliable for a quick characterization and prediction of ID of dry matter (DM) and CP.

The objectives of this study were (1) to determine and compare the *in situ* ruminal degradation of CP and AAs of DDGS from European ethanol plants and (2) to characterize the PPS of UDP of DDGS. We hypothesized that (1) it is possible to predict the ruminal degradation of individual AAs from CP degradation, (2) there exists a large variation of the PPS of UDP of DDGS and (3) there is a relationship between the PPS and in situ UDP.

4.4 MATERIAL AND METHODS

4.4.1 SAMPLES

Thirteen samples of DDGS obtained from ethanol facilities located in eight European countries were studied. Detailed nutrient composition, energy and protein values of the DDGS studied in this experiment were reported previously (Westreicher-Kristen *et al*., 2012). In brief, the samples originated from wheat, corn, barley and blends of different substrates. Ten samples were in pelleted form. Chemical fractions varied among samples and were (in g/kg DM ± standard deviation): 310 ± 33 CP, 86 ± 37 ether extract, 89 ± 18 crude fibre, 408 ± 39 neutral detergent fibre, 151 ± 39 acid detergent fibre and 62 ± 31 acid detergent lignin. The concentration of UDP, calculated on the basis of in situ data and using a passage rate of 8%/h, varied from 9% to 63% of CP. Metabolizable energy was estimated to vary between 10.9 and 12.8 MJ/kg DM.

4.4.2 *IN SITU* DEGRADATION OF CRUDE PROTEIN AND AMINO ACIDS

The rumen degradation of CP and AAs was determined using nylon bags incubated in the rumen. Four non-lactating cows fitted with a rumen cannula were used. The cows were fed twice daily a ration consisting of 2 kg of a mixed concentrate containing 30% wheat, 30% maize, 22% rapeseed cake, 15% field beans and 3% of a mineral and vitamin premix and hay. Hay and water were offered for *ad libitum* consumption. Around 8 g of sample, in original condition, was placed into nylon bags (10 × 20 cm, ~ 50 µm of pore size, Type R1020, Ankom Technology, NY, USA) to be ruminally incubated for 16 h. This incubation time was chosen as a universal standard that represents a rumen outflow rate of 6.3 %/h which corresponds to a feeding level of about 2.5 × maintenance (AFRC, 1993). In addition, Calsamiglia and Stern (1995) found no differences in the PPS of UDP of different plant and

animal protein sources when samples were pre-incubated in the rumen between 12 and 18 h. Immediately before placement in the rumen, the filled nylon bags were soaked in warm water (~39°C) for 15 min. The bags were introduced into the rumen immediately after the afternoon feeding. Three separate runs were required to incubate all the samples and to obtain enough DDGS residues (DDGS-r) for further analyses. After incubation, the bags were removed from the rumen and immersed in ice-cold water to minimize microbial activity. Thereafter, the bags were rinsed in cold tap water to remove excess ruminal contents and were stored frozen (-20°C) until the end of the experiment. The incubated bags were washed with cold water in a washing machine (extraKLASSE E12.18 Siemens, Germany) for 15 min without centrifugation, and subsequently freeze-dried, weighed and ground to pass a 0.5 mm screen for later analyses of DM, CP and AAs.

4.4.3 *IN VITRO* PEPSIN-PANCREATIN SOLUBILITY

PPS was determined according to the pepsin and pancreatin procedure described by Boisen and Fernández (1995). DDGS-r obtained after the ruminal incubation and dietary DDGS (d-DDGS) ground to pass a 0.5 mm sieve were used. For analysis, the DDGS-r were pooled for each cow, resulting in four repetitions for each DDGS sample. PPS analysis was carried out in triplicate. Briefly, samples (500 ± 5 mg) were suspended in 100 ml Erlenmeyer flasks with 25 ml phosphate buffer (0.1 M; pH 6.0) and incubated with 10 ml 0.2 M HCl and 1 ml pepsin solution (0.01 g/ml; Merk 7190, 200 FIP U/g) for 6 h at a pH of 2.0 to simulate abomasal digestion. Thereafter, 5 ml of 0.6 M NaOH and 10 ml of a phosphate buffer were added, and the pH of the samples was adjusted to 6.8 with 5 M HCl or 5 M NaOH using a pH meter. Immediately, samples were incubated with 1 ml pancreatin solution (0.05 g/ml; Sigma P-1750) for 18 h to simulate small intestinal digestion. The incubations were performed under constant stirring in an oven at 40°C. After *in vitro* incubation, samples were treated with 5 ml of solution containing 20% of sulfosalicylic acid for 30 min at room temperature. Then, the whole content of the Erlenmeyer flasks was filtered through nylon bags (4 × 12 cm, ~ 30 µm of pore size, No. 10-0127, C. Gerhardt GmbH & Co. KG, Germany) and washed with ethanol and acetone. The nylon bags containing the insoluble residues were weighed, dried for 4 h at 103°C and analysed for CP content. Simultaneously, in each incubation run, two blanks containing only incubation solutions and without samples were treated the same way and used for correction of PPS. Due to shortage in the sample size after rumen incubation, one sample of DDGS-r could not be analysed for PPS.

4.4.4 CHEMICAL ANALYSES, CALCULATIONS AND STATISTICAL ANALYSES

The chemical analyses followed the official analytical standards in Germany (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, VDLUFA, 2006). The d-DDGS, DDGS-r and PPS residues were analysed for DM and CP content (methods 3.1 and 4.1.1, respectively). The AA analysis of d-DDGS and DDGS-r was run in duplicate. Samples were hydrolysed with 6 M HCl for 24 h at 110°C after oxidation using performic acid and hydrogen peroxide (Rodehutscord *et al*., 2004). Norleucine was used as the internal standard. Separation and detection of AAs was done with an AA analyser (Hitachi L-8900). Photometric detection was at 570 nm (440 nm for proline) with post-column ninhydrin derivatization. Methionine and cysteine were determined as methionine sulfone and cysteic acid, respectively. Due to shortage in the amount after rumen incubation, only 11 samples of DDGS residues could be analysed for their AA contents.

In situ degradation of AAs was calculated by the difference of AA from DDGS weighed into the nylon bags and the AA remaining in the respective bags after rumen incubation. The milk protein score (MPS) was calculated as (g of essential AA/kg CP)/(g essential AA in milk/kg of milk protein) according to Schingoethe (1996), and estimates of the AA content of milk protein were according to Waghorn and Baldwin (1984). The PPS of d-DDGS and DDGS-r was calculated by the difference of original amounts of N, and N remaining in the nylon bags after incubation with pepsin and pancreatin. The intestinally absorbable dietary protein (IADP) was calculated as UDP × PPS/100, where IADP and UDP are in per cent of CP, and PPS in per cent.

All statistical analyses were run using the software package SAS (version 9.2, SAS Institute Inc., Cary, NC). Correlations and linear regressions were tested using the CORR and REG procedure, respectively. Data were subjected to ANOVA using the GLM procedure. Significant differences between individual means were identified using the least significance difference (LSD). All significant differences were declared at $P < 0.05$, with tendencies associated with the *P*-value between 0.05 and 0.10.

4.5 RESULTS

4.5.1 AMINO ACIDS CONTENT AND *IN SITU* DEGRADATION OF CRUDE PROTEIN AND AMINO ACIDS

The AA composition, the MPS and the ranking of the three most limiting essential amino acids (EAAs) of the d-DDGS are shown in Table 9. Among EAAs and according to the

coefficient of variation (CV), lysine, leucine and methionine were the most variable (32.7%, 29.2% and 13.2%, respectively). Lysine averaged 2.07 g/16 g N and ranged from 1.36 to 4.00 g/16 g N. Leucine had the highest concentration among the EAAs, averaging 8.45 g/16 g N. Methionine averaged 1.64 g/16 g N and ranged from 1.34 to 1.99 g/16 g N. Among non-essential amino acids (NEAAs), the most variable were alanine, cysteine, glutamic acid, tyrosine and arginine (CV = 31.2%, 22.1%, 20.5%, 16.3% and 16.0%, respectively). The MPS of DDGS averaged 0.43 and ranged from 0.36 to 0.51. According to MPS, lysine was the first limiting AA in all DDGS; isoleucine was the second limiting AA in 12 DDGS whereas methionine and valine were the third limiting AAs in six of the 13 DDGS. In all DDGS-r, the first and second limiting AAs were lysine and isoleucine, respectively (data not shown). DDGS-r contained slightly more EAAs and fewer NEAAs than the d-DDGS. Therefore, MPSs of DDGS-r were slightly higher, averaging 0.47 and ranging from 0.41 to 0.55.

After 16 h of ruminal incubation, CP degradation varied widely from 44.1% to 94.2% and averaged 69.5% (Table 10). This was similar for total AA degradation, which varied from 40.3% to 94.6% and averaged 69.0%. The mean rumen degradation of individual AAs varied from 64.8% (leucine) to 72.4% (glycine). *In situ* degradation of lysine and methionine varied from 38.7% to 90.0% and from 35.1% to 92.4%, respectively. Among all analysed samples (n = 11), the majority of the degradation values for individual AAs were statistically similar to their respective CP degradation ($P > 0.05$), with the exception of leucine, lysine, methionine and glutamic acid. For these AAs, in only four (leucine) or five samples, the AA degradation was statistically similar to CP degradation. The degradation of EAAs was slightly lower than CP degradation, with the exception of histidine. Among NEAAs, AA degradation tended to be similar or slightly higher than CP degradation (69.5%), with the exception of alanine, asparagine, cysteine and tyrosine.

Regression equations showed significant relationships between degradation of CP and degradation of lysine and methionine (R^2 = 0.92 and 0.97, respectively) (Figure 4), as well as between CP degradation and degradation of other EAAs and NEAAs with R^2 values from 0.95 to 0.99 and low root mean square error (RMSE) for all AAs (Table 11).

Table 9 Concentration of crude protein (CP) and amino acids (AAs) and milk protein score (MPS) of distillers' dried grains with solubles (DDGS) with coefficient of variation (CV)

	DDGS samples													
	Corn			Wheat					Barley	Blends				CV
Item	1	2	3[1]	4[1]	5[1]	6	7	8	9	10[2]	11[3]	12[4]	13[5]	(%)
CP[6] (g/kg DM)	281	274	311	339	320	302	335	358	247	312	331	273	349	10.6
EAAs (g/16 g N)														
His	3.41	3.39	3.29	2.70	2.82	2.74	2.84	2.87	2.62	2.87	3.25	3.64	2.94	10.7
Ile	3.28†	3.31†	3.29†	3.30†	3.32†	3.13†	3.14†	2.72†	3.38†	3.09†	3.23‡	3.26†	3.10†	5.4
Leu	12.3	12.3	12.1	8.05	6.83	6.69	6.70	6.53	6.47	7.44	6.08	11.0	7.43	29.2
Lys	1.86*	1.74*	2.60*	1.54*	1.99*	2.35*	1.39*	1.36*	4.00*	1.89*	2.03*	2.01*	2.17*	32.7
Met	1.97	1.99	1.99	1.54‡	1.54‡	1.63	1.43‡	1.46	1.51‡	1.72	1.34†	1.67‡	1.58‡	13.2
Phe	5.09	5.06	4.93	4.66	4.60	4.51	4.58	4.54	4.39	4.67	4.42	4.70	4.70	4.8
Thr	3.9	3.93	3.87	3.18	3.19	3.21	3.16	3.15	3.86	3.35	3.15	3.82	3.26	9.9
Val	4.52‡	4.59‡	4.61‡	4.08	4.30	3.98‡	3.99	3.62‡	4.60	4.00‡	4.12	4.51	4.08	7.4
MPS	0.50	0.50	0.51	0.40	0.40	0.39	0.38	0.36	0.43	0.40	0.38	0.48	0.41	12.3
NEAAs (g/16 g N)														
Ala	7.62	7.67	7.55	5.02	3.90	3.86	3.65	3.70	5.44	4.38	4.14	7.28	4.34	31.2
Arg	4.35	4.31	4.35	3.88	4.44	4.69	3.65	3.31	4.32	4.03	2.51	3.09	4.19	16.0
Asp	6.61	6.64	6.61	5.39	5.26	5.30	4.94	4.93	7.11	5.32	4.99	6.49	5.23	13.8
Cys	1.95	1.99	1.86	1.77	2.15	2.09	2.05	2.06	1.62	1.97	0.77	1.19	1.82	22.1
Glu	18.1	18.1	18.0	25.8	26.7	26.5	28.1	28.4	15.7	25.4	25.3	17.6	26.6	20.5
Gly	3.98	4.04	3.75	3.89	4.08	4.13	4.14	4.07	4.40	4.01	4.01	3.94	4.12	3.8
Pro	8.39	8.34	8.15	8.89	9.26	9.24	9.44	9.42	8.88	9.53	9.25	8.01	9.46	6.1
Ser	5.30	5.31	5.32	4.78	4.84	4.95	4.92	5.04	4.30	4.85	4.52	4.90	5.10	6.1
Tyr	3.78	3.85	3.75	2.90	2.73	2.65	2.62	2.53	2.69	2.84	2.64	3.41	2.86	16.3
Sum	96.4	96.6	96.0	91.4	92.0	91.7	90.7	89.7	85.3	91.3	85.7	90.4	93.0	3.8

[1] Not pelleted. [2] wheat:barley:corn:sugar beet syrup in unknown proportions. [3] wheat:barley malt 85:15. [4] corn:barley malt 85:15. [5] wheat:corn:triticale 68:25:7.

[6] Originally presented by Westreicher-Kristen *et al*. (2012).

*† ‡ indicate the apparent sequence of the first, second and third most limiting AA, respectively; based on estimates of the AA content of milk protein (Baghorn and Baldwin, 1984).

MPS = (g of EAAs/kg CP)/ (g EAAs in milk/kg of milk protein) according to Schingoethe (1996).

EAAs = essential amino acids; NEAAs = non-essential amino acids

Table 10 *In situ* degradation (%) of individual amino acids and crude protein (CP) after 16 h ruminal incubation of distillers' dried grains with solubles (DDGS, *n*=11) with means, standard deviation (s.d.) and ranges of values

	Mean	s.d	Min.	Max.	No. of samples not different to CP[1]
Crude protein	69.5	15.5	44.1	94.2	
Essential AAs					
His	71.9	14.7	43.5	94.4	8
Ile	66.1	17.6	37.7	93.6	8
Leu	64.8	19.2	32.4	93.4	4
Lys	65.8	15.3	38.7	90.0	5
Met	65.3	17.7	35.1	92.4	5
Phe	68.4	17.9	38.8	94.5	9
Thr	68.8	15.9	40.9	93.3	10
Val	67.7	16.7	38.2	93.7	9
Non-essential AAs					
Ala	66.7	18.1	34.0	93.4	7
Arg	71.6	13.3	45.1	91.7	7
Asp	67.7	16.1	38.3	92.6	8
Cys	66.6	16.3	35.0	90.6	7
Glu	70.6	18.0	43.2	96.4	5
Gly	72.4	14.0	47.1	93.8	8
Pro	69.9	19.2	41.1	96.4	6
Ser	69.7	16.6	41.4	94.7	11
Tyr	68.1	17.5	39.6	94.3	10
All AA	69.0	17.1	40.3	94.6	

[1] $P \geq 0.05$ between individual amino acid degradation and CP degradation, one value calculated on mean of four replicates (cows) per DDGS.

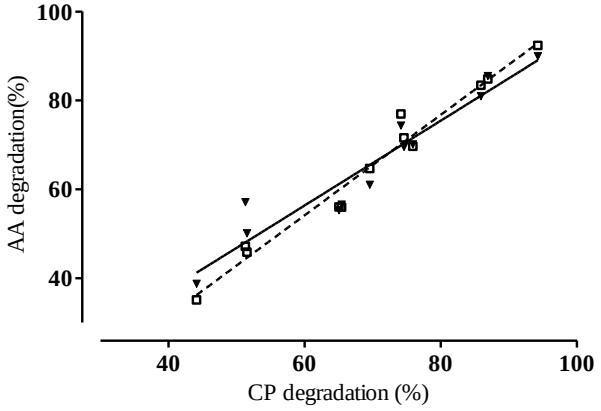

Figure 4 Relationship between ruminal degradation of methionine (□; $y = 1.131x + 12.13$; $R^2 = 0.97$; $P<0.0001$) and lysine (▼; $y = 0.954x - 0.94$; $R^2 = 0.92$; $P<0.0001$) and degradation of CP

Table 11 Relationship between ruminal degradation of individual amino acids and crude protein (CP) of distillers' dried grains with solubles

Amino acid	Equation	R^2	RMSE
Essential			
His	y = 0.93x + 7.25	0.97***	2.75
Ile	y = 1.12x – 11.49	0.98***	2.88
Leu	y = 1.23x – 20.62	0.99***	1.60
Lys	y = 0.95x – 0.94	0.92***	4.54
Met	y = 1.13x + 12.13	0.97***	3.13
Phe	y = 1.15x – 11.34	0.99***	1.68
Thr	y = 1.02x – 1.69	0.99***	2.43
Val	y = 1.06x – 5.76	0.97***	3.14
Non-essential			
Ala	y = 1.16x – 13.63	0.98***	2.35
Arg	y = 0.83x + 13.43	0.95***	3.08
Asp	y = 1.02x – 3.24	0.98***	2.49
Cys	y = 1.03x – 5.22	0.97***	3.16
Glu	y = 1.15x – 9.26	0.98***	2.53
Gly	y = 0.89x + 10.77	0.98***	2.25
Pro	y = 1.22x – 14.67	0.98***	3.06
Ser	y = 1.07x – 4.38	0.99***	1.21
Tyr	y = 1.12x – 9.76	0.99***	1.34

y = ruminal degradation of amino acid (%), x = ruminal degradation of CP (%)
RMSE, root mean square error
***P<0.001

4.5.2 *IN VITRO* PEPSIN-PANCREATIN SOLUBILITY OF CRUDE PROTEIN

The PPS of d-DDGS was higher than that of DDGS-r, with both of them varying from 69.8% to 89.0% and from 47.3% to 80.7%, respectively (Table 12). Considering the raw materials, corn-DDGS averaged the highest PPS (81%), followed by wheat-DDGS (66%) and blend-DDGS (63%). The PPS of blend-DDGS was similar to that of wheat-DDGS but was the most variable (47-76%). The PPS of barley-DDGS was 53%. IADP averaged 21% of CP and varied considerably from 2.8% to 40.3% of CP.

There was only a tendential relationship (P = 0.06) between the PPS of d-DDGS and PPS of DDGS-r (Figure 5) with low R^2 (0.31). Correlation coefficients between PPS of DDGS-r, and chemical constituents and protein fractions were determined. Chemical constituents and protein fractions of the d-DDGS previously published (Westreicher-Kristen *et al*., 2012) were used for this analysis. Among the chemical constituents, only crude fibre (CF) was correlated to the PPS of DDGS-r (r = -0.66, P = 0.02). Among protein fractions according to the Cornell Net Carbohydrate and Protein System (Licitra *et al*., 1996), the PPS

was negatively correlated with protein fraction A (r = -0.75) and positively with fractions B2 and B3 (r = 0.64 and 0.61, respectively), whereas protein fraction C or acid-detergent insoluble nitrogen (ADiN) only tended to correlate with PPS (r = -0.51, P = 0.09). Other chemical constituents neither had significant relations nor tended to correlate with PPS. When estimating the PPS of DDGS-r from chemical composition, only CF delivered a significant equation but with low coefficient of determination (PPS = -0.109CF + 149.5, R^2 = 0.43, P = 0.02), whereas ADiN delivered a non-significant one (PPS = -0.213ADiN + 106.1, R^2 = 0.26, P = 0.09). Estimation of the PPS of DDGS-r from the UDP value of d-DDGS was significant but with low relationship (PPS = 0.86UDP + 30.73, R^2 = 0.37, P = 0.04).

4.6 DISCUSSION

4.6.1 AA PROFILE AND *IN SITU* DEGRADATION OF CP AND AMINO ACIDS

The high variability of lysine concentration in DDGS that we found confirmed results from previous reports (Stein *et al*., 2006; Spiehs *et al*., 2002). In addition, lysine content in DDGS was similarly reported to be the most variable among AAs (Cozannet *et al*., 2010, 2011). High variability of lysine could be explained in part by the variable degree of heating during the drying process of DDGS (Kleinschmit *et al*., 2007), which could reduce the concentration and availability of lysine. Fontaine *et al*. (2007) found a linear decrease of lysine concentration in DDGS as a function of heat treatment time. Maillard reactions have been divided into early, advanced and final reactions (Mauron, 1981). Final reactions result in AA decomposition and resulting compounds are not detected by AA analysis (Classen *et al*., 2004), thus resulting in lower concentrations of AA in the sample. The methionine concentration was higher for corn-DDGS than for wheat-DDGS and similar values were reported before (Stein *et al*., 2006; Widyaratne and Zijlstra, 2007). Variability of AA content could be also explained due to the different raw materials and their mixtures used for ethanol production. Finally, the contribution of yeast used during ethanol production should be also considered as a source of AA and its variation. Li *et al*. (2012) found that the changes in AA profile from the original grain to its DDGS did not follow the same trend as the changes in CP, suggesting that yeast AA would have influenced the AA profile of DDGS. More specifically, Han and Liu (2010) found that yeast contributed about 20% to DDGS protein.

Concentrations of EAAs slightly increased after rumen incubation. Similar tendencies were found by Kleinschmit *et al*. (2007), but contradictory results were reported by Li *et al*. (2012), Boucher *et al*. (2009a) and O´Mara *et al*. (1997). The increase in the EAA portion within DDGS-r resulted from a slightly higher degradability of NEAAs relative to

EAAs. This increase was more obvious for lysine, which probably had a higher resistance to ruminal degradation due to heat exposure. Contrary to this, O´Mara *et al*. (1997) reported lysine as one of the more degradable AAs of DDGS in the rumen. Concentrations of isoleucine, leucine and methionine also increased in the DDGS-r, which is in concordance with the tendencies reported by O´Mara *et al*. (1997), reflecting their higher resistance to ruminal degradation. Boucher *et al*. (2009a) also found some resistance of methionine to ruminal degradation when compared to other AAs. However, the increase in lysine, methionine, isoleucine and leucine concentrations in DDGS-r compared to d-DDGS may not be necessarily advantageous regarding EAA supply to the animal. Boucher *et al*. (2009a) found lower ID of lysine in DDGS compared with other AAs and other feedstuffs. In addition, O´Mara *et al*. (1997) found lower intestinal disappearance of lysine than total AAs in DDGS. This suggests that heat treatment would positively increment the EAA content of UDP by reducing its degradability in the rumen but may simultaneously reduce its ID.

According to Boucher *et al*. (2009a), one reason for discrepancies in the AA profile between the intact feed and UDP could be the microbial contamination of DDGS during ruminal incubation. Diaminopimelic acid (DAPA) is a component of bacterial cell walls, and is therefore an indirect indicator of microbial protein (Rubio, 2003) that has been used for the measurement of bacterial contamination in biological materials (Puchala *et al*., 1992). We have used performic acid oxidation preceding hydrolysis of proteins for AA analysis. Even though oxidative hydrolysis was proved to not interfere in the determination of DAPA (Csapó *et al*., 1995), we observed that the appearance of DAPA peaks on the chromatogram was not sharp and well defined. We hypothesized that during oxidative hydrolysis, new substances originated which co-eluted with DAPA giving no reliable measurements, and this may be a particular problem for this group of feedstuffs. Consequently, DAPA was not used for correction of microbial AA contamination in the present study. Erasmus *et al*. (1994) studied 12 different feedstuffs after 16 h of ruminal incubation and reported that 0.8-8.6% of AAs came from bacterial contamination. They assumed this lower microbial contamination of the feed residues to the washing procedure (10 min with washing machine).

Table 12 UDP values, *in vitro* pepsin-pancreatin solubility of CP (PPS), and calculated intestinal absorbable dietary protein (IADP) of distillers' dried grains with solubles (DDGS) and DDGS residues with standard deviation (s.d.)

	DDGS sources												
		Corn			Wheat				Barley	Blends			
Item[6]	1	2	3[1]	4[1]	5[1]	6	7	8	9	10[2]	11[3]	12[4]	13[5]
UDP (% of dietary CP)	n.d.	49.9	48.7	56.0	19.2	30.6	34.7	37.1	13.5	28.1	5.8	16.5	26.3
s.d.	n.d.	2.10	2.40	2.95	2.1	3.63	2.24	4.25	0.83	1.01	0.48	2.28	2.38
In vitro PPS (%)													
Dietary DDGS	81.3	81.4	85.5	73.5	82.4	86.3	77.1	77.3	79.9	85.7	82.4	69.8	89.0
s.d.	0.18	0.18	0.53	0.63	1.35	0.50	1.42	0.15	1.10	0.55	0.69	1.37	0.31
DDGS residues	n.d.	80.7	80.3	60.8	65.2	77.2	60.0	65.3	52.8	71.5	47.3	57.7	75.8
s.d.	n.d.	0.91	0.69	1.07	1.75	0.89	0.67	0.98	1.77	0.86	2.02	1.27	1.77
IADP (% of dietary CP)	n.d.	40.3	39.1	34.0	15.5	23.6	20.8	24.2	7.1	20.1	2.8	9.5	20.0

DM = dry matter; n.d. not determined due to shortage in sample size

[1] Not pelleted; [2] wheat:barley:corn:sugar beet syrup inunknown proportions [3] wheat:barley malt 85:15 [4] corn:barley malt 85:15 [5] wheat:corn:triticale 68:25:7; [6] UDP = undegradable CP after 16 h ruminal incubation; *in vitro* PPS determined through pepsin-pancreatin method; DDGS residue obtained after 16 h of ruminal incubation; IADP = UDP x PPS/100.

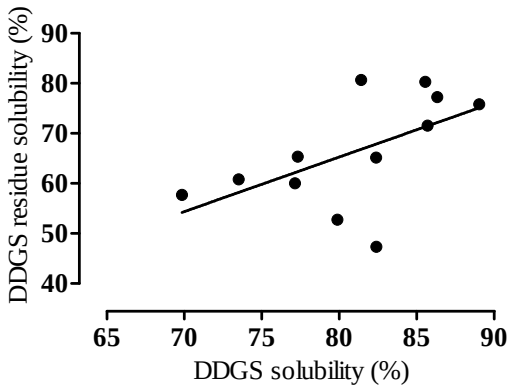

Figure 5 Relationship between *in vitro* CP pepsin-pancreatin solubility of DDGS and DDGS residues after rumen incubation in distillers' dried grains with solubles (y =1.090x – 21.9; R^2 = 0.31; P = 0.06; n =12).

In our experiment, bags were first intensively rinsed in cold tap water to remove excess rumen content until the water came clear and then were additionally washed in a washing machine for longer period (15 min). Steingass *et al*. (2013), using a similar approach as in the present study, found no need for correction of microbial AA with DAPA after 16 h of ruminal incubation of rapeseed meals due to low microbial contamination, which was estimated to be 3.4 ± 0.3% of total N. Thus, we assumed low microbial contamination in the DDGS residues, and data were interpreted considering that this aspect did not affect considerably the AA profile of DDGS-r.

Despite the high variation of *in situ* AA degradation, it reflected the overall pattern of CP degradation. Similar tendencies were found by Mjoun *et al*. (2010), Kleinschmit *et al*. (2007) and Li *et al.* (2012). However, lysine and methionine degradation values of the majority of the samples were significantly different to those of CP degradation (Table 10). Considering the variation around the regression line, deviation of the slopes from 1 and the low RMSE, linear regressions between degradation of CP and degradation of lysine and methionine show that *in situ* degradation of these AAs can be reliably predicted from *in situ* CP degradation. This approach allows also for the reliable prediction of degradation of the other AAs from CP degradation. The results show that CP degradation can be used to predict the AA content of UDP of DDGS. Even though these regressions are specific only to DDGS, similar results were published for the rapeseed meal (Steingass *et al*., 2013).

Since it can be assumed that AA composition of milk protein is indicative of the ideal AA balance for cows, the MPS may be a good indicator of protein quality of feedstuffs (Shingoethe, 1996). Due to its low lysine content, the MPS of DDGS is generally low but varied considerably due to the variability of lysine and isoleucine. The MPS of DDGS in the present study was higher than published before (Shingoethe, 1996; Kleinschmit *et al*., 2007),

which may be related to the different types of DDGS used in different studies. Lysine and isoleucine, like in our study, were also reported as the first and second limiting EAAs, respectively, in d-DDGS and DDGS-r (Kleinschmit *et al*., 2007).

Ten out of the 13 DDGS samples were delivered as pellets and no sample was ground as often done in the standard *in situ* approach. Kleinschmit *et al*. (2007) proposed that grinding DDGS may be an issue that may affect its degradation in the rumen because particle size varies widely among samples. Additionally, they reported from five DDGS a geometric mean diameter of 0.78 mm and that more than 95% of the DDGS are composed by particles less than 2 mm, proving that it may not be necessary to grind DDGS for *in situ* incubations. We did not measure particle size distribution in the current study, but assume that it was similar as reported by Kleinschmit *et al*. (2007), since grains are ground before fermentation for ethanol production. Moreover, since more of the sample is exposed to the bacteria in the rumen due to grinding, this could overestimate the rumen degradation of CP (Kleinschmit *et al*., 2007).

4.6.2 *IN VITRO* PEPSIN-PANCREATIN SOLUBILITY OF CRUDE PROTEIN

ID of CP is an important factor that determines the amount of CP and AAs available for the animal. The values for PPS of DDGS-r and IADP found in the present study are lower and more variable to those reported by Kleinschmit *et al*. (2007). Carvalho *et al*. (2005) and Mjoun *et al*. (2010) reported *in vitro* digestibility of DDGS-r at 51% and 92%, respectively. These differences may be attributed to the different DDGS sources used. Processing and drying techniques were found to affect the intestinal CP digestibility of DDGS (Cao *et al*., 2009) and were postulated to affect CP degradability in the rumen (Kleinschmit *et al*., 2007). Therefore, we hypothesized to find a relationship between PPS of DDGS-r and *in situ* UDP and expected to find increased UDP with a corresponding decreased PPS of DDGS-r. However, there was only a moderate relationship (R^2 = 0.37, P = 0.04) between *in situ* UDP and PPS of DDGS-r in our study. This suggests that PPS is not well predictable from UDP content, perhaps due to differences in processing and drying methods between ethanol production plants. In addition, different substrates and their mixtures used in the plants should be also considered as contributing to the variability observed in the present data set.

Since ADiN seems to be indigestible in the intestine (Lanzas *et al*., 2008), it was proposed as an indicator for CP indigestibility. Even when the content of ADiN was highly variable and could be considered high enough to have negative effects (3-16 g/kg DM), there was only a weak relationship between ADiN and PPS of DDGS-r (R^2 = 0.26, P = 0.09). This

suggests that ADiN is not a good indicator for unavailable CP in DDGS-r. Woods *et al.* (2003) found poor relationship between ADiN of different feedstuffs and unavailable protein in the small intestine. Similarly, Kleinschmit *et al*. (2007) and Li *et al*. (2012) reported that neutral-detergent insoluble CP (NDiCP) did not reflect the unavailable CP of DDGS in the intestine. The higher variation of PPS obtained in the present study compared to those published could be due to a great variability of the raw materials and their mixtures used for the ethanol production, due to differences in drying processes among samples and due to differences in the techniques used to estimate the digestibility of UDP.

Although DDGS may be considered as a good source of UDP, the PPS values showed that ID of UDP may be low compared to soybean meal and corn gluten meal (Kleinschmit *et al*. 2007; Maiga *et al*., 1996) but can be higher than rapeseed meal (Steingass *et al*., 2013). Considering that *in situ* incubations are time consuming and labour intensive, the direct determination of CP digestibility of d-DDGS was proposed as a simplified and more cost-effective approach to predict the digestibility of UDP from DDGS. However, we found that the PPS of d-DDGS was higher than from DDGS-r, and they were lowly correlated with each other. Similar results were reported by Boucher *et al*. (2009b). Our results suggest that the previous *in situ* incubation is always necessary for the determination of ID of UDP from DDGS and that it is not possible to estimate with sufficient accuracy the PPS of DDGS-r from the digestibility of CP in d-DDGS. The NRC (2001) model assumes a UDP digestibility of 80% for DDGS and the UK Feeding System 90% with adjustment based on ADiN (AFRC, 1993) for all feeds, whereas the German Protein System assumes a UDP intestinal digestibility of 85% for all feedstuffs (GfE, 2001). Our *in vitro* data indicate that ID of UDP from DDGS is lower compared to the values currently used in these protein systems and that the contributions of UDP to the intestinal AA supply among all the DDGS samples analysed are overestimated. Similar overestimation was observed by Steingass *et al*. (2013) for rapeseed meal. This emphasized the need to update the values of UDP digestibility in several protein evaluation systems. Moreover, consideration of the variability of UDP digestibility within one feedstuff should be a main concern.

Digestibility of individual AAs can vary widely with AAs and feeds (Li *et al*., 2012; Mjoun *et al*., 2010). Therefore, in further studies, when considering a high variability of DDGS types and protein values like the European DDGS here described, the aim should be to estimate the digestibility of each AA instead of the digestibility of UDP to better characterize the variation of UDP quality.

To conclude, ruminal degradation of AAs can be predicted from the degradation of CP. Lysine was the first limiting EAA in DDGS. Therefore, including DDGS as a major protein source in rations for high-yielding dairy cows may not meet the requirement of lysine. The PPS of DDGS-r was lower compared to d-DDGS and to constant values used in some protein evaluation systems. Therefore, an estimation of the intestinal UDP digestibility of DDGS should consider previous ruminal incubation. The PPS of DDGS-r was not related with in situ UDP or chemical constituents; therefore, predictions are not reliable through this approach. More research is required and recommended to assess the digestibility of individual AAs from DDGS in the intestine. Validation of PPS of DDGS with *in vivo* data is also suggested.

REFERENCES

AFRC (Agricultural and Food Research Council), 1993. Energy and protein requirements of ruminants. An Advisory Manual Prepared by the AFRC Technical Committee on Response to Nutrients. CAB International, Wallingford, UK

Boisen S and Fernández JA 1995. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by *in vitro* analyses. Animal Feed Science and Technology 51, 29-43

Boucher SE, Calsamiglia S, Parsons CM, Stein HH, Stern MD, Erickson PS, Utterback PL and Schwab CG 2009a. Intestinal digestibility of amino acids in rumen-undegraded protein estimated using a precision-fed cecectomized rooster bioassay: II. Distillers dried grains with solubles and fish meal. Journal of Dairy Science 92, 6056-6067

Boucher SE, Calsamiglia S, Parsons CM, Stern MD, Ruiz Moreno M, Vásquez-Añon M and Schwab CG 2009b. *In vitro* digestibility of individual amino acids in rumen-undegraded protein: The modified three-step procedure and the immobilized digestive enzyme assay. Journal of Dairy Science 92, 3939-3950

Calsamiglia S and Stern MD 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. Journal of Animal Science 73, 1459-1465

Cao ZJ, Anderson JL and Kalscheur KF 2009. Ruminal degradation and intestinal digestibility of dried or wet distillers grains with increasing concentrations of condensed distillers solubles. Journal of Animal Science 87, 3013-3019

Carvalho LPF, Melo DSP, Pereira CRM, Rodrigues MAM, Cabrita ARJ and Fonseca AJM 2005. Chemical composition, in vitro digestibility, N degradability and enzymatic intestinal digestibility of five protein supplements. Animal Feed Science and Technology 119, 171-178

Classen HL, Newkirk RW and Maenz DD 2004. Effects of conventional and novel processing on the feed value of canola meal for poultry. Proceedings of Australian Poultry Science Symposium 16, 1-8

Cozannet P, Primot Y, Gady C, Métayer JP, Callu P, Lessire M, Skiba F and Noblet J 2010. Ileal digestibility of amino acids in wheat distillers dried grains with soluble for pigs. Feed Animal Science and Technology 158, 177-186

Cozannet P, Primot Y, Gady C, Métayer JP, Lessire M, Skiba F and Noblet J 2011. Standardised amino acid digestibility of wheat distillers' dried grains with solubles in force-fed cockerels. British Poultry Science 52, 72-81

Csapó J, Csapó-Kiss Z, Csordás E, Martin TG, Folestad S, Tivesten A and Némethy S 1995. Rapid method for the determination of diaminopimelic acid using ion exchange column chromatography. Analytical Letters 28, 2049-2061

Erasmus LJ, Botha PM and Cruywagen CW 1994. Amino acid profile and intestinal digestibility in dairy cows of rumen-undegradable protein from various feedstuffs. Journal of Dairy Science 77, 541-551

Fontaine J, Zimmer U, Moughan PJ and Rutherfurd SM 2007. Effect of heat damage in an autoclave on the reactive lysine contents of soy products and corn distillers grains with solubles. Use of the results to check on lysine damage in common qualities of these ingredients. Journal of Agricultural and Food Chemistry 55, 10737-10743

GfE (Gesellschaft für Ernährungsphysiologie), 2001. Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrindern. DLG-Verlag, Frankfurt am Main, Germany (in German)

Han J and Liu K, 2010. Changes in composition and amino acid profile during dry grind ethanol processing from corn and estimation of yeast contribution toward DDGS proteins. Journal of Agricultural and Food Chemistry 5 8, 3430-3437

Kleinschmit DH, Anderson JL, Schingoethe DJ, Kalscheur KF and Hippen AR 2007. Ruminal and intestinal degradability of distillers grains plus solubles varies by source. Journal of Dairy Science 90, 2909-2918

Lanzas C, Broderick GA and Fox DG 2008. Improved protein fractionation schemes for formulating rations with the Cornell Net Carbohydrate and Protein System. Journal of Dairy Science 91, 4881-4891

Li C, Li JQ, Yang WZ and Beauchemin KA 2012. Ruminal and intestinal amino acid digestion of distiller´s grain vary with grain source and milling process. Animal Feed Science and Technology 175, 121-130

Licitra G, Hernandez TM and van Soest PJ 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57, 347-358

Maiga HA, Schingoethe DJ and Henson JE 1996. Ruminal degradation, amino acid composition, and intestinal digestibility of the residual components of five protein supplements. Journal of Dairy Science 79, 1647-1653

Mauron J 1981. The maillard reaction in food; a critical review from the nutritional standpoint. Progress in Food and Nutrition Science 5, 5-35

Mjoun K, Kalscheur KF, Hippen AR and Shingoethe DJ 2010. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grains products. Journal of Dairy Science 90, 4144-4154

NRC (National Research Council), 2001. Nutrient requirement of dairy cattle. 7th revised edition, National Academy Press. Washington, DC

O´Mara FP, Murphy JJ and Rath M 1997. The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. Journal of Animal Science 75, 1941-1949

Puchala P, Piór H and Kulasek GW 1992. Determination of diaminopimelic acid in biological materials using high-performance liquid chromatography. Journal of Chromatography 623, 63-67

Rodehutscord M, Kapocius M, Timmler R and Dieckmann A 2004. Linear regression approach to study amino acid digestibility in broiler chicken. British Poultry Science 45, 85-92

Rubio LA 2003. Determination of diaminopimelic acid in rat feces by high-performance liquid chromatography using the Pico Tag method. Journal of Chromatography 784, 125-129

Schingoethe DJ 1996. Balancing the amino acid needs of the dairy cow. Animal Feed Science and Technology 60, 153-160

Spiehs MJ, Whitney MH and Shurson GC 2002. Nutrient database for distiller's dried grains with solubles produced from new ethanol plants in Minnesota and South Dakota. Journal of Animal Science 80, 2639-2645

Stein HH, Gibson ML, Pedersen C and Boersma MG 2006. Amino acid and energy digestibility in ten samples of distillers dried grains with solubles fed to growing pigs. Journal of Animal Science 84, 853-860

Steingass H, Kneer G, Wischer G and Rodehutscord M 2013. Variation of *in situ* degradation of crude protein and amino acids and *in vitro* digestibility of undegraded feed protein in rapeseed meals. Animal, doi: 10.1017/S175173111300030X, Published online by Cambridge University Press 11 March 2013.

VDLUFA (Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten), 2006. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany

Waghorn GD and Baldwin RL 1984. Model of metabolite flux with mammary gland of the lactating cow. Journal of Dairy Science 67: 531-544

Weisbjerg MR, Hvelplund T, Hellberg S, Olsson S and Sanne S 1996. Effective rumen degradability and intestinal digestibility of individual amino acids in different concentrates determined *in situ*. Animal Feed Science and Technology 62, 179-188

Westreicher-Kristen E, Steingass H and Rodehutscord M 2012. Variations in chemical composition and *in vitro* and *in situ* ruminal degradation characteristics of dried distillers' grains with solubles from European ethanol plants. Archives of Animal Nutrition 66, 458-472

Widyaratne GP and Zijlstra RT 2007. Nutritional value of wheat and corn distillers dried grain with soluble: Digestibility and digestible contents of energy, amino acids and phosphorus, nutrient excretion and growth performance of grower-finisher pigs. Canadian Journal of Animal Science 87, 103-114

Woods VB, Moloney AP, Calsamiglia S and O´Mara FP 2003. The nutritive value of concentrate feedstuffs for ruminants animals Part III. Small intestinal digestibility as measured by *in vitro* or mobile bag techniques. Animal Feed Science and Technology 110, 145-157

CHAPTER 5

EFFECT OF FEEDING DRIED DISTILLERS' GRAINS WITH SOLUBLES ON MILK YIELD AND MILK COMPOSITION OF COWS IN MID-LACTATION AND ON DIGESTIBILITY IN SHEEP [1, 2]

Edwin Westreicher-Kristen, Kaiser R, Herbert Steingass and M. Rodehutscord

Institut für Tierernährung, Universität Hohenheim, Emil-Wolff-Str. 10, 70599 Stuttgart, Germany

[1] Revised version submitted to the Journal of Animal Physiology and Animal Nutrition on May 15, 2013

[2]Presented in part at the annual Congress of the Association of German Agricultural Analytic and Research Institutes (VDLUFA), Passau, Germany, September 18-21, 2012: Westreicher E, Kaiser R, Steingaß H and Rodehutscord M 2012. Vergleich von drei verschiedenen Trockenschlempen (DDGS) in Rationen für Milchkühe. 124. VDLUFA-Kongress. Kurzfassungen der Referate S. 126

5.1 ABSTRACT

We evaluated the effect of three sources of dried distillers' grains with solubles (DDGS) in diets of mid-lactating dairy cows on milk production and milk composition and on digestibility in sheep. DDGS from wheat, corn and barley ($DDGS_1$), wheat and corn ($DDGS_2$) and wheat ($DDGS_3$) were studied and compared with a rapeseed meal (RSM). RSM and DDGS were characterized through *in situ* crude protein (CP) degradability. Nutrient digestibility was determined in sheep. Twenty-four multiparous cows were used in a 4 × 4 Latin square design with 28-day periods. Treatments included total mixed rations containing as primary protein sources RSM (control), $DDGS_1$ (D1), $DDGS_2$ (D2) or $DDGS_3$ (D3). RSM contained less rapidly degradable CP (fraction a), more potentially degradable CP (fraction b) and more rumen undegradable CP (UDP) than the three DDGS. *In vivo* digestibility of RSM organic matter was similar to DDGS. Calculated net energy for lactation (NE_L) was lower for RSM (7.4 MJ/kg DM) than for DDGS, which averaged 7.7 MJ/kg DM. Cows' dry matter intake did not differ between diets (21.7 kg/d). Cows fed D1 yielded more milk than those fed D3 (31.7 vs. 30.4 kg/d); no differences were found between control and DDGS diets (31.3 vs. 31.1 kg/d). Energy-corrected milk was similar among diets (31.2 kg/d). Diets affected neither milk fat concentration (4.0%) nor milk fat yield (1.24 kg/d). Milk protein yield of control (1.12 kg/d) was significantly higher than D3 (1.06 kg/d) but not different form D1 and D2 (1.08 kg/d each). Feeding DDGS significantly increased milk lactose concentration (4.91%) compared to control (4.81%). DDGS can be a suitable feed compared to RSM and can be fed up to 4 kg dry matter per day in rations of dairy cows in mid-lactation. However, high variation of protein and energy values of DDGS should be considered when included in diets of dairy cows.

Keywords: protein sources, digestibility, milk protein, milk fat, lactose

5.2 INTRODUCTION

Increased ethanol production in the European Union has led to an increased availability of dried distillers' grains with solubles (DDGS), most of it used for ruminant feeding. While in general DDGS appears a valuable source of energy and protein for ruminants, it is a feedstuff that greatly varies in nutrient content and protein and energy values (Westreicher-Kristen *et al*., 2012). Some studies using dairy cows investigated the effect of partial substitution of protein sources of the diets by one corn-DDGS (Anderson *et al*., 2006; Janicek *et al*., 2008; Grings *et al*., 1992), or by three different corn-DDGS (Kleinschmit *et al*., 2006; Powers *et al*., 1995). However, to fully explore the potential of using DDGS, they

should be included as the sole protein-rich feed in dairy cow diets. Liu *et al*. (2000) and Mulrooney *et al*. (2009) studied the total substitution of soybean meal (SBM) and fish meal or canola meal by one DDGS source, respectively. Nevertheless, these studies included only corn-DDGS and did not consider blend-DDGS or DDGS originating from other grains like those mainly produced under European conditions. In Europe, Dunkel *et al*. (2010) studied the effect of wheat-DDGS in dairy cows. However, again, they only considered one DDGS source. Only Urdl *et al*. (2006) totally replaced SBM and rapeseed cake by Austrian corn-DDGS or wheat-DDGS in dairy rations. To the best of our knowledge, it seems that there is no more available information published in scientific journals considering the simultaneous evaluation of various DDGS sources as the main protein supplement in diets of dairy cows.

To evaluate the suitability of DDGS for dairy cows, feeding trials are required. Nevertheless, in addition to chemical analyses, characterization of feeds in regard to their energy and protein values is needed. One important key variable for the characterization of feed crude protein (CP) is the rumen undegradable crude protein (UDP) value, which can be calculated on the basis of *in situ* procedures. The energy value of feeds can be calculated based on digestibility determined with sheep (GfE, 1991) and nutrient content. Our objectives were (1) to evaluate the effect of a complete replacement of rapeseed meal (RSM) as the main protein source by three different sources of DDGS on dry matter intake (DMI), milk production and milk composition in dairy cows in mid-lactation and (2) to compare and characterize the three DDGS from different ethanol production plants in terms of feed value based on *in vivo* digestibility and *in situ* CP degradability and to compare them with RSM. We hypothesized (1) to confirm the feasibility of the use of DDGS as the primary protein source in dairy cow rations and (2) to find differences between DDGS from different origins in terms of CP degradation, digestibility of nutrients and performance when fed to dairy cows. All animal studies reported herein were in accordance with the animal welfare legislation and approved either by the Animal Welfare Commissioner of the University of Hohenheim or by the Provincial Government of Stuttgart, Germany.

5.3 MATERIALS AND METHODS

5.3.1 FEEDSTUFFS

Three DDGS from different European ethanol production plants and one RSM were used. $DDGS_1$ was delivered as pellets and originated from 65% wheat, 15% corn and 20% barley. $DDGS_2$ was delivered as pellets and originated from 85% wheat and 15% corn. $DDGS_3$ was delivered unpelleted and originated from 100% wheat (all values according to the

producers). Information concerning processing and drying technologies was not available. Chemical composition, essential amino acids (EAA) and milk protein scores (MPS) of DDGS and of RSM are shown in Table 13. The RSM and DDGS differed in chemical composition. RSM had higher CP than DDGS (391 vs. 355 g/kg dry matter (DM)). DDGS had higher ether extract (EE) content than RSM but lower sugar and starch. Fibre fractions also differed between feeds. RSM had lower neutral-detergent insoluble nitrogen (NDiN) content than DDGS (13 vs. 18 g N/kg DM). RSM had a more favourable EAA composition than DDGS, with higher content of lysine, methionine, histidine, isoleucine, threonine and valine, thus resulting in a higher MPS for RSM than DDGS.

Table 13 Analysed chemical composition, essential amino acids (EAA) and milk protein score (MPS) of dried distillers' grains with solubles (DDGS) and rapeseed meal (RSM)

	RSM	$DDGS_1$	$DDGS_2$	$DDGS_3$
Nutrient (g/kg DM)				
Crude ash	79	49	55	42
Crude protein	391	336	367	363
Ether extract	33	71	75	66
Crude fibre	133	80	77	78
Sugar	107	53	34	34
Starch	69	24	38	38
$aNDF_{om}$	318	387	394	392
ADF_{om}	179	132	111	129
ADL	73	47	41	47
NDiN	13	18	20	17
ADiN	4	6	3	4
Ca	7.1	0.9	1.3	1.0
P	11.7	7.6	8.8	7.6
Na	0.9	2.2	3.4	0.6
Cl	0.2	1.3	1.4	1.5
Mg	4.6	2.7	3.1	2.3
K	13.2	9.3	10.0	8.9
EAA (g AA/16 g N)				
His	3.14	2.63	2.58	2.64
Ile	3.84*	3.33†	3.42†	3.44†
Leu	7.36‡	7.52	7.36	7.00
Lys	5.52†	1.99*	2.49*	2.17*
Met	2.00	1.62‡	1.63‡	1.64‡
Phe	4.07	4.84	4.60	4.63
Thr	4.79	3.43	3.29	3.21
Val	5.03	4.35	4.30	4.39
MPS	0.50	0.41	0.41	0.40

$DDGS_1$ = originated from wheat:corn:barley = 65:15:20; $DDGS_2$ = originated from wheat:corn = 85:15; $DDGS_3$ = originated from wheat; DM = dry matter; $aNDF_{om}$ = neutral detergent fibre; ADF_{om} = acid detergent fibre; ADL = acid detergent lignin; NDiN = neutral detergent insoluble nitrogen; ADiN = acid detergent insoluble nitrogen; MPS = (g of EAA/kg CP)/ (g EAA in milk/kg of milk protein) according to Schingoethe (1996). * † ‡ Indicate the apparent sequence of the first, second and third most limiting AA, respectively; based on estimates of the AA content of milk protein (Waghorn and Baldwin, 1984).

According to MPS, lysine, isoleucine and methionine were the first, second and third most limiting amino acids (AA) among DDGS, respectively. Among DDGS, substantial

variations were observed in sugar, starch, acid-detergent insoluble nitrogen (ADiN), Na and lysine content.

5.3.2 CHARACTERIZATION OF RUMEN DEGRADATION

Ruminal degradation of CP was determined using the nylon bag technique according to Ørskov and McDonald (1979) in four non-lactating cows fitted with a rumen cannula. Cows were fed a daily ration consisting of 2 kg of concentrate and hay for *ad libitum* consumption. Around 1.5 g of each protein feed was placed into nylon bags (5 × 10 cm, ~50 µm pore size, Type R510, Ankom Technology, NY, USA) to be incubated in duplicate in the rumen of each cow for 1, 2, 4, 8, 16, 32 and 72 h. Protein feeds were incubated in original condition. Grinding DDGS was proposed to overestimate rumen degradation of CP (Kleinschmit *et al.*, 2007). Additionally, these authors reported from five DDGS a geometric mean diameter of 0.78 mm and that more than 95% of the DDGS are composed by particles less than 2 mm, proving that it may not be necessary to grind DDGS for *in situ* incubations. RSM was composed by fine particles and not required to be ground. The samples were introduced into the rumen immediately after the morning feeding. After each incubation time, the bags were removed from the rumen, immersed in ice-cold water, rinsed in cold tap water and stored frozen (-20°C). Six additional nylon bags were filled with each of the protein feeds for determination of zero time disappearance. Bags were washed together with the rumen-incubated bags with cold water in a washing machine for 15 min without centrifugation and were subsequently dried at 65°C for 24 h, and weighed. Finally, the dried residues were analysed for their CP content. The values of CP disappearance were corrected for the loss of small particles from the bags according to Weisbjerg *et al*. (1990). The small particle fraction escaping from the bags was calculated by subtracting the water-soluble fraction from zero-disappearance values. Parameters of *in situ* ruminal CP degradation were estimated according to Ørskov and McDonald (1979).

5.3.3 *IN VIVO* DIGESTIBILITY TRIAL

As suggested by the German standard of energy evaluation in ruminants (GfE, 1991), digestibility of the four protein feeds was determined in sheep. The 24 wethers had an average body weight of 69.3 ± 4.6 (SD) and 68.7 ± 4.3 kg at the start and end of the trial, respectively. Sheep were randomly divided into groups according to body weight. Animals were kept in metabolic cages and fed 1145 ± 19 g DM/d of diets containing decreasing rates of chopped meadow hay (85%, 70%, 55% and 40%) complemented with increasing rates (15%, 30%, 45%

and 60%) of DDGS or RSM (n = 8 sheep per feedstuff; 2 per inclusion level). This design was chosen to proof linearity of digestibility response of the test feeds at different inclusion levels in the total diet. Feed allowance was chosen at the maintenance requirement level of metabolizable energy (ME) according to GfE (1991). Diets contained a minimum of 12 g CP/MJ ME and were offered in two meals per day at about 0800 h and 1600 h. The experiment was performed in four periods, each comprising a 14 d adaptation phase followed by a 7 d faeces collection phase. Faeces were collected and composited by sheep and stored frozen for later analyses. At the end of the collection period, pooled faeces were homogenized and weighed and fresh samples were taken for determination of N content. Additional faeces samples were dried at 60°C in a forced-air oven for 48 h and ground through a 1-mm screen for other chemical analyses.

5.3.4 DAIRY COW FEEDING TRIAL

The feeding experiment was carried out with 24 multiparous Holstein cows in mid-lactation at the Agriculture Experiment Station, location Meiereihof, of the University. Cows averaged 32.2 kg/d of energy-corrected milk (ECM) and 121 days of lactation at the beginning of the experiment. The trial was based on a Latin square design (4 × 4), with each of the four periods lasting 28 d. Adaptation periods comprised 7 d to assure cows to be still in mid-lactation stage at the end of the experiment. The measurement periods comprised 21 d. Cows were allocated at random to each group according to milk yield, lactation number and lactation day. Cows were housed in a free stall barn equipped with Calan gates (American Calan Inc.) and balance troughs (Westfalia Surge, Bönen, Germany), which allowed for the monitoring of individual feed intake. Each group comprised six cows and had access to three feed troughs. Cows were milked twice a day starting at 0500 and 1600 h. During the measurement phase, milk yield was recorded daily by calibrated electronic milk meters. Milk samples were collected once a week at morning and afternoon milking and pooled for one day proportional to the milk yield of each milking for milk composition analyses. Milk analyses were carried out by Milchprüfring Baden-Württemberg for fat, protein, lactose and urea by mid-infrared spectroscopy using Bentley FTS (Bentley Instruments Inc., Minnesota, USA). Body weight was recorded daily. Cows were offered the feed once a day at approximately 0800 h for *ad libitum* consumption. Water and mineral blocks were available at all times. The TMR were mixed in a mixer wagon (Seko SpA, Curtarolo, Italy). All diets were based on the same ingredients: hay, corn silage, grass silage, corn, barley and a mixture of vitamins and minerals. For the control diet, RSM was used as the main protein source (Table 14). For the

other three diets, RSM was completely replaced by DDGS sources ($DDGS_1$, $DDGS_2$ or $DDGS_3$) and dietary urea was added to make the diets isonitrogenous. The TMR were aimed to be equal in the concentrations of CP, utilizable CP (uCP), minerals and vitamins and were formulated to meet the nutritional requirements of dairy cows eating 21 kg DM and producing 31 kg of ECM per day as recommended by GfE (2001). During the measurement phase, around 400 g of TMR samples were collected daily and stored frozen at -20°C. At the end of the experiment, TMR samples were dried at 65°C in a forced-air oven for 48 h, weighed for calculation of DMI, ground through a 1-mm screen and pooled per TMR and period for chemical analyses. *In vitro* estimation of energy values of the TMR was performed using the Hohenheim gas test method according to Menke and Steingass (1988) following the official method 25.1 (Verband Landwirtschaftlicher Untersuchungs- und Forschungsanstalten, VDLUFA, 2006). Energy values of the diets were calculated based on gas production at 24 h (G24) of incubation, corrected for blank and standard values, and nutrient composition of the TMR samples according to Menke and Steingass (1988).

Table 14 Ingredient composition of the total mixed rations (TMR) used in the feeding trial with dairy cows (% of DM)

	TMR			
	Control	D1	D2	D3
Basal mix				
Corn silage		15.8		
Grass silage		21.3		
Meadow hay		15.9		
Premix[1]		2.3		
Concentrate[2]				
Corn grain:barley grain (1:1)	29.80	26.00	28.20	27.80
RSM	14.90	-	-	-
$DDGS_1$	-	18.58	-	-
$DDGS_2$	-	-	16.45	-
$DDGS_3$	-	-	-	16.83
Urea	-	0.12	0.05	0.07

[1] Beta carotene premix (0.2%), sodium chloride (0.3%), calcium carbonate (0.9%), monosodium phosphate (0.1%) and trace elements premix (0.8%).
[2] RSM = rapeseed meal; $DDGS_1$ =dried distillers' grains with soluble originated from wheat:corn:barley = 65:15:20; $DDGS_2$ originated from wheat:corn = 85:15; $DDGS_3$ originated from wheat.

5.3.5 CHEMICAL ANALYSES

All analyses followed the official analytical methods in Germany (VDLUFA, 2006). Samples of DDGS, RSM, TMR and faeces of sheep were analysed for DM, crude ash (CA), CP, EE and crude fibre (CF) (methods 3.1, 8.1, 4.1.1, 5.1.1 and 6.1.1, respectively). Neutral detergent fibre was assayed with a heat-stable amylase and expressed without residual ash ($aNDF_{om}$; method 6.5.1). Acid detergent fibre was determined and expressed without residual

ash (ADF_{om}) in the four protein feeds (method 6.5.2), whereas ADF was determined in faeces and TMR (method 6.5.2). Acid detergent lignin (ADL) was determined in DDGS, RSM and TMR (method 6.5.3). Starch and sugar were analysed in the four protein feeds according to methods 7.2.1 and 7.1.1, respectively. Ca, P, Mg, K, Na and Cl content were determined (methods 10.2.1, 10.6.1, 10.4.1, 10.2.1, 10.1.1 and 10.5.1, respectively) in the four protein feeds. AA contents in the DDGS and RSM were measured with an amino acid analyser L8900 (VWR/Hitachi). After oxidation with performic acid, samples were hydrolysed with 6 M HCl for 24 h at 110°C (Rodehutscord *et al*., 2004). The AA were separated with ion-exchange chromatography and quantified by post-column derivatization with ninhydrin using photometric detection at 570 nm (440 nm for proline). NDiN and ADiN were determined according to Licitra *et al*. (1996).

5.3.6 CALCULATIONS AND STATISTICAL ANALYSES

Percentage digestibility was calculated by the difference between quantities of nutrient intake and their excretion with faeces, divided by intake and multiplied with 100. Energy values (MJ/kg DM) of DDGS and RSM based on digestibility were calculated using the equations given by GfE (2001).

The uCP and ruminal nitrogen balance (RNB) of the TMR and ECM were estimated according to the equations of the GfE (2001). For uCP calculations of TMR, UDP and ME values of DDGS and RSM obtained from the *in situ* and digestibility trials were used. UDP and ME of the other TMR ingredients (hay, corn silage, grass silage, corn and barley) were taken from published values of DLG (1997). The MPS was calculated according to Schingoethe (1996) as (g of sum of EAA/kg CP)/(g of sum of EAA in milk/kg of milk protein), and estimates of the AA content of milk protein were according to Waghorn and Baldwin (1984).

Model parameters for the *in situ* experiment were estimated using the software GraphPad Prism 5.00 for Windows (GraphPad Software Inc., San Diego, CA). Other statistical analyses were run using the software package SAS (version 9.2, SAS Institute Inc., Cary, NC). Multiple linear regressions for calculation of digestibility were estimated using the GLM procedure. Calculations were based on a common intercept model and slopes of multiple linear regressions (Kluth *et al*., 2005b). The following equation was used: $y = a + b_n x_n$, where y = digestibility (%) of a given constituent in the diet, a = intercept, b_n = slope for test source n and x_n = a given concentration originating from test source n (%). To calculate the digestibility of the respective protein feed, equations were extrapolated to x = 100.

Differences in digestibility between protein feeds were tested based on the significance of the slopes using the ESTIMATE statement (*t*-test). Data of *in situ* trial were subjected to ANOVA using the MIXED procedure. Data of the feeding trial were analysed according to a 4 × 4 Latin square design using the MIXED procedure. For all variables of the feeding trial, statistical analyses were conducted on data averaged per cow and period. The model included rations and periods as fixed effects and cows as random effect. Significant differences between individual means were identified using the Tukey–Kramer method. All significant differences were declared at $P < 0.05$.

5.4 RESULTS

5.4.1 *IN SITU* CP DEGRADABILITY

Estimated parameters of *in situ* ruminal CP degradation and UDP values of RSM and DDGS are given in Table 15. $DDGS_1$ had the highest rapidly degradable fraction (16.8%) and RSM the lowest (5.3%), while $DDGS_2$ and $DDGS_3$ were statistically not different. RSM had a higher potentially degradable fraction (b) and potential degradability (a + b) (88.9% and 94.2%, respectively) compared to DDGS. UDP (% of CP) calculated for a passage rate of 8%/h was significantly different between RSM and DDGS and also between the DDGS.

Table 15 Characteristics of *in situ* ruminal CP degradation and UDP values of dried distillers' grains with solubles (DDGS) and rapeseed meal (RSM)

Item	RSM	$DDGS_1$	$DDGS_2$	$DDGS_3$	Pooled s.e.
In situ parameters[1]					
a	5.3^{c}	16.8^{a}	12.3^{b}	14.6^{b}	0.81
b	88.9^{a}	71.4^{c}	77.6^{b}	72.5^{c}	0.93
a + *b*	94.2^{a}	88.2^{bc}	89.9^{b}	87.1^{c}	0.91
c	11.6^{b}	12.4^{b}	28.7^{a}	26.2^{a}	0.22
UDP (5 %/h)	32.7^{a}	32.5^{a}	22.1^{c}	24.6^{b}	0.52
UDP (8 %/h)	42.2^{a}	39.9^{b}	27.6^{d}	30.0^{c}	0.68

$DDGS_1$ = originated from wheat:corn:barley = 65:15:20; $DDGS_2$ = originated from wheat:corn = 85:15; $DDGS_3$ = originated from wheat.

[1] Calculated from the fitted equation $P = a + b\ (1\text{-}e^{-ct})$, where P = degradation after t hours, a = rapidly degradable fraction (%), b = potentially degradable fraction (%), c = rate of degradation of b fraction (%/h) and t = time (h); UDP = undegraded CP (% of CP), calculated as 100 – ED, ED calculated from the equation a + [(b x c)/(c + k)] with rate of passage of 5 and 8%/h (k).

[abcd] Different superscripts within rows indicate significant differences ($P<0.05$)

5.4.2 *IN VIVO* DIGESTIBILITY AND CALCULATED ENERGY VALUES

Results of multiple linear regression analysis, digestibility and ME and NE_L values are summarized in Table 16. Based on the statistical comparison of estimated slopes, the digestibility of OM, EE, NFE and $aNDF_{om}$ was similar between RSM and the three DDGS.

Some significant differences were found for the digestibility of CF and ADF. DDGS2 obtained the highest CF and ADF digestibility. The calculated NE_L value of RSM was the lowest (7.40 MJ/kg DM) and that of DDGS varied from 7.65 to 7.73 MJ/kg DM.

5.4.3 FEEDING TRIAL

The content of CP, EE, NFE, CA and fibre fractions was similar among the four TMR (Table 17). Calculated uCP and RNB were also similar among the TMR, averaging 156 and -1.8 g/kg DM, respectively. Calculated UDP (g/kg DM) and estimated NE_L varied among the TMR. Results of feed intake, milk yield and milk composition of the feeding trial are shown in Table 18. The DMI (21.7 kg/d) was similar between the TMR. Cows fed D1 had a higher milk yield than those fed D3 (31.7 vs. 30.4 kg/d), but no differences were found between cows fed control and diets containing DDGS. Diets did not affect the ECM yield (31.2 kg/d). Diets affected neither the concentration of milk fat (4.0%) nor the milk fat yield (1.24 kg/d) or milk urea concentration (20.9 mg/100 ml). Milk protein concentration was higher for cows receiving the control diet (3.59%) than those with diets D1 and D2. Cows fed control diet yielded more milk protein (1.12 kg/d) than those getting D3 (1.06 kg/d). Feeding DDGS significantly increased the milk lactose concentration (4.91%) compared to the control diet (4.81%).

5.5 DISCUSSION

5.5.1 COMPOSITION, DIGESTIBILITY AND *IN SITU* CP DEGRADABILITY

A similar nutrient composition of a larger number of RSM samples compared to our sample was reported (Kluth *et al*., 2005a; Steingass *et al*., 2013). DDGS came from different ethanol production plants. They mainly originated from wheat with variable proportions of other grains and showed similar nutrient profiles and content of fibre fractions with few exceptions (CP, sugar, starch, ADiN, Na and lysine). However, variability in the nutrient composition of European DDGS was reported to be much higher than in the present study (Westreicher-Kristen *et al*., 2012). In dairy cows, the UDP fraction supplies a substantial amount of AA; therefore, a good characterization of feedstuffs in terms of AA profile in UDP and hence in dietary CP is highly relevant. Variability of the lysine content of DDGS used in the present study could be explained due to the variability of the raw materials used for ethanol production, the contribution of yeast used for fermentation or the interactions between these factors.

Table 16 Nutrient digestibility and calculated energy values of the dried distillers' grains with soluble (DDGS) and rapeseed meal (RSM) based on common intercepts and slopes determined by multiple linear regression analysis (n = 8 per feed, estimate ± SE)

Item	Common Intercept	Slope							
		RSM	$DDGS_1$	$DDGS_2$	$DDGS_3$	RSM	$DDGS_1$	$DDGS_2$	$DDGS_3$
						Estimated digestibility[1] (%)			
Organic matter	67.0±0.13	0.12±0.01	0.10±0.01	0.12±0.01	0.10±0.01	79	77	79	77
Ether extract	21.6±1.71	0.64±0.04	0.69±0.03	0.64±0.03	0.67±0.03	86	91	86	89
Crude fibre	66.1±0.51	-0.25^{b}±0.03	-0.26^{b}±0.05	-0.14^{a}±0.05	-0.24^{b}±0.05	41	40	52	42
N-free extract	70.8±0.38	0.14±0.02	0.09±0.02	0.12±0.02	0.10±0.02	85	80	80	83
$aNDF_{om}$	64.3±0.86	-0.08±0.05	0.04±0.04	0.06±0.04	-0.03±0.04	56	68	70	61
ADF	56.5±1.30	-0.03^{b}±0.07	-0.03^{b}±0.07	0.10^{a}±0.08	0.02^{b}±0.07	54	54	67	59
Energy content[2] (MJ/kg DM)									
	Hay								
ME	9.18					12.1	12.5	12.5	12.7
NE_L	5.38					7.40	7.65	7.68	7.73

RSM = rapeseed meal; $DDGS_1$ = originated from wheat:corn:barley = 65:15:20; $DDGS_2$ = originated from wheat:corn = 85:15; $DDGS_3$ = originated from wheat; $aNDF_{om}$ = neutral detergent fibre; ADF = acid detergent fiber; ME = metabolizable energy ; NE_L = net energy for lactation

[1] Digestibility calculated on the basis of the multiple linear regression analysis and extrapolated to x = 100 by the following equation: $y = a + b_n x_n$, where y = digestibility (%) of a given parameter in the diet, a = intercept, b_n = slope for test source n and x_n a given parameter concentration originating from test source n (100%)

[2] Based on digestibility using the equations given by GfE (2001): GE = 0.0239 CP + 0.0398 EE + 0.0201 CF + 0.0175 NFE; ME = 0.0312 dEE + 0.0136 dCF + 0.0147 (dOM – dEE – dCF) + 0.00234 CP; NE_L = 0.6 [1 + 0.004 (q – 57)] ME; where CP, EE, CF, N-free extracts (NFE) are in g/kg DM; dEE, dCF and dOM are digestible ether extract, crude fibre and organic matter, respectively, and are in g/kg DM; NFE was calculated as 1000 – CA – CP – EE – CF; q = ME/GE x 100.

[ab] Different superscripts within rows indicate significant differences between estimated slopes (P<0.05).

Table 17 Chemical composition, protein and energy values of the total mixed rations (TMR) used in the feeding trial (average of 4 samples, 1 in each period)

	TMR[1]			
	Control	D1	D2	D3
DM (g/kg)	412	406	408	410
Nutrient (g/kg DM)				
Crude ash	80	77	75	73
Crude protein	144	143	145	147
Ether extract	31	36	37	36
Crude fibre	162	157	150	152
$aNDF_{om}$	328	338	332	328
ADF	201	199	189	189
ADL	26	26	24	24
uCP	156	155	155	157
RNB	-1.9	-1.9	-1.6	-1.6
UDP[2]	46	43	37	39
UDP[2] (% of CP)	32	30	26	26
Energy[3] (MJ/kg DM)				
ME	11.1[b]	11.2[b]	11.5[a]	11.5[a]
s.d.	0.13	0.11	0.08	0.09
NE_L	6.94[b]	7.00[b]	7.16[a]	7.19[a]
s.d.	0.10	0.08	0.06	0.06

DM = dry matter; $aNDF_{om}$ = neutral detergent fibre; ADF= acid detergent fibre; ADL = acid detergent lignin; uCP = utilizable CP; RNB = ruminal nitrogen balance.
[1]Control = diet with rapeseed meal as primary protein source; D1, D2 and D3 = diets formulated with the three different dried distillers' grain with solubles (DDGS).
[2] Estimated from the results of the *in situ* degradation of the protein sources (passage rate 5 %/h) and from values from the DLG (1997) for other components of the TMR.
[3] ME = metabolizable energy and NE_L = net energy for lactation with standard deviation (s.d.) and estimated through the gas production method (Menke and Steingass, 1988) from pooled TMR samples over the four periods of the feeding trial (n = 4).
[ab] Different superscripts within rows indicate significant differences (P<0.05).

Additionally, this variability could be influenced by the degree of heating during the drying process of the DDGS, which could reduce the concentration and availability of lysine (Kleinschmit *et al*., 2007). This could be supported by the fact that there is tendentially a negative correlation between the lysine content (g/16 g N) and UDP5 (% of CP) of the three DDGS (1.99 and 32.5, 2.17 and 24.6, 2.49 and 22.1, respectively).

The MPS was proposed as a good indicator of the protein quality of feedstuffs (Schingoethe, 1996), assuming that AA composition of milk protein is indicative of the ideal AA balance for high-yielding cows when milk production dominates total AA requirements. The MPS of DDGS was lower than of RSM, explained by their lower content of EAA, especially lysine, which was around the half of RSM. Additionally, RSM had a more favourable protein value than DDGS in terms of higher methionine content. Low content of lysine and methionine and low MPS value of DDGS may negatively influence the supply of essential AA, affecting production parameters when feeding dairy cows, provided that the AA composition of UDP is similar to that of original DDGS.

Table 18 Dry matter intake, milk production and milk composition

	TMR[1]				Pooled
	Control	D1	D2	D3	s.e
DMI (kg/d)	22.0	21.6	21.7	21.5	0.43
Milk production (kg/d)					
Milk	31.3[ab]	31.7[a]	31.3[ab]	30.4[b]	1.03
ECM	31.2	31.4	31.3	30.6	0.98
Fat	1.23	1.26	1.25	1.23	0.05
Protein	1.12[a]	1.08[ab]	1.08[ab]	1.06[b]	0.03
Lactose	1.50[ab]	1.56[a]	1.54[ab]	1.50[b]	0.05
Milk composition					
Fat (%)	3.99	4.02	4.06	4.12	0.14
Protein (%)	3.59[a]	3.43[b]	3.48[b]	3.50[ab]	0.05
Lactose (%)	4.81[b]	4.93[a]	4.90[a]	4.90[a]	0.04
Urea (mg/100 ml)	20.1	21.2	21.3	21.0	0.54

DMI = dry matter intake; ECM = Energy-corrected milk.
[1]Control = diet with rapeseed meal as protein source; D1, D2 and D3 = diets formulated with the three different dried distillers' grain with solubles (DDGS).
[ab] Different superscripts within rows indicate significant differences (P<0.05).

Digestibility was calculated by a multiple linear regression method with a common intercept, an approach that can be applied when at least two supplementary levels of the feedstuffs under study are used. The stepwise substitution of hay by the respective test feeds resulted in a linear response of digestibility over the whole range of inclusion levels. The standard errors of the estimated slopes varied from 0.01 to 0.08. Since this variation is relatively low, the accuracy of the models for the determination of digestibility of chemical fractions of DDGS and RSM can be regarded high. Digestibility of DDGS and RSM was generally in accordance with that in the literature. Similar digestibility of OM from RSM was reported (Kluth *et al*., 2005a). Digestibility of OM, CP, CF and NFE is similar to one corn-DDGS and one wheat-DDGS published by Urdl *et al*. (2006). Between the DDGS, the lower digestibility of ADF of $DDGS_1$ could be associated to the fact that it originated from 20% of barley, which normally has an enriched proportion of low-digestible hulls.

The calculated NE_L of RSM was higher than published by Kluth *et al*. (2005a) and similar to DLG (1997). Energy values of DDGS were similar to *in vitro* results previously published (Westreicher-Kristen *et al*., 2012). Higher energy values of DDGS compared to RSM reflect how valuable DDGS is for ruminants' diets in terms of energy content. Due to no differences in digestibility of most of the crude nutrient fractions, differences in energy values between RSM and DDGS may be mostly explained by differences in nutrient composition rather than in digestibility results. Thus, lower energy values of RSM seem to be a result of a combination of lower content of EE and OM and higher content of CF.

RSM and $DDGS_1$ obtained the highest UDP values associated with the lowest rates of CP degradation (11.6%/h and 12.4%/h, respectively). The variation in UDP observed among the

DDGS samples (22.1-32.5% at outflow rates of 5%/h) may be due to differences in the nutritional composition of raw materials and differences in processing methods between the different ethanol production plants. We have investigated DDGS based mainly on wheat. In the German feed tables (DLG, 1997) there is no information for UDP of wheat-DDGS. Values are available only for corn-DDGS (50% UDP) and barley-DDGS (40% UDP). Even when no major differences of nutrient and fibre fractions between DDGS were found, the observed differences of UDP values confirm the high relevance of this key variable for the characterization of feedstuffs in terms of protein quality and highlights the necessity for the update of protein values of DDGS in official feed tables.

5.5.2 FEEDING TRIAL

Observed DMI was similar among the TMR when feeding DDGS or RSM at inclusion levels of approximately 17% and 15% of DM, respectively. Similarly, Mulrooney *et al*. (2009), Liu *et al*. (2000) and Urdl *et al*. (2006) found no differences in DMI of dairy cows when replacing protein supplements by DDGS in rations at levels similar to this study. Slight differences were observed in milk yield among the TMR containing DDGS, but yield was not different when DDGS diets were compared to RSM. Other studies have evaluated the effect of feeding DDGS to dairy cows with controversial results. Mulrooney *et al*. (2009), Liu *et al*. (2000) and Urdl *et al*. (2006) found no differences on yield and milk composition of cows when replacing completely the protein sources by DDGS in rations. In addition, Nichols *et al*. (1998) found higher milk yield, higher protein yield and similar fat yield when cows were fed DDGS diets compared with SBM diets. Since raw materials used for ethanol production may influence the feed value of DDGS and thus feeding trial results, comparison of our results with trials where corn based DDGS were fed should be considered with care. In the present study, no differences in milk fat yield and concentration were found, which rejects the general perception that feeding DDGS results in milk fat depression (Urdl *et al*., 2006; Kleinschmit *et al*., 2006). All DDGS sources used in the present study had only moderate contents of EE between 66 g/kg DM and 75 g/kg DM. Inclusion levels of 15-17% in total DM of TMR and DMI between 21.5 kg/d and 21.7 kg/d resulted in EE intakes between 240 g/d and 290 g/d from DDGS. These low quantities and the moderate intake of EE from the total diets ranging between 775 g/d and 800 g/d makes a milk fat depression caused by EE intake less probable.

Since no major differences were observed in DMI and nutrient composition between the TMR, the effect of diets on animal performance may be mainly expected due to differences in the content of UDP, AA profile of UDP or intestinal digestibility of UDP of the

protein supplements. Protein supplements influenced the milk protein yield and concentration. Similar to our results, Kleinschmit *et al*. (2006) and Powers *et al*. (1995) observed lower milk protein percentages when DDGS substituted protein supplements like SBM or blood meal. Reduced concentration of milk protein obtained with diets D1 and D2 may be attributed to an unbalanced supply of AA from UDP of DDGS, particularly lysine, compared to control, which delivered probably more AA to the mammary gland for milk protein synthesis due to more UDP and a more favourable AA composition. RSM contained almost twofold more lysine and 25% more methionine than DDGS. According to Powers *et al*. (1995), depressed milk protein percentage, like in the case of the cows fed D1, could be an indicator of poor quality or heat damage of $DDGS_1$, which is reflected on its highest unavailable dietary protein in the form of ADiN (11.1% of CP) compared to RSM (6.4% of CP). However, Kleinschmit *et al*. (2007) and Li *et al*. (2012) reported that NDiN did not reflect the unavailable CP of DDGS in the intestine. Moreover, Nakamura *et al*. (1994) and Kajikawa *et al*. (2012) found partial degradation of ADiN in the rumen, suggesting that ADiN is not a reliable measure for protein indigestibility of DDGS and other plant protein by-product sources. Kajikawa *et al*. (2012) proposed a partial degradation of NDiN, due to low levels of lysine and xylose in DDGS, which are involved in the Maillard reaction.

In addition, the combination of the highest UDP and the lowest lysine content of $DDGS_1$ resulted probably in a lower supply of lysine influencing the lower protein milk percentage. Schingoethe (1996), Nichols *et al*. (1998) and Kleinschmit *et al*. (2006) indicated lysine as the first limiting AA for diets containing DDGS. Mulrooney *et al*. (2009) found decreased lysine concentration in blood when increasing the amounts of DDGS in the diet, proving the lysine deficiency of DDGS. Similar reduction of lysine in blood was also reported when feeding DDGS (Palmquist and Conrad, 1982; Kleinschmit *et al*., 2006; Nichols *et al*., 1998).

Since the UDP, besides the microbial protein, supplies the AA required by the cows, differences of protein digestibility and AA availability in the intestine should also be considered when differences on milk protein production are discussed. Boucher *et al*. (2009) and O'Mara *et al*. (1997) found lower intestinal digestibility of lysine from DDGS compared to other AA and other feedstuffs. According to Kleinschmit *et al*. (2007), excessive heat during drying process could increase the portion of damaged protein unavailable to the animal, and lysine is particularly the most susceptible AA to such damage.

The lactose concentration of milk usually shows only little variation in healthy cows and it is rarely influenced by the feeding. However, protein supplements seemed to have significantly

influenced the milk lactose yield and concentration in the present trial. Higher concentration of milk lactose when fed DDGS may confirm our hypothesis that lysine was unbalanced and insufficient for milk protein synthesis, leading to a relative surplus of other AA which were used for gluconeogenesis. Similarly, Liu *et al*. (2000) found tendentially higher content of lactose for DDGS diets linked to a lower content of lysine in blood. Also, Nichols *et al*. (1998) found significant increment of lactose when replacing SBM by DDGS. Nevertheless, presuming that lactose is the dominant osmoregulatory compound in milk, the fact that higher production of lactose increased its concentration in milk and not milk yield at unchanged concentration remains unexplained. Moreover, the possible surplus of other AA than lysine should theoretically result in deamination of unused AA and urea synthesis in the liver, thus incrementing the urea content of milk. Nevertheless, milk urea was only numerically higher and not statistical significant when cows were fed DDGS compared to control diet. Thus, it may be hypothesized that gluconeogenesis from AA was not high enough to quantitatively influence milk urea content and/or that the surplus of urea resulting from this process might be compensated by increased use of nitrogen for ruminal microbial protein synthesis. As reported by Baker *et al*. (1995), milk urea is sensitive to changes in CP, rumen degraded protein and UDP but insensitive to difference in AA balance. Similarly, Nousiainen *et al*. (2004) reported higher impact of CP content or ruminal ammonia on milk urea than absorbed AA not utilized for milk protein.

5.6 CONCLUSIONS

Overall, the DDGS sources had no effects on DMI and milk fat concentration and production of dairy cows compared to RSM. Differences in milk protein concentration when feeding different DDGS sources indicate a variation in the availability of lysine in UDP of DDGS. This variation should be considered when DDGS is fed to dairy cows. We suggest that DDGS can be a suitable feed when compared to RSM in diets of dairy cows in mid-lactation. A limiting factor of our study is that the DDGS sources used did not reflect the overall variability of nutrient composition and protein and energy values of DDGS already found in previous studies. Even higher differences in production results should be expected when considering more variable sources of DDGS.

REFERENCES

Anderson, J.L.; Schingoethe, D.J.; Kalscheur, K.F.; Hippen, A.R., 2006: Evaluation of dried and wet distillers grains included at two concentrations in the diets of lactating dairy cows. Journal of Dairy Science 89, 3133–3142.

Baker, L.D.; Ferguson J.D.; Chalupa W., 1995: Response in urea and true protein of milk to different protein feeding schemes for dairy cows. Journal of Dairy Science 78, 2424-2434.

Boucher, S.E.; Calsamiglia, S.; Parsons, C.M.; Stein, H.H.; Stern, M.D.; Erickson, P.S.; Utterback, P.L.; Schwab, C.G., 2009: Intestinal digestibility of amino acids in rumen-undegraded protein estimated using a precision-fed cecectomized rooster bioassay: II. Distillers dried grains with soluble and fish meal. Journal of Dairy Science 92, 6056–6067.

DLG (Deutsche Landwirtschafts-Gesellschaft),1997: DLG Futterwerttabellen-Wiederkäuer. 7., erweiterte und überarbeitete Auflage, Herausgeber Universität Hohenheim-Dokumentationsstelle, DLG-Verlag, Frankfurt.

Dunkel, S.; Potthast, C.; Eggers, J.; Trauboth, K.; Früh, G., 2010: Trockenschlempe und Rapsextraktionsschrot als alleiniger Proteinergänzer in Futterrationen für Hochleistungskühe. 122 VDLUFA-Kongress 66, 613–622.

GfE, 1991: Leitlinien für die Bestimmung der Verdaulichkeit von Rohnährstoffen an Wiederkäuern. Journal of Animal Physiology and Animal Nutrition 65, 229–234.

GfE, 2001: Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt.

Grings, E.E.; Roffler, R.E.; Deitelhoff, D.P., 1992: Responses of dairy cows to additions of distillers dried grains with soluble in alfalfa-based diets. Journal of Dairy Science 75, 1946–1953.

Janicek, B.N.; Kononoff, P.J.; Gehman, A.M.; Doane, P.H., 2008: The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. Journal of Dairy Science 91, 3544–3553.

Kajikawa, H.; Miyazawa, K.; Yanase, A.; Tanabe, Y.; Tsuchida, Y.; Mitsumoto, Y.; Kozato, Y.; Mitsumori, M., 2012: Variation in chemical composition of corn dried distillers grains with solubles in relation to *in situ* protein degradation profiles in the rumen. Animal Science Journal 83, 299–304.

Kleinschmit, D.H.; Schingoethe, D.J.; Kalscheuer, K.F.; Hippen, A.R., 2006: Evaluation of various sources of corn dried distillers grains plus solubles for lactating dairy cattle. Journal of Dairy Science 89, 4784–4794.

Kleinschmit, D.H.; Anderson, J.L.; Schingoethe, D.J.; Kalscheur, K.F.; Hippen, A.R., 2007: Ruminal and intestinal degradability of distillers grains plus solubles varies by source. Journal of Dairy Science 90, 2909–2918.

Kluth, H.; Engelhard, T.; Rodehutscord, M., 2005a: Zum Ersatz von Sojaextraktionsschrot durch Rapsextraktionsschrot in der Fütterung der Hochleistungskuh. Züchtungskunde 77, 58–70.

Kluth, H.; Mantei, M.; Elwert, C.; Rodehutscord, M., 2005b: Variation in precaecal amino acid and energy digestibility between pea (Pisum sativum) cultivars determined using a linear regression approach. British Poultry Science 46, 325–332.

Li, C.; Li, J.Q.; Yang, W.Z.; Beauchemin, K.A., 2012: Ruminal and intestinal amino acid digestion of distiller's grain vary with grain source and milling process. Animal Feed Science and Technology 175, 121–130.

Licitra, G.; Hernandez, T.M.; Van Soest, P.J., 1996: Standarization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57, 347–358.

Liu, C.; Schingoethe, D.J.; Stegeman, G.A., 2000: Corn distillers grains versus blend of protein supplements with or without ruminally protected amino acids for lactating cows. Journal of Dairy Science 83, 2075–2084.

Menke, K.H.; Steingass, H., 1988: Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development 28, 7–55.

Mulrooney, C.N.; Schingoethe, D.J.; Kalscheur, K.F.; Hippen, A.R., 2009: Canola meal replacing distillers grains with soluble for lactating dairy cows. Journal of Dairy Science 92, 5669–5676.

Nakamura, T.; Klopfenstein, T.J.; Britton, R.A., 1994: Evaluation of acid detergent insoluble nitrogen as an indicator of protein quality in nonforage proteins. Journal of Animal Science 72, 1043–1048.

Nichols, J.R.; Schingoethe, D.J.; Maiga, H.A.; Brouk, M.J.; Piepenbrink, M.S., 1998: Evaluation of corn distillers grains and ruminally protected lysine and methionine for lactating dairy cows. Journal of Dairy Science 81, 482–491.

Nousiainen, J.; Schingfield, K.J; Huhtanen, P., 2004: Evaluation of milk urea nitrogen as a diagnostic of protein feeding. Journal of Dairy Science 87, 386-398.

O'Mara, F.P.; Murphy, J.J.; Rath, M., 1997: The amino acid composition of protein feedstuffs before and after ruminal incubation and after subsequent passage through the intestines of dairy cows. Journal of Animal Science 75, 1941–1949.

Ørskov, E.R.; McDonald, I., 1979: The estimation of protein degradability in the rumen from incubation measurements weighted according to the rate of passage. Journal of Agricultural Science 92, 449–503.

Palmquist, D.L.; Conrad, H.R., 1982: Utilization of distillers dried grains plus soluble by dairy cows in early lactation. Journal of Dairy Science 65, 1729–1733.

Powers, W.J.; Van Horn, H.H.; Harris, Jr. B.; Wilcox, C.J., 1995: Effects of variable sources of distillers dried grains plus soluble on milk yield and composition. Journal of Dairy Science 78, 388–396.

Rodehutscord, M.; Kapocius, M.; Timmler, R.; Dieckmann, A., 2004: Linear regression approach to study amino acid digestibility in broiler chicken. British Poultry Science 45, 85–92.

Schingoethe, D.J., 1996: Balancing the amino acid needs of the dairy cow. Animal Feed Science and Technology 60, 153–160.

Steingass, H.; Kneer, G.; Wischer, G.; Rodehutscord, M. 2013: Variation of *in situ* degradation of crude protein and amino acids and *in vitro* digestibility of undegraded feed protein in rapeseed meals. Animal, in press

Urdl, M.; Gruber, L.; Häusler, J.; Maierhoffer, G.; Schauer, A., 2006: Influence of distillers dried grains with soluble (Starprot) in dairy cow feeding. Slovak Journal of Animal Science 39, 43–50.

Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (VDLUFA) (ed.) 2006. Handbuch der Landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch), Bd. III Die chemische Untersuchung von Futtermitteln. VDLUFA-Verlag, Darmstadt, Germany.

Waghorn, G.D.; Baldwin, R.L., 1984: Model of metabolite flux with mammary gland of the lactating cow. Journal of Dairy Science 67, 531–544.

Weisbjerg, M.R.; Bhargava, P.K.; Hvelplund, T.; Madsen, J., 1990: Anveldelse af Nedbrydningsprofiler I Fordemiddelvurderingen (Use of Degradation Curves in Feed Evaluation), Report 679, National Institute of Animal Science, Denmark, 33 pp.

Westreicher-Kristen, E.; Steingass, H.; Rodehutscord, M., 2012: Variations in chemical composition and *in vitro* and *in situ* ruminal degradation characteristics of dried distillers' grains with solubles from European ethanol plants. Archives of Animal Nutrition 66, 458–472

CHAPTER 6

GENERAL DISCUSSION

6.1 VARIATION OF CHEMICAL COMPOSITION AND ENERGY AND PROTEIN VALUE OF DDGS WITH SPECIAL CONSIDERATION OF PRACTICAL FEEDING

Determination of the chemical composition is the basis for the evaluation of the nutritive value of feedstuffs. The nutritive values of a feedstuff is a concept which combines information on the content and availability of nutrients, with considerations of the characteristics such as level of intake, palatability, and the effects of the feed on animal health and the quality of animal products (Gordon, 2008). Nutrients are those components of feedstuff capable of being utilised by animals (McDonald *et al*., 2002), which are released by digestion from their combination in food and absorbed from the digestive tract, or are the products of the metabolism of these constituents in the digestive tract (Gordon, 2008). Nutrients can be summarized in six major groups: water, carbohydrates, lipids, proteins, vitamins and minerals. Structural carbohydrate is not a nutrient as itself; however, it is indirectly used by ruminants through the ruminal microorganisms, moreover, supply of structural carbohydrates is essential to maintain a physiological rumen function. Therefore, fibre is considered and determined as CF according to the "proximate analyses" or is divided into its different fractions (NDF, ADF and ADL) based on its solubility according to the detergent fibre system (van Soest, 1991). The latter combined with the "proximate analysis" results in a better and more complete chemical evaluation of feedstuffs for ruminants. Energy content is also an important criterion to evaluate the feed value of feedstuffs and depend on the digestibility of the nutrients in the digestive tract, especially in the rumen. Net energy is not a nutrients but the product of nutrients use. It is the chemical energy of a feedstuff which is released when AA, fatty acids and/or carbohydrates are oxidized in the bodies' cell, yielding ATP (Gordon, 2008). An extension of the CP content of feed is the protein fractionation into A, B1, B2, B3 and C according to the CNCPS (Sniffen *et al*., 1992), which is gaining more attention for the evaluation of protein value of feeds for ruminants.

Nutrient concentration of DDGS is expected to reflect a proportional increase compared to the original substrate due to removal of starch and sugar during ethanol production. This proportional increase was observed in general for most of the chemical components and were duplicated (CA, EE, CF, CP) or more than triplicated (NDF and ADF) compared to published values of corn, wheat and barley (DLG, 1997; NRC, 2001). Reasons for variation of chemical composition of DDGS were widely discussed in individual chapters of this work and can be summarized as effects of substrates used and process technology applied.

Conventional strategies to improve intake and performance of dairy cows focus on increasing the energy density of the diet (Penner and Oba, 2009). The latter may result in a high proportion of starch and/or sugar in the diet which may as well increment the risk of sub-acute rumen acidosis (SARA). Starch content of DDGS in the present study varied from 19 to 185 g/kg DM with a CV of 100%. Without considering the extreme value, starch content would vary from 19 to 58 g/kg DM with a CV of 39%. The variation of starch content of DDGS reflects the variation of efficiency of ethanol production among production plants, which supports the theory that technological and processing methods vary between production plants. Moreover, starch variation also explains part of the variability of chemical constituents of DDGS. On the other hand, sugar content of DDGS ranged from 11 to 49 g/kg DM with a CV of 39%. Although, DDGS have relative high variability of sugar and starch content, values along DDGS are low compared to table values of original grains (DLG, 1997), which give probably no evidence of SARA risk when feeding dairy cows with high proportion of DDGS.

Highly fermentable diets like those for high producing cows require the inclusion of adequate amount of fibre fractions to reduce the risk of SARA (Zebeli *et al*., 2012). According to NRC (2001), the recommended concentration of total NDF for cows may be set at 25% of dietary DM with the condition that 19% must be NDF from forage. However, this recommendation may vary depending on the particle size of forages, rumen availability of starch sources or amount of NDF in forage and presentation of the diet (e.g. TMR). In addition, Zebeli *et al*. (2012) suggested that 31.2% of physically effective NDF ($_{pe}$NDF, particle size >1.18 mm) or 18.5% $_{pe}$NDF (particle size >8 mm) in the diet on DM basis is needed to ensure prevention of SARA. In this study, DDGS was shown to have relative high content of NDF (33.4–47.5% of DM) which may easily result in an "apparent" optimal content of NDF in a formulated total ration. Nevertheless, DDGS contain relatively low content of $_{pe}$NDF (3.4–19.8%; Kleinschmit *et al*., 2007), since the small particle size of DDGS means that its "effective fibre" is not as great as that of forage (Schingoethe, 2006). Therefore, special care should be taken regarding to NDF when formulating diets with high levels of DDGS. Furthermore, it is recommended that DDGS replace NDF of concentrate and not of forages (Schingoethe *et al*. 2009). Neglecting this may result in low $_{pe}$NDF content resulting as well in low fat content of the milk or SARA problems.

Along minerals variation in contents of P and Mg was less (CV 6.3 and 15%, respectively) and their concentration in DDGS was duplicated compared to original grains from tabular data of NRC (2001). Concentration of P in DDGS is high (about 8 g/kg DM) and

should be considered when formulating diets. Depending on the concentration of P of other rations ingredients, it might result in excessive P intake causing probably high P excretion when supplementing with DDGS. Nowadays P is of main concern when feeding animals due to its high cost and its potential as pollutant. Walter *et al*. (2012) found linear increase of P excretion when feedlot heifers were fed wheat or corn-DDGS due to increased P intake. Similar was observed by Spiehs and Varel (2009) when corn was replaced with corn-WDGS in cattle. This aspect should be considered when developing nutrient management based on DDGS diets to minimize P loss to the environment and allow maximum P use (Walter *et al*., 2012).

Cl and Ca concentration in DDGS was incremented only by 1.4 and 1.7 fold compared to NRC values of grains, respectively. This may suggest that Cl and Ca concentration declined compared to original grains and probably were washed out somehow along some steps of the ethanol production. Explanation for such results is not known. The Ca:P ratio is not of concern when feeding ruminants (Spiekers *et al*., 2009). However, the low Ca:P of the DDGS (0.11) here studied, may be of main concern when feeding monogastric animals and probably high amounts of Ca need to be supplemented to match the P content in DDGS and to maintain the optimal Ca:P ratio between 1.5:1 to 2:1 (Belyea *et al*., 1998). K concentration of DDGS was incremented by around two fold compared to table values of original grains (NRC, 2001). However, this increment was exceptionally triplicated in two DDGS samples (e.g. one wheat-DDGS and one blend-DDGS). Variation of K content of DDGS was suggested as a result of the addition of pH regulatoring substances added during ethanol production or due to the use of raw materials for ethanol production with high content of K (e.g. sugar beet syrup) (NRC, 2001) .On the other hand, Na was both the most variable mineral (CV = 83%) in DDGS and the most incremented compared to NRC table values of original grains (12 fold). Such an increment was suggested to be due to addition of exogenous sources such as buffers or NaOH as pH regulators during ethanol production. Nevertheless, all these interpretations regarding to mineral concentration of DDGS should be carefully considered, since differences in nutrient composition within the same grain type may be expected between production factories and they may not necessarily match with the NRC tabular values. High concentration or variation of Mg, Na and K may not be of main concern, because tolerance of ruminants is very high (Belyea *et al*., 1998). Due to expected increment of DDGS production in Europe, the relative favourable economic substitution of conventional feed proteins and relative good animal performance responses when feeding DDGS, a trend to formulate dairy cows and especially ruminant-finishing diets at higher inclusion levels of

DDGS may be expected. Therefore, mineral content of DDGS should be considered when formulating diets, and mineral analysis is required as there is no possibility for prediction.

Ammonia emission from cattle farms is also of concern because it can contribute to air and water pollution. Practices to mitigate ammonia emission include reducing CP content in rations and ruminal protein degradability; therefore, it is important to match dietary protein supplies as closely as possible to microbial and animal needs (Hristov *et al*., 2011). Walter *et al*. (2012) found linear increase of N excretion when feedlot heifers were fed wheat or corn-DDGS due to increased N intake. Moreover, this was probably influenced by a high supply of UDP from DDGS. For a correct diet formulation, actualized information of the nutrient composition of feedstuffs is a prerequisite. However, in practice, ration formulation is not always based on analytical results but based on tabular values. Thus, when formulating diets including DDGS, a feedstuff with high variation in nutrient composition, mis-formulation can be arising regarding to CP and/or UDP content of the ration resulting in high excretion of N, or N deficiency on the other hand.

Feeds with relative high energy content are needed to match the requirements of energy for milk production. The latter is especially important and difficult with high producing cows due to high requirements and decreased feed intake at the beginning of the lactation. Moreover, accurate estimates of energy values of feedstuffs and diets are important because of the large quantities required by dairy cows (Weiss, 1998). Therefore, the enhancement of energy density is a conventional approach to overcome low feed intake during the first few weeks of lactation (Xu *et al.*, 1998). DDGS averaged 7.3 MJ NE_L/kg DM based on *in vitro* gas production (n = 13) and 7.6 MJ NE_L/kg DM based on *in vivo* digestibility with sheep (n = 3). These values confirm DDGS to be a good supplement of energy for ruminants. At least a part of the high energy content in DDGS is due to the fat content, whereas some can be attributed to the highly digestible fibre (NDF). Energy value (MJ NE_L/kg DM) of DDGS is comparable to other feed proteins like RSM (7.3) and rapeseed cake (7.5), but lower than SBM (8.6) (DLG, 1997).

In dairy cows, the UDP fraction supplies a substantial amount of AA. Since high yielding cows require higher content of UDP in the diet than mid or low yielding cows, a good characterization of feedstuffs in terms of AA profile in UDP and hence in dietary CP is highly relevant. AA pattern of UDP is closely linked with AA pattern of the DDGS. *In situ* ruminal degradation of AA was proved to be suitable predicted from CP degradation (Chapter 4). In addition, lysine and methionine content in DDGS and in UDP varied widely. Lysine and methionine are commonly considered to be the first and second limiting AA for milk

protein synthesis (Xu *et al*., 1998; Schwab *et al*., 1992). In addition, special interest is currently focused on AA supply for milk production because of the growing emphasis on milk protein. Since all grains, especially corn have a substantially low content of lysine (Xu *et al*., 1998), it was expected that lysine was the most limiting AA both in DDGS and in UDP along all DDGS types. Additionally, we proposed that heating during the drying process may further reduce the concentration and availability of lysine in DDGS, so that original grain has no longer a dominant effect on the concentration of lysine in the resulting DDGS. Grass silage and corn silage are the main forage sources for dairy cows. Since corn silage has also relative poor content of lysine (Xu *et al*., 1998) and lysine appears to be first-limiting when corn-based rations are supplemented (Schwab *et al*., 1992), diets based on high proportions of corn silage supplemented with DDGS could lead to even lower supply of lysine and limit milk production especially in high producing cows.

Linked to AA content of UDP, AA digestibility in the duodenum is also relevant, since digestibility determines the amount of AA available for the animal. Whether the individual AA digestibility of DDGS reflects the overall pattern of CP digestibility was not investigated and is still not known. However, UDP digestibility was proved to be highly variable (see Chapter 4) and the same may be assumed for individual AA. In recent times, balancing diets of high yielding dairy cows based on intestinal available or digestible AA and not only on UDP and uCP is taking more importance. Variability of AA digestibility of DDGS may suggest that AA supply may not always match correctly the AA demand of the animal if required information is not available for diet formulation. Therefore, to fully complement this study, further studies should be focused on assessing the digestibility of individual AA of UDP of DDGS in the intestine and characterize its variation.

Management strategies to reduce the negative impact of the high variability of nutrients and feed value of DDGS should be considered in the field. Availability of adequate and updated feed information may enable to know and manage this variation. Thus, reducing the risk of mis-formulation of diets. Limiting or reducing the inclusion rate of DDGS in diets of ruminants, especially in high producing dairy cows, may be also considered. Difference of nutrient content of DDGS between production plants was already proved among different sources. Therefore, purchasing DDGS from a single source may be also considered as an alternative strategy.

Due to high variability of many parameters of chemical composition and feed value of DDGS, it was attempted in this work to predict complex parameters (e.g. effective degradability of DM and CP, UDP, ruminal AA degradation and intestinal digestibility of CP)

through simple methods to be used in practice for rapid DDGS evaluation and estimates of feeding value for its further possible consideration in practical feeding and diets formulation.

6.2 ESTIMATION OF *IN SITU* PARAMETERS FROM CHEMICAL COMPOSITION AND *IN VITRO* STUDIES

It was studied whether there are relationships between *in situ* and *in vitro* experiments and the potential to predict *in situ* results through simple *in vitro* and chemical characteristics. Correlation analyses between *in vitro* gas production at different incubation times and chemical constituents were performed to explain differences of *in vitro* results. Moreover, regression analyses were performed between *in vitro* and *in situ* results. It was attempted to predict *in situ* values from *in vitro* gas production parameters combined with chemical composition through stepwise multiple linear regressions. Linear regressions with the highest coefficient of determination were chosen. Relationships were described using R^2, RMSE and regression coefficients. Correlation coefficients between gas production (G) at different incubation times and chemical constituents are shown in Table 19. EE and NDF were negatively correlated with G at all incubation times. Sugar was positively correlated to G at all incubation times; whereas, starch started to correlate with G at 8h of incubation. Hemicellulose was negatively correlated with G at 2 and 4 h, and tended to correlate with higher incubation times (8 and 12h). Although CP was not correlated with G at any incubation time, protein fraction A tended to correlate with G2 (r = 0.48, P = 0.09), and fraction B3 was negatively correlated to G2 (r = -0.62, P = 0.02) and tended to correlate with G4 (r = -0.50, P = 0.08) and with G12 (r = -0.50, P = 0.08). Other chemical constituents (CA, CF, ADF, NDiN and ADiN) and protein fractions neither had significant relations, nor tended to correlate, with G at different incubation times. Since gas production is mainly the result of fermentation of carbohydrates, the most degradable carbohydrates (sugar and starch) were positively correlated to G. Whereas, low degradable fractions like NDF_{om} and hemicelluloses were negatively correlated.

It was also attempted to assess whether *in situ* effective degradability of dry matter (EDDM) could also be predicted from chemical constituents and *in vitro* gas production. Stepwise linear multiple regression was used to predict EDDM values from chemical constituents and *in vitro* gas production.

Table 19 Correlation coefficients (r) between chemical constituents and *in vitro* gas production of dried distillers´ grains with soluble at different incubation times (based on results of Chapter 3)

	EE	Sugar	Starch	$aNDF_{om}$	Hemicellulose
G2[1]	-0.91****	0.69*	0.18	-0.63*	-0,68*
G4	-0.89****	0.78**	0.38	-0.76**	-0.61*
G8	-0.76**	0.77**	0.61*	-0.84***	$-0.51^{(*)}$
G12	-0.62*	0.65*	0.62*	-0.84***	$-0.53^{(*)}$
G24	-0.56*	0.59*	0.63*	-0.79**	-0.43
G36	-0.68*	0.71**	0.66*	-0.76**	-0.36
G48	-0.77**	0.74**	0.63*	-0.72**	-0.32
G72	-0.79**	0.75**	0.62*	-0.69**	-0.33

EE = ether extract; $aNDF_{om}$ = neutral detergent fibre; amylase pre-treated, ash free; where EE, sugar, starch, $aNDF_{om}$ and hemicelluloses is expressed in g/kg DM.

[1] Gas production (G, ml) estimated through the gas production method (Menke and Steingass, 1988) at 2, 4, 8, 12, 24, 36, 48, and 72 h, respectively.

****P<0.0001; ***P < 0.001; **P < 0.01; *P < 0.05; $^{(*)}$ 0.10 < P > 0.05; otherwise P > 0.10.

Equations were estimated at different passage rate (2, 5 and 8%/h; i.e., $EDDM_2$, $EDDM_5$ and $EDDM_8$, respectively). Linear regressions with the highest R^2 were chosen and presented. Relationships were described using R^2, RMSE and regression coefficients. Equations for the prediction of EDDM are given in Table 20. For each passage rate, between two and four equations were significant. Considering the variables used, equations were not consistent between different passage rates. For $EDDM_2$, the equation based on chemical constituents was only tendencially significant. The use of G24 improved the significance of the equation; however, the coefficient of determination was reduced. When G4, G8 and G24 were included, the equation was improved explaining 82% of the variation of $EDDM_2$ value with lower RMSE. For $EDDM_5$, significant equations were not found using chemical constituents, and the better equation was obtained with a combination of starch content and G24 with relative high R^2 and low RMSE (0.86 and 7.40, respectively). For $EDDM_8$, two significant equations using different combinations of chemical constituents were found; however, R^2 were only moderate (0.49 and 0.69). Similar to $EDDM_5$, when starch was combined with G24, the equation was even better, explaining 86% of the variation of $EDDM_8$ with a lower RMSE (10.7). Based on the linear multiple regression analysis, chemical composition or/and *in vitro* gas production have a good potential as predictors of EDDM of DDGS at different passage rates. However, variables used in the equation would depend on the passage rate. For $EDDM_2$, the best equation for prediction is based on *in vitro* gas production parameters. Whereas, for $EDDM_5$ and $EDDM_8$, the best equations include a combination of chemical constituents and *in vitro* gas production.

Table 20 Equations calculated to predict effective degradability of dry matter (EDDM) of dried distillers´ grains with solubles from chemical constituents and *in vitro* gas production (based on results of Chapter 3)

	Regression								
	$EDDM_2$			$EDDM_5$		$EDDM_8$			
Item	1	2	3	1	2	1	2	3	4
Intercept	105.2***	31.3*	22.8*	-5.46	-38.8*	104.7***	68.4**	-23.3	-65.82**
CP	0.82(*)						1.58*		
Sugar	-3.62*								
Starch	-1.13*				-1.06**				-1.35**
$aNDF_{om}$									
Hemic						-1.42*	-1.62**		
ADL						-1.34	-2.64*		
G4			2.94*						
G8			-5.61**						
G24		0.99**	3.74***	1.69**	2.58***			2.01**	3.18***
P_{global}	(*)	**	***	**	***	*	*	**	***
R^2	0.55	0.53	0.82	0.61	0.86	0.49	0.69	0.58	0.86
RMSE	9.18	9.56	3.63	20.6	7.40	39.3	24.1	32.1	10.7

$EDDM_2$, $EDDM_5$ and $EDDM_8$ (%) at passage rate of 2, 5 and 8%/h, respectively. CP= crude protein; $aNDF_{om}$ = neutral detergent fibre; amylase pre-treated, ash free; Hemic = Hemicellulose; ADL = acid detergent fibre; where CP, sugar, starch, $aNDF_{om}$ ADL and hemicelluloses is expressed in g/kg DM.
G4, G8 and G24 = Gas production (ml) estimated through the gas production method (Menke and Steingass, 1988) at 4, 8 and 24 h, respectively.
RMSE = root mean square error.
***P<0.001; **P<0.01; *P<0.05; (*) 0.10<P>0.05; otherwise P>0.10.

Similar to EDDM, it was attempted to predict effective degradability of CP (EDCP) of DDGS based on chemical composition, CP fractions and *in vitro* gas production. EDCP was estimated at different passage rates (2, 5 and 8%/h; i.e., $EDCP_2$, $EDCP_5$ and $EDCP_8$, respectively). EDDM was additionally used in the equations. Similar statistical analyses like those for estimation of EDDM were considered. Equations for prediction of EDCP of DDGS are shown in Table 21. Similar to EDDM predictions, different equations were simultaneously significant and equations were not consistent between different passage rates resulting significant either using variables of chemical constituents, EDDM or gas production as well as combinations. For estimation of $EDCP_2$, the equation based on CP fraction A was significant but with moderate coefficient of determination (0.68). Other CP fraction did not deliver siginificant equations. The use of cellulose and G48 improved the accuracy of the prediction, explaining 83% of the variation in $EDCP_2$. $EDCP_5$ was only possible to be predicted based on $EDDM_5$ with a relative good R^2 (0.74); however, the RMSE was relatively high (62.6). $EDCP_8$ was either possible to be significantly estimated based on $EDDM_8$ (R^2 = 0.78) or in a better way using cellulose and $EDDM_8$ (R^2 = 0.85). The use of cellulose and G48 improves the accuracy of the prediction. In terms of coefficient of determination and RMSE, when considering the parameters starch, cellulose and G48, the equation was even more

suitable for predicting $EDCP_8$, explaining 91% of the variation of EDCP. Protein fractions did not deliver significant equations neither for prediction of $EDCP_5$ nor for $EDCP_8$. Based on the linear multiple regression analysis, chemical composition, protein fractions, *in vitro* gas production and/or EDDM have a good potential as predictors of EDCP of DDGS at different passage rates. However, variables used in the equation would depend on the target passage rate or feedstuff under study. Umucalilar *et al*., (2002) estimated $EDDM_5$ of grains from *in vitro* gas production at different incubation times and found even lower correlations (R^2 < 0.18).

Table 21 Equations calculated to predict effective degradability of crude protein (EDCP) of dried distillers´ grains with solubles from chemical constituents, protein fractions and *in vitro* gas production (based on results of Chapter 3)

	Regression						
	$EDCP_2$		$EDCP_5$	$EDCP_8$			
Item	1	2	1	1	2	3	4
Intercept	58.4****	-72.2**	-56.2*	-50.5*	-62.02**	-190.3***	-206.4***
Starch							-1.29*
Cellulose		2.51***			1.59$^{(*)}$	5.30***	4.54***
A	0.10***						
G48		2.42***				3.91***	4.51***
$EDDM_2$							
$EDDM_5$			1.82***				
$EDDM_8$				1.77***	1.67***		
P_{global}	***	***	***	***	***	***	***
R^2	0.68	0.83	0.74	0.78	0.85	0.86	0.91
RMSE	6.04	17.8	62.6	65.6	46.1	42.4	26.4

$EDCP_2$, $EDCP_5$ and $EDCP_8$ (%) at passage rate of 2, 5 and 8%/h, respectively.
$EDDM_2$, $EDDM_5$ and $EDDM_8$ (%) at passage rate of 2, 5 and 8%/h, respectively.
Starch and cellulose expressed in g/kg DM.
A, protein fractions (g/kg CP) according to the Cornell Net Carbohydrate and Protein System (CNCPS).
G48 = Gas production (ml) estimated through the gas production method (Menke and Steingass, 1988) at 48 h.
RMSE = root mean square error.
****P<0.0001; ***P<0.001; **P<0.01; *P<0.05; $^{(*)}$ 0.10<P>0.05; otherwise P>0.10.

6.3 INNOVATIVE ASPECTS OF EVALUATION AND PREDICTION OF PROTEIN VALUES OF FEEDS FOR RUMINANTS

Nutrient composition of feeds is the basis for the evaluation of the nutritive value of feedstuffs and is determined through chemical analysis. However, this alone does not provide all the information required to determine the feed value of a feedstuff or to precisely formulate rations for dairy cows. Besides proximal analyses, determination of UDP, utilizable CP (uCP) and their digestibility are relevant. *In vivo* studies are costly and time consuming. Therefore, special attention should be turned to the potential and limits of laboratory methods for the determination of protein values, because such methods compared to *in vivo* ones, may be used for easy routinely procedures (Steingass and Südekum, 2012).

6.3.1 ESTIMATION OF UDP

Rumen degradable protein (RDP) and UDP fractions of dietary protein are important considerations in formulating diets for dairy cattle, especially for high producing dairy cows (Schingoethe *et al*., 2009). The development of performance of dairy cows requires an increasing supply of UDP (Rodehutscord, 2012). UDP is one of the most important key variables used in many protein evaluation systems for ruminants for the evaluation of protein value of a feed. In Chapter 3, estimated parameters of *in situ* ruminal kinetic and UDP fractions of DDGS with a passage rate of 8%/h are given (UDP_8) (see Table 5). However, this rumen outflow rate corresponds to a feeding level of high yielding cows. In addition, information of UDP values for lower feeding levels, those like for dry cows and fattening cattle or low yielding dairy cows is always required for ration formulation purposes. Moreover, when different passage rates are considered, the protein value of a feed is not constant (Steingass and Südekum, 2012). Therefore, Table 22 additionally shows the UDP values of DDGS at passage rates of 2 and 5%/h (i.e., UDP_2 and UDP_5, respectively). UDP values varied widely within each passage rate. UDP values ranged from 7.2 to 40.2% and from 7.9 to 54.6% of CP for UDP_2 and UDP_5, respectively. UDP tended to be higher for corn-DDGS, followed by wheat-DDGS and blend-DDGS for both passage rates. UDP values of DDGS seem to be related to the degradability of their respective grain substrates (Schingoethe, 2006; Nuez-Ortín and Yu, 2009). However, other factors like processing methods which influence ruminal degradation of CP and consequently UDP must be considered. If UDP value of DDGS is usually high, it may be advisable to check for heat damaged indigestible protein (Schingoethe *et al*., 2009).

UDP is commonly calculated on the basis of procedures, which require rumen-fistulated animals and the method is time consuming and labour intensive. Prediction of protein value of feeds on the basis of simple methods may quickly and cost-effectively deliver information. Therefore, as an alternative method was proposed the prediction of UDP values of DDGS based on the protein fractions according to the CNCPS. In Chapter 3, correlations and estimation of UDP values of DDGS from proximate constituents and protein fractions were presented and discussed. Results were calculated for a passage rate of 8%/h. In addition, correlation coefficients between UDP, and chemical constituents and protein fractions at passage rates 2 and 5%/h are given in Table 23.

Table 22 Estimated UDP of distillers' dried grains with solubles (DDGS) at different passage rates based on parameters of *in situ* ruminal kinetic (mean with standard deviation) (based on results of *in situ* stuy of Chapter 3)

	DDGS samples												
		Corn		Wheat					Barley	Blends			
Item	1	2	3[1]	4[1]	5[1]	6	7	8	9	10[2]	11[3]	12[4]	13[5]
UDP_2	40.2	37.5	36.3	37.7	17.1	18.6	25.9	25.6	14.4	22.7	7.2	23.7	18.3
s.d.	2.62	1.84	1.87	1.57	2.00	0.99	1.25	1.10	0.39	1.19	0.54	1.56	0.56
UDP_5	54.3	52.9	54.6	53.0	21.7	28.8	34.2	35.9	17.6	28.3	7.9	25.1	27.4
s.d.	1.38	1.26	1.90	1.49	1.60	1.75	1.79	1.12	0.59	0.96	0.51	1.33	0.53

[1] Not pelleted; [2] wheat:barley:corn:sugar beet syrup = unknown proportions; [3] wheat:barley malt = 85:15; [4] corn:barley malt = 85:15; [5] wheat:corn:triticale = 68:25:7; [6] Calculated from the fitted equation $P = a + b\ (1\text{-}e^{-ct})$, where P = degradation after t hours, a = rapidly degradable fraction, b = potentially degradable fraction, c = rate of degradation of b fraction and t = time.

UDP = undegradable CP (% of CP), calculated as 100 – ED, ED calculated from the equation $a + [(b \times c)/(c + k)]$ according to rate of passage 2, 5 and 8%/h (i.e., UDP_2 and UDP_5, respectively

Significance of correlation coefficients between UDP and chemical fractions varied among passages rates. EE was not correlated to UDP_8; however, it was tendencially significant and *r* increased when rumen outflow was reduced. Similar was for NDiN which was not correlated with UDP at UDP_5 and UDP_8 but was at the lowest passage rate (UDP_2) (r = 0.57). For CF, it was the other way around, correlation was reduced when reducing rumen outflow rate and was not significant for UDP_2. Similar to results at passage rate 8%/h (see Table 6), protein fraction A and B3 were correlated to UDP_5 and UDP_2. However, the coefficient of correlation between B3 and UDP at the lowest passage rate was numerically reduced (r = 0.56). Whereas, CP, $aNDF_{om}$, ADF_{om}, ADL and ADiN and protein fractions B1, B2 and C were not correlated with UDP values at all passages rates.

Equation for the prediction of UDP values from nutritional composition and protein fractions at passage rates 2 and 5%/h are show in Table 24. Similar to the result of correlation coefficients, significance of equations differed among passages rates. The equations based on the proximate constituents (EE and CF) were significant at passage rates 2 and 5%/h. However, the moderate coefficient of determination and high RMSE between EE and CF, and UDP values, indicate that these chemical fractions are not reliable for UDP prediction for passage rates 2 and 5%/h (R^2 = 0.49, RMSE = 24.7 and r = 0.52, RMSE = 36.5, respectively). Similar result was found for UDP_8. Similar to UDP_8, prediction equation of UDP_5 was better when considering parameters B3 + C and NDiN x 6.25/(A+B1) with higher coefficient of determination (0.95) and lower RMSE (11.5). However, when these variables were considered to predict UDP_2 values, coefficient of determination was reduced and RMSE was incremented, reducing its capability of prediction. The use of fractions B2, B3 and C resulted in improved accuracy of prediction, explaining 94% of the variation of the UDP value, with a lower RMSE. Which protein fraction can be used for prediction of the UDP value will differ among passage rate, since the rate and extent of ruminal degradation differs among the different protein fractions. Moreover, since proportion of protein fractions varies between feedstuffs, which protein fraction can be used for UDP predictions will also depend on the feed under study. Therefore, the discussed equations for prediction of UDP are specific for DDGS. Shannak *et al*. (2000) and Kirchhof (2007) predicted satisfactorily UDP values for concentrates and forages, respectively, from protein fractions and NDF. As demonstrated in chapter 5 these equations were not at all suitable for prediction of UDP of DDGS. However, parameters used between equation (see Table 7) varied widely, probably due to variation in CP fractions among concentrate, forages and DDGS. The equations obtained show that protein fractionation have also good potential as predictor of UDP in DDGS at low ruminal

outflows rates (2 and 5%/h). Shannak *et al*. (2000) and Kirchhof (2007) obtained also reliable equation for prediction of UDP in concentrates and forages at low rumen outflows.

Table 23 Correlation coefficient (r) between UDP and chemical fractions considering different passage rates (based on chemical composition and protein fractions shown in Chapter 3)

Item	UDP_2	UDP_5	UDP_8
Chemical profiles (g/kg DM)			
Crude protein	0.15	0.20	0.24
Ether extract	0.51$^{(*)}$	0.48$^{(*)}$	0.44
Crude fibre	-0.46	-0.52$^{(*)}$	-0.55*
$aNDF_{OM}$	0.20	0.21	-0.39
ADF_{OM}	0.01	-0.08	-0.09
ADL	0.02	-0.05	-0.06
NDiN	0.57*	0.55*	0.54$^{(*)}$
ADiN	0.27	0.16	0.12
Protein fractions (g/kg CP)[1]			
A	-0.82***	-0.82***	-0.83***
B1	-0.19	-0.22	-0.20
B2	0.14	0.19	0.22
B3	0.56*	0.67*	0.70**
C	0.24	0.11	0.06

UDP = undegradable CP (g/kg DM) with a rate of passage of 2, 5 and 8 %/h (i.e., UDP_2, UDP_5 and UDP_8, respectively); $aNDF_{om}$ = neutral detergent fibre, amylase pre-treated, ash free; ADF_{om} = acid detergent fibre, ash free; ADL = acid detergent lignin; NDiN = neutral detergent insoluble N; ADiN = acid detergent insoluble nitrogen.
[1] According to the Cornell Net Carbohydrate and Protein System (CNCPS).
***P < 0.001; **P < 0.01, *P < 0.05; $^{(*)}$ 0.10 < P> 0.05; otherwise P > 0.10.

Table 24 Equations calculated to predict UDP at passage rates 2 and 5%/h from nutrient composition and protein fractions (based on chemical composition and protein fractions showed in Chapter 3)

	Regression							
	UDP_2				UDP_5			
	1	2	3	4	1	2	3	4
ßo	115.2*	202.4***	199.9***	-224.2***	177.3*	223.7**	-337.8***	35.6*
EE	4.49*				6.55*			
CF	-8.57$^{(*)}$				-14.36*			
A			-0.43***			-0.56***		
B2			-0.17**	0.28***		-0.20*	0.39***	
B3			0.06	0.48***		0.22†	0.77***	
C				0.44***			0.59***	
ß1								-0.0003*
ß2								107.4***
ß3		-29.57***						
ß4		-0.0008**						
P_{global}	0.03	***	***	***	0.03	***	***	***
R^2	0.49	0.89	0.90	0.94	0.52	0.90	0.94	0.95
RMSE	24.7	11.2	11.5	9.23	36.5	17.5	14.1	11.5

UDP = undegradable CP (g/kg DM) with a rate of passage of 2 and 5 %/h (UDP_2 and UDP_5, respectively); EE = ether extract; CF = crude fibre; A, B2, B3 and C are protein fractions (g/kg CP) according to the Cornell Net Carbohydrate and Protein System (CNCPS).
ßo, intercept; ß1, (B3+C) x A ; ß2, NDiN x 6.25/(A + B1); ß3, CP/NDiN x 6.25; ß4, CP x (A + B1).
RMSE = root mean square error
***P<0.001; **P<0.01; *P<0.05; $^{(*)}$ <0.10P>0.05; otherwise P > 0.10

6.3.2 ESTIMATION OF UCP

A very important extension of UDP determination as feed value of feedstuffs considers the knowledge and quantification of uCP. This is of great significance and represents an important key variable of the German Protein Evaluation Systems. The uCP represents the CP reaching the duodenum and is the sum of UDP and microbial CP (MCP). Ruminal microorganisms are the most important supplier of protein to the small intestine, accounting for 50 to 80% of total absorbable protein (Storm and Ørskov, 1983). However, in most of the Protein Systems, UDP and MCP are estimated separately (Zhao and Lebzien, 2000). The German Protein System (GfE, 2001) estimates uCP based on CP, UDP and ME content of feed. However, the equations do not consider that UDP and uCP values are also influenced by passage rates (Steingass and Südekum, 2012). The determination of uCP was suggested to be more accurate than the separate determination of UDP and MCP (Lebzien *et al.*, 1996) since it gives a direct estimate of both CP fractions at the duodenum (Edmunds *et al.*, 2012). However, direct determination of uCP using *in vivo* trials is expensive, labour intensive and time consuming.

Regarding to DDGS, it seems that there is no available information published in scientific journals considering the estimation and the variation of uCP content of European sources. Therefore, the uCP (g/kg DM) content of DDGS were estimated according to GfE (2001): [11.93 - (6.82 x (UDP/CP))] x ME + 1.03 x UDP, for DDGS with EE ≤ 70 g/kg DM and, [13.06 - (8.41 x (UDP/CP))] x (ME – MEEE) + 1.03 x UDP, for DDGS with EE > 70 g /kg DM ; where UDP is in g/kg DM and estimated by *in situ* method, CP is in g/kg DM, ME and MEEE (ME without ether extract consideration) is in MJ/kg DM and was calculated through the standard Hohenheim gas test. The uCP values were calculated with passage rates 2, 5 and 8%/h (i.e., uCP_2, uCP_5 and uCP_8, respectively).

The uCP content of DDGS are shown in Table 25. Estimated uCP of DDGS was high and varied considerably within all passage rates. Values averaged 195, 216 and 229 g/kg DM for uCP_2, uCP_5 and uCP_8, respectively. Values ranged from 148 to 231, from 153 to 279 and from 158 to 302 g/kg DM for uCP_2, uCP_5 and uCP_8, respectively. The CV for uCP was high and incremented with incrementing passages rates, resulting in 12.3, 16.6 and 18.3% for uCP_2, uCP_5 and uCP_8, respectively. Estimated uCP_5 of DDGS (216 g/kg DM) was lower to table values of SBM (324 g/kg DM) but similar to RSM and rapeseed cake (219 and 236 g/kg DM, respectively) (DLG, 1997). Regarding to uCP content, DDGS have a good feeding value for ruminants.

6.3.2.1 Correlation and prediction of uCP

Correlation coefficients between calculated uCP at different passage rates and chemical constituents and protein fractions are shown in Table 26. The EE, $aNDF_{om}$, and ADiN were not correlated with uCP at all rates of passage. The CP and ADF_{om} tended to correlate only with uCP_2. The CF and NDiN were negatively correlated with uCP at all rates of passage. Protein fractions B1, B2 and C had no relationship with uCP at all rates of passage, whereas protein fraction B3 was correlated with uCP_5 and uCP_8 (r = 0.63 and 0.65, respectively). Protein fraction A was negatively correlated with uCP at all rate of passage ($r <$ -0.73).

Innovative *in vitro* approaches have been developed for estimation of uCP. The *in vitro* method of Zhao and Lebzien (2000) based on the classic two steps method of Tilley and Terry (1963) was proved to be related to *in vivo* data for uCP determination in feeds. Steingass *et al.* (2001) proposed the *in vitro* modified Hohenheim gas test (moHGT), based on the standard Hohenheim gas test (Menke and Steingass, 1988) and modifications according to Raab *et al*. (1983). The difference to the previous method is that in the moHGT uCP is not directly determined as precipitant in the incubation residue but calculated by subtracting NH_3-N from total N of the *in vitro* batch (Melesse *et al*., 2013) and only requires the knowledge of CP of feed prior to incubation (Edmunds *et al*., 2012).

The method has shown high potential use. The moHFT method showed promising results when estimating uCP of concentrates (Leberl *et al*., 2007), forages and grasses (Edmunds *et al*., 2012, Leberl and Schenkel, 2012), RSM (Steingass *et al*., 2013), brewer´s grains (Seifried *et al*., 2009), alfalfa (Leberl *et al*., 2010) and different silages, protein feeds, grains and mixed rations (Richardt, 2012). Although *in vitro* methods cannot precisely simulate *in vivo* situation, the proposed method is sound to be reliable for a quick characterization and prediction of uCP of feedstuffs. Howerer, it seems that the method still stays in a state that needs validation with *in vivo* data to be later on routinely and reliable used in laboratories for uCP estimation (Edmunds *et al*., 2012; Richardt, 2012). Even when *in vitro* methods may release quickly valuable information, it requires the use of rumen fistulated animals. Looking for alternative approaches when rumen fistulated animals are not available is imperative. One proposed technique relies on the basis of the protein fractionation according to the Cornell Net Carbohydrate and Protein System (CNCPS).

Table 25 Estimated utilizable CP (uCP) and CP content of distillers dried grains with soluble (DDGS) at different passage rates with means, standard deviation (SD) and range (Min., Max.)

	DDGS sources																
	Corn			Wheat					Barley	Blends							
	1	2	3[1]	4[1]	5[1]	6	7	8	9	10[2]	11[3]	12[4]	13[5]	Mean	SD	Min.	Max
CP[2]	281	274	311	339	320	302	335	358	247	312	331	273	349	310	34	247	358
uCP_2	208	201	212	237	181	191	206	219	148	197	165	177	189	195	24	148	237
uCP_5	237	232	256	279	193	213	229	248	153	210	167	180	213	216	36	153	279
uCP_8	252	247	275	302	203	230	244	268	158	221	169	183	231	229	42	158	302

[1] Not pelleted; [2] wheat:barley:corn:sugar beet syrup in unknown proportions; [3] wheat:barley malt = 85:15; [4] corn:barley malt = 85:15; [5] wheat:corn:triticale = 68:25:7.

[2] CP = crude protein; uCP = estimated at passage rate 2, 5 and 8%/h (uCP_2, uCP_5 and uCP_8, respectively); where uCP estimated according to GfE (2001): [11.93 - (6.82 x (UDP/CP))] x ME + 1.03 x UDP, for DDGS with EE ≤ 70 g/kg DM and, [13.06 - (8.41 x (UDP/CP))] x (ME – MEEE) + 1.03 x UDP, for DDGS with EE > 70 g/kg DM ; where UDP is in g/kg DM and estimated by *in situ* method, CP is in g/kg DM, ME and MEEE (ME without ether extract consideration) is in MJ/kg DM and was calculated through the standard Hohenheim gas test (menke and Steingass, 1988). Calculations based on results presented in Chapter 3.

Table 26 Correlation coefficient (*r*) between calculated utilizable CP (uCP) and chemical composition and protein fractions (based on chemical composition and protein fractions showed in Chapter 3)

Item	uCP_2[1]	uCP_5	uCP_8
Nutrient [g/kg DM]			
Crude protein	0.50(*)	0.45	0.46
Ether extract	0.42	0.25	0.23
Crude fibre	-0.66*	-0.64*	-0.66*
$aNDF_{om}$	0.05	0.12	0.13
ADF_{om}	0.49(*)	0.43	0.40
ADL	0.59*	0.49(*)	0.47
NDiN	0.69**	0.65*	0.63*
ADiN	0.36	0.24	0.20
Protein fractions[2] [g/kg CP]			
A	-0.73**	-0.77**	-0.77**
B1	-0.21	-0.24	-0.21
B2	0.09	0.14	0.17
B3	0.50	0.63*	0.65*
C	0.26	0.14	0.09

$aNDF_{om}$ = neutral detergent fiber, amylase pre-treated, ash free; ADF_{om} = acid detergent fiber, ash free; ADL = acid detergent lignin; NDiN = neutral detergent insoluble N; ADiN = acid detergent insoluble nitrogen.
[1] uCP = calculated with rate of passage of 2, 5 and 8 %/h (uCP_2, uCP_5 and uCP_8, respectively) and expressed in g/kg DM
[2] According to the Cornell Net Carbohydrate and Protein System (CNCPS).
**$p < 0.01$, *$p < 0.05$; (*) $0.10 < p > 0.05$; otherwise $p > 0.10$

Protein fractions have been previously proved to be correlated to *in vitro* estimated uCP (Zhao and Cao, 2004) and to duodenal utilizable true protein (uTP) (Zhao *et al*., 2008). Therefore, it was attempted to estimate the uCP values of DDGS based on protein fractions. Equation for prediction of calculated uCP at different passage rates from protein fractions are resumed in Table 27. The equations based on CP fraction A were significant for the estimation of uCP at all passage rates. However, equations shown overall only moderate levels of coefficient of determination (0.54, 0.59 and 0.60; i.e., uCP_2, uCP_5 and uCP_8, respectively). The use of protein fractions A and B2 improved the accuracy of the prediction, explaining 75, 76 and 74% of the variation of uCP_2, uCP_5 and uCP_8, respectively. In terms of coefficient of determination and RMSE, when considering protein fraction A, B2 and C, the equation was even more suitable for uCP8 (R^2 = 0.85, RMSE = 19.0). The present calculations show that uCP values of DDGS was significantly correlated with CP fractions (A, B2, C) in a multiple way. CP fractions have a good potential as predictors of uCP in DDGS, and a similar approach may be followed for other types of protein feedstuffs.

Table 27 Equations calculated to predict utilizable CP (uCP) from protein fractions considering different passage rates fractions (based on chemical composition and protein fractions showed in Chapter 3)

	Regression						
	uCP_2		uCP_5		uCP_8		
	1	2	1	2	1	2	3
ßo	228.7***	297.6***	270.4***	366.8***	293.6***	398.3***	476***
A	-0.21**	-0.31**	-0.34**	-0.48***	-0.40**	-0.55***	-0.48***
B2		-0.13*		-0.18*		-0.20*	-0.254**
C							-6.14*
P_{global}	**	***	**	***	**	***	***
R^2	0.54	0.75	0.59	0.76	0.60	0.74	0.85
RMSE	16.6	12.8	24.3	19.5	28.1	23.0	19.0

uCP (g/kg DM) = estimated with a rate of passage of 2 and 5 %/h (uCP_2, uCP_5 and uCP_8, respectively).
ßo, intercept; A, B2 and C are protein fractions (g/kg CP) according to the Cornell Net Carbohydrate and Protein System (CNCPS).
RMSE = root mean square error
***P<0.001; **P<0.01; *P<0.05

6.3.3 INTESTINAL DIGESTIBILITY (ID) AND AA CONTENT OF UDP

The NRC (2001) model assumes UDP digestibility of 80% for DDGS, whereas in the German Protein System UDP digestibility in the intestine of 85% is generally used for all feedstuffs (GfE, 2001). It was proved in the Chapter 4, that these assumptions overestimate the intestinal digestibility of UDP of DDGS. Similar results were found for RSM (Steingass *et al*., 2013). Additionally, there is a necessity to consider the AA composition of the CP of the feed and the ruminal degradation of individual AA (Rodehutscord, 2012). This will be even more important in the future, due to the necessity of using more efficiently our scarce feed protein resources and the continuous improvement of animal performance.

6.3.3.1 *IN VITRO* METHOD

Since *in vivo* determination of intestinal digestibility of protein fractions in ruminants is expensive, labour-intensive and time consuming, a need for alternative *in vitro* methods to predict digestibility has emerged. The method of Boisen and Fernández (1995) was proposed to be suitable and was used to characterize the intestinal digestibility of UDP of DDGS (Chapter 4). However, the question whether the protein digestibility is related to the digestibility of individual AA still remains open. Since DDGS are exposed to heating during drying process, it may be hypothesized that AA would be resistant to enzymatic breakdown reducing in consequence the digestibility of individual AA, especially lysine. Thus, CP will not reflect the digestibility of individual AA. Therefore, assessment of the digestibility of individual AA of UDP of DDGS was suggested to complement the present study. The method of the *in vitro* pepsin-pancreatin solubility (Boisen and Fernández, 1995) was primarilly

conceived to estimate the intestinal digestibility of CP of feedstuffs. However, an extension of this approach might predict the digestibility of individual AA. Similar to calculation of digestibility of CP, *in vitro* intestinal digestibility of AA may be calculated by difference of AA content from weighed ruminally-incubated materials and the AA remained after *in vitro* PPS incubation. Future works may focus using this method for prediction of ID of individual AA. However, some limitation should be considering with such an extension. Normally around 500 mg of the feed are *in vitro* incubated, and depending on the factor of digestibility, around 50 and 250 mg are revovered for CP analyses. Since many repetitions would be required to achieve the required mass residue for DM, CP and AA analyses (around 20g) after the *in vitro* incubation, this extension will again mean a labour-intensive and time consuming procedure. However, it may probably reduce experiment cost compared to *in vivo* technique. Another option may consider a macro-*in vitro* incubation, where higher amount of the respective feed is incubated in a higher volume of pepsin or pancreation containing solutions. Consequently reducing the number of repetions to obtain sufficient feed residue for analyses and simplifying again the method. Finally, if such an extension is feasible, validation of *in vitro* digestibility of AA with *in vivo* data is required.

6.3.3.2 COLORIMETRIC METHOD

A colorimetric procedure was proposed to evaluate CP quality and to predict amino acid digestibility in DDGS. The extent of drying and temperature that undergoes DDGS may significantly affect the nutrients content, damage considerably portion of the protein and affect digestibility of nutrients, resulting as well in darker color products. Therefore, darker color may probably indicate overheating during dry process. Color scores are determined using a Lab-Scan XE spectrocolorimeter, asessing the lightness (L), redness (a), and yellowness (b) color scales (McNaughton *et al*., 1981). The method is called Hunterlab color score L (Hunter Associates Laboratory, 2002). The Hunterlab L score ranges from 0 (black) to 100 (white). Values a and b indicate redness and yellowness, respectively; higher scores of a or b indicates a greater degree of each color (Cromwell *et al*., 1993). In addition, the same authors found color scores of DDGS ranging from very light to very dark and odor scores from normal to burnt or smoky. Color was highly related to nutritional properties of DDGS, in that dark-colored DDGS was lower in nutritional value than light-colored DDGS in terms of performance of chickens (Cromwell *et al*., 1993). They also found higher lysine and lower ADiN x 6.25 concentrations in the lightest-colored DDGS. Similarly, McNaughton *et al*. (1981) reported similar relationship between color and performance of broilers fed heated

soybean meal. Pahm *et al*. (2009) found that Hunterlab L scores may be used to estimate the concentration of bio-available lysine in DDGS (R^2 = 0.90) for chicks. Harty *et al*. (1998) and Kleinschmit *et al.* (2007) found no correlation between color scores and ADiN x 6.25 concentration in DDG. Furthermore, Kleinschmit *et al*. (2007) suggested that using color as an indicator of DDGS CP quality, in relation to ADiN x 6.25, may be appropriate only when the feed contains a high concentration of ADiN x 6.25, and additionally proposed that it may have been more appropriate to regress with nonlinear model to investigate if color score may have a curvilinear relationship. However, it is important to emphasize, that those evaluations were carried out in corn-DDGS. Since other grains have different colors, the latter may influence the color of the DDGS obtained after grain processing. Therefore, it may not be reliable when characterizing the color score of DDGS from different grains like those produced under EU conditions. Although colorimetric procedure shows some contradictory results, it is an alternative parameter to evaluate DDGS quality and may give evidence of heat damage. But use of this approach would be only recommendable with same DDGS type.

6.4 GENERAL CONCLUSIONS AND OUTLOOK

The chemical composition, energy and protein values of DDGS differed among samples studied. These variations were due to large variations in raw materials and their combinations in different proportions, and probably also due to different processing methods between ethanol production plants.

The variation of *in vitro* gas production values, IVDOM and EM may be explained by differences in nutrient composition among DDGS samples. Although the energy value of DDGS are expected to be inferior than of the corresponding original grains due to an increase in fibre fractions and reduction in starch after ethanol production, DDGS studied were proved to be not so much different or similar to their respective raw materials when compared to tabular values, confirming the good quality of DDGS as energy source for ruminants.

The CP fractionation based on the CNCPS was shown to have a good potential as predictors of UDP value of DDGS. Similar results were published by other authors with other feedstuffs. The good potential of protein fractions relay in the fact that they have different rates and extents of ruminal degradation. The CP fractionation provides a better characterization of the protein value than only the CP content. This study also suggests that protein fractionation may be successfully used to predict UDP of other protein feedstuffs. However, specific studies and adaptations should be carefully considered for each type of

feedstuff. Moreover, the use of protein fractionation is encouraged to be used as a standard for nutrient characterization of feedstuffs for ruminants.

The UDP value of DDGS was proved to be widely variable. There is a normal perception that DDGS have aroud 40% of UDP of CP. In the German feed tables (DLG) values of UDP are available at passage rates of 5%/h only for corn-DDGS (50%) and barley-DDGS (40%). This study suggests that feed table values may drastically either overestimate or underestimate the UDP value of DDGS. The observed difference of UDP values confirm also the high relevance of this key variable for the characterization of feedstuffs in terms of protein quality, and highlight the necessity for the update of protein values of DDGS in official feed tables.

Although DDGS may be considered as a good source of UDP, PPS values showed that intestinal digestibility of UDP may be highly variable and can be low compared to SBM. In addition, this study suggest that intestinal digestibility of UDP from DDGS is lower compared to values currently used in protein evaluation systems and that the contribution of UDP to the intestinal AA supply may be overestimated. This emphasizes again the need to update the values of UDP digestibility in several protein evaluation systems. Since UDP substantially contributes to the supply of AA at the duodenum especially in high producing dairy cows, the AA composition of UDP and the intestinal digestibility of UDP and individual AA should be important charachteristics considered in the future for the evaluation of feed value of feedstuffs for ruminants.

DDGS was proved to be suitable as a sole protein feed in diets of dairy cows in mid-lactation, especially in terms of DMI milk fat concentration and production. However, differences in milk protein concentration when feeding different DDGS sources indicate a variation in the availability of lysine in UDP of DDGS. Moreover, DDGS sources used in the feeding trial did not reflect the overall variability of nutrient composition and protein and energy values of DDGS already found in previous studies. Therefore, differences in production parameters might be expected when considering more variable sources of DDGS.

Specific information about technical process was not provided by processing plants, and this represents an important limiting factor in the present survey and in further data interpretations. However, this lack of information is also a reality in the feed market. The latter and the variation of nutrient value of DDGS should be of major concern for the ethanol industry and reveal the challenging importance of more standardized products to be used in practice. Ethanol plants should deliver very specific information about production processes

in further studies to better comprenhence the effects of different technologies or/and steps within the production process of ethanol on the feed value of DDGS.

Nutrient content and feed value of DDGS may vary even within the same factory. High variation of nutrient content and feed value of DDGS suggests that ethanol producers should consider more routinely analysis for product specification both from specific batches and not limited only to proximal analysis results. This should be delivered to farmers and to feed producers for monitoring variablitiy, application of management strategies of nutrient variability and adjustment of diets to updated nutrient information.

Variability of feed value of DDGS should be considered when feeding to dairy cows. Strategies to manage this variability in farms may include sampling and analysing more routinely the shipments, demanding ethanol producers for more detailed and updated nutritional information, limiting or reducing the proportion of DDGS in the diets and when possible buying DDGS from the same producer.

More routinely analyses of feeds to manage the variability may increment the costs. Therefore, scientists and nutritionists should put more emphasis in looking for easier, cost-effective methods to quickly deliver information of characterization of feed value of feedstuffs for ruminants.

REFERENCES

Belyea R, Eckhoff S, Walling M and Tumbleson M 1998. Variability in the nutritional quality of distillers solubles. Bioresource Technology 66, 207-212

Cromwell GL, Herkelman KL and Stahly TS 1993. Physical, chemical, and nutritional characteristics of distillers dried grains with solubles for chicks and pigs. Journal of Animal Science 71, 679-686

DLG (Deutsche Landwirtschafts-Gesellschaft),1997: DLG Futterwerttabellen-Wiederkäuer. 7., erweiterte und überarbeitete Auflage, Herausgeber Universität Hohenheim-Dokumentationsstelle, DLG-Verlag, Frankfurt.

Edmunds B, Südekum KH, Spiekers H, Schuster M and Schwarz FJ 2012. Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. Animal Feed Science and Technology 175, 106-113

GfE, 2001: Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder. DLG-Verlag, Frankfurt.

Gordon MD 2008. Animal Nutrition Science. Cambridge Univeryity Press. Cambridge, UK. 302 p.

Harty SR, Akayezu JM, Linn JG and Cassady JM 1998. Nutrient composition of distillers grains with added soluble. Journal of Dairy Science 81(Suppl. 1), 1201

Hristov AN, Hanigan M, Cole A, Todd R, McAllister TA, Ndegwa PM and Rotz A 2011. Ammonia emissions from dairy farms and beef feedlots. Canadian Journal of Animal Science 91, 1-35

Kirchhof S. 2007. Untersuchungen zur Kinetik des ruminalen in situ-Nährstoffabbaus von Grünlandaufwüchsen des Alpenraumes unterschiedlicher Vegetationsstadien sowie von Maissilagen und Heu- ein Beitrag zur Weiterentwicklung der Rationsgestaltung für Milchkühe. Doctoral thesis, University of Kiel, Germany.

Kleinschmit, D.H.; Anderson, J.L.; Schingoethe, D.J.; Kalscheur, K.F.; Hippen, A.R., 2007: Ruminal and intestinal degradability of distillers grains plus solubles varies by source. Journal of Dairy Science 90, 2909–2918

Leberl P, Gruber L, Steingass H and Schenkel H 2007. Comparison of the methods modified Hohenheimer Futterwerttest (moHFT) and Cornell system for determination of nXP-content of concentrates. 16th International Science Symposium on Nutrition of Domestic Animals. Radenci, Slovenia, 8-9 November, pp. 171-176

Leberl P and Schenkel H 2012. Einsatzmöglichkeiten des modifizierten Hohenheimer Futterwerttestes (moHFT) zur Proteinbewertung von Gras- und Leguminosenprodukten. Forum angewandte Forschung in der Rinder- und Schweinefütterung. Verband der Landwirtschaftskammern-Verlag, Bonn. Tagungsband S. 22-26

Lebzien P, Voigt J, Gabel M and Gädeken D 1996. Zur Schätzung der Menge and nutzbaren Rohprotein am Duodenum von Milchkühen. Journal of Animal Physiology and Animal Nutrition 76, 218-223

McDonald P, Edwards RA, Geenhalgh JFD and Morgan CA 2002. Animal Nutrition. Sixth edition. Pearson Education Limited. UK. 693 p

Melesse A, Steingass H, Boguhn J and Rodehutscord M 2013. *In vitro* fermentation characteristics and effective utilisable crude protein in leaves and green pods of Moringa stenopetala and Moringa oleifera cultivated at low and mid-altitudes. Journal of Animal Physiology and Animal Nutrition 97, 537-546

Menke KH, Steingass H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development 28, 7-55

McNaughton JL, Reese FN and Deaton JW 1981. Relationship between color, trypsin inhibitor, contents and urease index of soybean meal and effects on broilers performance. Poultry Science 60, 393-400

NRC (National Research Council), 2001. Nutrient requirement of dairy cattle. 7th revised edition, National Academy Press. Washington, DC

Nuez-Ortín WG and Yu P 2009. Nutrient variation and availability of wheat DDGS, corn DDGS and blend DDGS from bioethanol plants. Journal of Science Food and Agriculture 89, 1754-1761

Pahm AA, Scherer CS, Pettigrew JE, Baker DH, Parsons CM and Stein HH 2009. Standardized amino acid digestibility in cecectomized roosters and lysine bioavailability in chicks fed distillers dried grains with soluble. Poultry Science 88, 571-578

Penner GB and Oba M 2009. Increasing dietary sugar concentration may improve dry matter intake, ruminal fermentation, and productivity of dairy cows in the postpartum phase of the transition period. Journal of Dairy Science 92, 3341-3353

Raab L, Cafantaris B, Jilg T and Menke H 1983. Rumen protein degradation and biosynthesis: A new method for determination of protein degradation in rumen fluid *in vitro*. British Journal of Nutrition 50, 569-582

Richardt W 2012. Erfahrungen und praktische Umsetzung der nXP-Analytik mit Hilfe des modifizierten Hohenheimer futterwerttests (moHFT) und der Rohproteinfraktionierung. Forum angewandte Forschung in der Rinder- und Schweinefütterung. Verband der Landwirtschaftskammern-Verlag, Bonn. Tagungsband S. 27-30

Rodehutscord M 2012.Einsatz von Aminosäuren in der Tierernährung. 24. Hülsenberger Gespräche 2012: Zusatzstoffe in der Ernährung. H.-Wilhelm Schaumann Stiftung-Verlag, Hamburg. (Kurzfassung)

Schwab CG, Bozak CK and Whitehouse NL 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence and methionine limitation. Journal of Dairy Science 75, 3486-3502

Schingoethe DJ 1996. Balancing the amino acid needs of the dairy cow. Animal Feed Science and Technology 60, 153-160

Schingoethe DJ 2006.Utilization of DDGS by cattle. Proccedings of the 27th Western Nutrition Conference. Manitoba, Canada. 19-20 September 2006. pp 61-74

Schingoethe DJ, Brouk MJ and Birkelo CP 1999. Milk production and composition from cows fed wet corn distillers grains. Journal of Dairy Science 82, 574-580

Schingoethe DJ, Kalscheur KF, Hippen AR and Garcia AD 2009. Invited review: The use of distillers products in dairy cattle diets. Journal of Dairy Science 92, 5802-5813

Shannak S, Südekum K-H, Susenbeth A. 2000. Estimating ruminal crude protein degradation with in situ and chemical fractionation procedures. Anim Feed Science and Technology. 85:195-214.

Sniffen CJ, O´Connor JD, van Soest PJ, Fox DG, Russell JB. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J Anim Sci. 70:3562-3577.

Spiehs MJ and Varel VH 2009. Nutrient excretion and odorant production in manure from cattle fed corn wet distillers grains with solubles. Journal of Animal Science 87, 2977-2984

Spiekers H, Nußbaum H and Potthast V 2009. Erfolgreiche Milchviehfütterung. DLG-Verlag. Frankfurt am Main, Germany. 576 p

Steingass H, Kneer G, Wischer G and Rodehutscord M 2013. Variation of *in situ* degradation of crude protein and amino acids and *in vitro* digestibility of undegraded feed protein in rapeseed meals. Animal, doi: 10.1017/S175173111300030X, Published online by Cambridge University Press 11 March 2013

Steingass H, Nibbe D, Südekum KH, Lebzien P and Spiekers H 2001. Schätzung des nXP-Gehaltes mit Hilfe des modifizierten Hohenheimer Futterwerttests und dessen Anwendung

zur Bewertung von Raps- und Sojaextraktionsschroten. VDLUFA-Kongress 113, Berlin, Kurzfassungen der Vorträge, 114 (Abstract)

Steingass H and Südekum KH 2012. Proteinbewertung beim Wiederkäuer-Grundlagen, analytische Entwicklungen, Ausblick. Forum angewandte Forschung in der Rinder- und Schweinefütterung. Verband der Landwirtschaftskammern-Verlag, Bonn. Tagungsband S. 11-21

Storm E and Ørskov 1983. The nutritive value of rumen microorganisms in ruminants. 1. Large-scale isolation and chemical composition of rumen microorganisms. British Journal of Nutrition 50, 463-470

Umucalilar HD, Coskun B and Gülsen N 2002. *In situ* rumen degradation and *in vitro* gas production of some selected grains from Turkey. Journal of Animal Physiology and Animal Nutrition 86, 288-297

Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74: 3583-3597

Walter LJ, McAllister TA, Yang WZ, Beauchemin KA, He M and McKinnon JJ 2012. Comparison of wheat or corn dried distillers grains with solubles on rumen fermentation and nutrient digestibility by feedlot heifers. Journal of Animal Science 90, 1291-1300

Weiss B and St-Pierre N 2012. Trying to make sense of feed composition data: within farm variation. The Mid-South Ruminant Nutrition Conference. Grapevine, Texas. pp 33-44

Weiss WP 1998. Estimating the available energy content of feeds for dairy cattle. Journal of Dairy Science 81, 830-839

Xu S, Harrison JH, Chalupa W, Sniffen C, Julien W, Sato H, Fujieda T, Watanabe K, Ueda T and Suzuki H 1998. The effect of ruminal bypass lysine and methionine on milk yield and composition of lactating cows. Journal of Dairy Science 81, 1062-1077

Zebeli Q, Aschenbach JR, Tafaj M, Boguhn J, Ametaj BN and Drochner W 2012. Role of physically effective fiber and estimation of dietary fiber adecuacy in high-producing dairy cattle. Journal of Dairy Science 95, 1041-1056

CHAPTER 7

SUMMARY

The increasing demand of energy together with the implementation of the European Program for the use of energy from renewable sources are favourable scenarios to increment the ethanol production in the coming years in the EU. Ethanol production yields dried distillers´ grains with soluble (DDGS) as the main by-product, a valuable feedstuff for ruminants. A great number of publications mainly form USA and Canada has demonstrated the great variability of the feed value of corn-DDGS, the main by-product from ethanol production in these countries. In the EU, different and diverse technological conditions predominate and little was investigated to evaluate the feed value of DDGS. The variability of feeding value in conjunction with expected increase of DDGS production demands for further and more specific characterization of this by-product in the EU. Therefore, a project was conceived to characterize the chemical composition and evaluate the protein and energy value for ruminants of DDGS from different European countries. Thirteen samples of DDGS originating from wheat, corn, barley, and blends of different substrates were used.

In the first study, the objective was to characterize variations in the composition and nutritive value of DDGS, and to estimate the undegradable crude protein (UDP) in DDGS. The rumen degradation of crude protein (CP) was determined using the nylon bag technique. Samples were incubated for 0, 1, 2, 4, 8, 16, 32, and 72 h, and *in situ* degradation kinetics were determined. UDP was estimated using a passage rate of 8 %/h. *In vitro* gas production was measured to estimate the metabolizable energy (ME), net energy for lactation (NE_L) and *in vitro* digestibility of organic matter (IVDOM). Chemical profiles varied among samples (in g/kg dry matter (DM) ± standard deviation, the values were 310 ± 33 CP, 86 ± 37 ether extract, 89 ± 18 crude fibre, 408 ± 39 neutral detergent fibre, 151 ± 39 acid detergent fibre, and 62 ± 31 acid detergent lignin), as well as in protein fractions according to the Cornell Net Carbohydrate and Protein System (in g/kg CP, the values were 161 ± 82 for fraction A, 24 ± 11 for fraction B1, 404 ± 105 for fraction B2, 242 ± 61 for fraction B3, and 170 ± 87 for fraction C). ME, NE_L (MJ/kg DM) and IVDOM (%), also varied among samples: 12.1 ± 0.59, 7.3 ± 0.39, and 72.5 ± 4.30, respectively. The *in situ* rapidly degradable CP fraction (a) varied from 10.2 to 30.6%, and the potentially degradable fraction (b) averaged to 66.8%. UDP varied from 8.6 to 62.6% of CP. This first study suggests significant variations in composition and nutritive value among different sources of DDGS. UDP could be predicted on the basis of analysed CP fractions, but the accuracy of UDP prediction improved upon the inclusion of neutral-detergent insoluble nitrogen, explaining 94% of the variation in the UDP values. To conclude, chemical protein fractions may be used to predict the UDP values of DDGS and the

variability in the protein fractions of DDGS should be considered when formulating diets for dairy cows.

To provide additional information on the nutritional value of DDGS, a second study was carried out to determine and compare the *in situ* ruminal degradation of CP and amino acids (AAs) of DDGS and to characterize the *in vitro* pepsin-pancreatin solubility of CP (PPS) from dietary DDGS (d-DDGS) and DDGS residue (DDGS-r) obtained after 16-h ruminal incubation. The rumen degradation of AAs and CP was determined using nylon bag incubations in the rumen of cows. Lysine and methionine content of d-DDGS varied from 1.36 to 4.00 and 1.34 to 1.99 g/16 g N, respectively. The milk protein score (MPS) of d-DDGS was low and ranged from 0.36 to 0.51, and lysine and isoleucine were estimated to be the most limiting AAs in d-DDGS and DDGS-r. DDGS-r contained slightly more essential AAs than did the d-DDGS. Rumen degradation of CP after 16 h varied from 44% to 94% between DDGS samples. Rumen degradation of lysine and methionine ranged from 39% to 90% and from 35% to 92%, respectively. Linear regressions showed that ruminal degradation of individual AAs can be predicted from CP degradation. The PPS of d-DDGS was higher than that of DDGS-r and it varied from 70% to 89% and from 47% to 81%, respectively. There was no significant correlation between the PPS of d-DDGS and PPS of DDGS-r (R^2 = 0.31). The estimated intestinally absorbable dietary protein (IADP) averaged 21%. Moderate correlation was found between the crude fibre content and PPS of DDGS-r (R^2 = 0.43). This study suggests an overestimation of the contribution of UDP of DDGS to digestible protein supply in the duodenum in currently used protein evaluation systems. More research is required and recommended to assess the intestinal digestibility of AAs from DDGS.

Finally, in a third study, three sources of DDGS were evaluated in diets of mid-lactating dairy cows on milk production and milk composition and on digestibility in sheep. DDGS from wheat, corn and barley ($DDGS_1$), wheat and corn ($DDGS_2$) and wheat ($DDGS_3$) were studied and compared with a rapeseed meal (RSM). RSM and DDGS were characterized through *in situ* CP degradability. Nutrient digestibility was determined in sheep. Twenty-four multiparous cows were used in a 4 × 4 Latin square design with 28-day periods. Treatments included total mixed rations containing as primary protein sources RSM (control), $DDGS_1$ (D1), $DDGS_2$ (D2) or $DDGS_3$ (D3). RSM contained less rapidly degradable CP (fraction a), more potentially degradable CP (fraction b) and more UDP than the three DDGS. *In vivo* organic matter digestibility of RSM was similar to DDGS. Calculated NE_L was lower for RSM (7.4 MJ/kg DM) than for DDGS, which averaged 7.7 MJ/kg DM. Cows' dry matter intake did not differ between diets (21.7 kg/d). Cows fed D1 yielded more milk than those fed

D3 (31.7 vs. 30.4 kg/d); no differences were found between control and DDGS diets (31.3 vs. 31.1 kg/d). Energy-corrected milk was similar among diets (31.2 kg/d). Diets affected neither milk fat concentration (4.0%) nor milk fat yield (1.24 kg/d). Milk protein yield of control cows (1.12 kg/d) was significantly higher than D3 (1.06 kg/d) but not different from D1 and D2 (1.08 kg/d each). Feeding DDGS significantly increased milk lactose concentration (4.91%) compared to control (4.81%). DDGS can be a suitable feed compared to RSM and can be fed up to 4 kg dry matter per day in rations of dairy cows in mid-lactation.

To conclude, DDGS is a suitable feedstuff for ruminants in terms of chemical composition, energy and protein value. However, the variability should be considered when included in diets of ruminants, especially in animals with high performance. For this purpose, prediction approaches initated in this study should be further developed into tools for routine application for rapid DDGS evaluation and estimation of feed values. These approaches might also be usefull for the evaluation of other feed protein sources and taked into consideration for practical feeding and diets formulation.

CHAPTER 8

ZUSAMMENFASSUNG

Die steigende Nachfrage nach Energie sowie die Umsetzung des europäischen Programmes für die Nutzung von Energie aus erneubaren Ressourcen sind günstige Szenarien für das Wachstum der Ethanolproduktion der EU in den nächsten Jahren. Bei der Ethanolproduktion fällt Trockenschlempe (DDGS) als wichtigstes Nebenprodukt an, welche ein hochwertiges Futtermittel für Wiederkäuer darstellt. Zahlreiche Publikationen, überwiegend aus den USA und Kanada, haben die große Variabilität des Futterwertes von Mais-DDGS gezeigt, welche in diesen Ländern das wichtigste Nebenprodukt bei der Ethanolproduktion bildet. In der EU liegen verschiedene und vielfältige technologische Bedingungen vor, wobei es kaum Untersuchungen zur Evaluierung des Futterwertes von DDGS gibt. Die Variabilität des Futterwertes in Verbindung mit der erwarteten Zunahme der DDGS Produktion fordert eine weitere und spezifischere Charakterisierung dieses Nebenproduktes in der EU. Deshalb wurde ein Projekt konzipiert, um die chemische Zusammensetzung von DDGS aus verschiedenen europäischen Ländern zu charakterisieren und deren Protein- und Energiewert für Wiederkäuer zu evaluieren. Dreizehn DDGS-Proben, hergestellt aus Weizen, Mais, Gerste oder Mischungen verschiedener Substrate, wurden untersucht.

Ziel der ersten Studie war es, die Unterschiede in der Zusammensetzung und im Futterwert von DDGS zu beschreiben und die Gehalte der DDGS an nicht abbaubarem Rohprotein (UDP) zu schätzen. Der ruminale Abbau des Rohproteins (CP) wurde mittels der Nylonbeutel-Technik bestimmt. Die Proben wurden über 0, 1, 2, 4, 8, 16, 32 und 72h inkubiert und es wurde die *in situ* Abbaukinetik bestimmt. Der UDP Gehalt wurde für eine Passagerate von 8%/h geschätzt. Über die Ermittlung der Gasbildung *in vitro* erfolgte die Schätzung des Gehaltes an Umsetzbarer Energie (ME), Nettoenergie Laktation (NE_L) und der *in vitro* Verdaulichkeit der organischen Substanz (IVDOM). Die chemische Zusammensetzung variierte zwischen Proben (in g/kg Trockensubstanz (TS) ± Standardabweichung (SD), die Werte betrugen 310 ± 33 CP, 86 ± 37 Rohfett, 89 ± 18 Rohfaser, 408 ± 39 Neutral-Detergenz-Faser, 151 ± 39 Säure-Detergenz-Faser und 62 ± 31 Säure-Detergenz-Lignin) sowie zwischen den Proteinfraktionen, die nach dem Cornell Net Carbohydrate and Protein System bestimmt wurden (in g/kg CP, die Werte betrugen 161 ± 82 für Fraktion A, 24 ± 11 für Fraktion B1, 404 ± 105 für Fraktion B2, 242 ± 61 für Fraktion B3 und 170 ± 87 für Fraktion C). ME, NE_L (MJ/kg TS) sowie IVDOM (%) schwankten ebenfalls zwischen den Proben: 12.1 ± 0.59, 7.3 ± 0.39 und 72.5 ± 4.30. Die *in situ* lösliche Fraktion des CP (a) variierte zwischen 10.2 und 30.6%, die potentiell abbaubare Fraktion (b) lag im Mittel bei 66.8%. Der UDP Gehalt betrug 8.6-62.6% des CP. Diese erste Studie weist auf

signifikante Unterschiede zwischen den verschiedenen DDGS-Proben in der Zusammensetzung sowie im Futterwert hin. UDP konnte aus den analysierten Rohprotein Fraktionen geschätzt werden, jedoch wurde die Genauigkeit der UDP Schätzung durch die Einbeziehung des Neutral-Detergenz-unlöslichen N verbessert, wodurch 94% der Variation der UDP Werte erklärt werden konnten. Hieraus lässt sich schlussfolgern, dass die chemischen CP Fraktionen für die Schätzung der UDP Werte von DDGS verwendet werden können und die Variabilität der Proteinfraktionen von DDGS bei der Gestaltung von Milchviehrationen berücksichtigt werden sollte.

Für die Gewinnung weiterer Informationen zum Futterwert von DDGS wurde eine zweite Studie durchgeführt. Hierin sollte der ruminale *in situ* Abbau von Rohprotein und Aminosäuren verschiedener DDGS bestimmt und verglichen werden. Desweiteren sollte eine Charakterisierung der *in vitro* Pepsin-Pankreatin Löslichkeit des CP (PPS) der verfütterten DDGS (d-DDGS) und der nach 16 stündiger Inkubation verbleibenden DDGS-Rückstände (DDGS-r) erfolgen. Der ruminale Abbau von AS und CP wurde mithilfe von Nylonbeuteln in Pansen fistulierten Kühen bestimmt. Die Lysin- und Methioningehalte verschiedener d-DDGS schwankten zwischen 1.36 und 4.00 bzw. 1.34 und 1.99 g/16 g N. Der Milcheiweiß Score (MPS) von d-DDGS fiel gering aus und schwankte zwischen 0.36 und 0.51. Lysin und Isoleucin wurden als meist limitierende AS in d-DDGS und DDGS-r geschätzt. In DDGS-r wurde ein etwas höherer Gehalt an essentiellen AS als in d-DDGS ermittelt. Der ruminale CP-Abbau nach 16h Inkubationszeit schwankte zwischen den DDGS-Proben im Bereich von 44-94%. Der ruminale Abbau von Lysin und Methionin betrug 39-90% bzw. 35-92%. Anhand linearer Regressionen konnte gezeigt werden, dass der ruminale Abbau einzelner AS aus dem CP-Abbau geschätzt werden kann. Die PPS von d-DDGS war höher als die der DDGS-r und variierte zwischen 70 und 89% bzw. 47 und 81%. Es wurde keine signifikante Korrelation zwischen der PPS von d-DDG und der PPS von DDGS-r ermittelt (R^2 = 0.31). Der geschätzte Gehalt an intestinal absorbierbarem Futterprotein (IADP) lag im Durchschnitt bei 21%. Zwischen dem Gehalt an Rohfaser und PPS von DDGS-r wurde eine mittlere Korrelation ermittelt (R^2 = 0.43). Die Ergebnisse dieser Studie weisen auf eine Überschätzung des Beitrags vom UDP der DDGS zur Versorgung mit verdaulichem Eiweiß am Duodenum in den aktuell verwendeten Protein-Bewertungssystemen hin. Weitere Studien zur Bestimmung der intestinalen Verdaulichkeit von AS aus DDGS sind erforderlich.

Schließlich wurden in einer dritten Studie drei DDGS-Quellen in Rationen von Milchkühen in der Mittellaktation hinsichtlich der Milchproduktion und Milchzusammensetzung evaluiert. Desweiteren wurde die Verdaulichkeit dieser DDGS-

Quellen bei Schafen ermittelt. DDGS aus Weizen, Mais und Gerste ($DDGS_1$), Weizen und Mais ($DDGS_2$) und Weizen ($DDGS_3$) wurden untersucht und mit einem Rapsextraktionsschrot verglichen (RES). RES und DDGS wurden zunächst anhand der *in situ* CP-Abbaubarkeit charakterisiert. Die Nährstoffverdaulichkeit wurde bei Schafen bestimmt. Die Versuchsanordnung erfolgte in einem 4 × 4 Lateinischen Quadrat mit 24 multiparen Kühen in 28-tägigen Versuchsperioden. Die Versuchsrationen bestanden aus Total-Misch-Rationen, die als Hauptproteinquelle RES (Kontrolle), $DDGS_1$ (D1), $DDGS_2$ (D2) oder $DDGS_3$ (D3) enthielten. Im RES war weniger lösliches CP (Fraktion a), mehr potentiell abbaubares CP (Fraktion b) und mehr UDP enthalten verglichen mit den drei DDGS. Die *in vivo* Verdaulichkeiten der organischen Substanz von RES und DDGS waren vergleichbar. Die für RES (7.4 MJ/kg TS) kalkulierte NE_L fiel geringer aus verglichen mit DDGS, bei der die NE_L im Mittel 7.7 MJ/kg TS betrug. Zwischen den Rationen wurden keine Unterschiede in der TS-Aufnahme der Kühe festgestellt (21.7 kg/d). Die Milchleistung der Kühe, die Ration D1 erhielten, war höher verglichen mit Ration D3 (31.7 vs. 30.4 kg/d); wobei zwischen der Kontrollration und den DDGS Rationen kein Unterschied ermittelt wurde (31.3 vs. 31.1 kg/d). Es wurden keine Unterschiede in der Energie-korrigierten Milch zwischen den Rationen festgestellt (31.2 kg/d). Die Rationen zeigten keinen Effekt auf den Milchfettgehalt (4.0%) oder die Milchfettmenge (1.24 kg/d). Die Fütterung der Kontrollration erbrachte einen signifikant höheren Micheiweißmenge (1.12 kg/d) verglichen mit D3 (1.06 kg/d), aber keinen signifikant verschiedenen Milcheiweißmenge verglichen mit D1 oder D2 (1.08 kg/d bei beiden). DDGS führte zu einer signifikanten Erhöhung der Milchlaktosekonzentration (4.91%) verglichen zur Kontrolle (4.81%). Demzufolge kann DDGS ein adäquates Futtermittel zu RES sein und kann mit bis zu 4 kg Trockenmasse pro Tag in Rationen von Milchkühen in der Mittellaktation eingesetzt werden.

Abschließend kann geschlussfolgert werden, dass DDGS hinsichtlich der chemischen Zusammensetzung sowie des Energie- und Proteinwertes ein geeignetes Futtermittel für Wiederkäuer darstellt. Allerdings sollte die Variabilität beim Einsatz in Wiederkäuerrationen berücksichtig werden, insbesondere bei Tieren mit hoher Leistung. Zu diesem Zweck sollten die in dieser Studie entwickelten Ansätze zu den Schätzungen weiterentwickelt werden, mit dem Ziel einer routinemäßigen Anwendung für eine schnelle Bewertung und Schätzung der Futterwerte von DDGS. Diese Ansätze könnten auch für die Bewertung von anderen Proteinträgern nützlich sein und in der praktischen Fütterung und Rationsgestaltung berücksichtigt werden.

ACKNOWLEDGEMENTS

The realization of this work was only possible with the contribution of many especial persons which I would like thanks without any special order of importance:

First, I would like to thanks Prof. Dr. Markus Rodehutscord for giving me the great opportunity to do this work at the Institute of Animal Nutrition of the University of Hohenheim, for his supervision, guidance and continuous support throughout my Doctor study.

Special thanks and my deepest gratefulness to Dr. Herbert Steingass, my daily supervisor, for his outstanding supervision, special patience and his exceptional work besides me during the development of this project.

Thanks to the "Ministerium für Wissenschaft, Forschung und Kunst Baden-Württemberg" for the financial support through a doctoral scholarship.

For his help, the good moments and the hard work during the realization of the feeding trial with dairy cows I would like to thanks to my colleague Ralf Kaiser.

Thanks to Birgit Sprott and Ingrid Neff for their support and guidance during my stay in the Institute of Animal Nutrition.

Special thanks to my good friend "Enzo" for his emotional support and for carrying out the digestibility trial with sheep.

Thanks to my colleagues Ellen Zeller and Dr. Yauheni Shastak for their contribution with this thesis.

Thanks to Dr. Jeannette Boguhn for her help during the preparation of the doctoral proposal.

Thanks to Sussanne Plank, Sibylle Rupp and Dr. Margit Schollenberger for their continuously support during the laboratory analyses.

Finally, I would like to thanks to my wife Ana for her continuously support; and, to my children Marianne, Kirsten and Karsten.

PERSÖNLICHE ANGABEN

Edwin Westreicher Kristen
geb. am 17.12.1978 in Lince Peru
deutsch
verheiratet, 3 Kinder (12, 8 und 6 Jahre alt)

AKADEMISCHE WERDEGANG

02.2010-05.2013 **Doktorand**
Institut für Tierernährung, Universität Hohenheim
Promotionsstipendium der Landesgraduiertenförderung

10.2007-01.2010 **M. Sc. Agricultural Sciences in the Tropics & Subtropics**
Spezialisierung: Tierproduktion
Universität Hohenheim
Stipendium vom DAAD

01.1997–12.2001 **Diplom-Agraringenieur**
Spezialisierung: Tierwissenschaften
Universidad El Zamorano, Honduras
Stipendium von DEZA und USAID

BERUFSERFAHRUNG

05.2005-07.2007 **Universidad El Zamorano, Honduras**
Abteilung Milchvieh
Wissenschaftlicher Assistent

03.2004–04.2005 **Universidad El Zamorano, Honduras**
Projekt: „Intensivierung der Milchproduktion in Honduras“
Instrukteur Milchvieh

06.2002–02.2004 **Landwirtschaftlicher Betrieb „Agua Blanca“, Honduras**
Betriebsleiter

01.2002-05.2002 Selbständiger Berater im Bereich Milchproduktion

01.2000–12.2001 **Universidad El Zamorano, Honduras**
Projekt: „Milchproduktion und Verarbeitung Zamorano-USAID“
Instrukteur Milchvieh

…….
Stuttgart-Hohenheim, 29. Mai 2013

ERKLÄRUNG

Hiermit erkläre ich, Edwin Westreicher-Kristen, geboren am 17.12.1978, dass die vorliegende Dissertation selbständig und ausschließlich unter Zuhilfenahme der im Literaturverzeichnis genannten Quellen angefertigt wurde.

…….
Stuttgart-Hohenheim, 29. Mai 2013