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Effects of supplementing amylase and protease to ruminant diet on rumen fermentation characteristics and the rumen microbiota

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Mirko Deml

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List of Abbreviations

a	Soluble fraction
ADF	Acid-detergent fibre
Ala	Alanine
Amy	Amylase treatment
Amy+Prot	Treatment with amylase and protease
ANOVA	Analysis of variance
b	Not soluble, but ruminal degradable fraction
bp	Base pair
c	Constant rate of degradation of fraction b
CA	Crude ash
CF	Crude fibre
CH_4	Methane
cm	Centimetre
Co	Cobalt
Con	Control treatment
CP	Crude protein
Cu	Copper
$CuSO_4$	Copper sulphate
d	Degradable fraction
DM	Dry matter
DMD	Dry matter disappearance
DNA	Deoxyribonucleic acid
ED	Effective degradability
EDTA	Ethylenediaminetetraacetic acid
g	Gramme
h	Hour
H_2	Hydrogen
H_2O_2	Hydrogen peroxide

H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid
I	Iodine
IU	International unit
k	Passage rate
K_2SO_4	Potassium sulphate
kg	Kilogram
KNU	Kilo novozymes unit (activity of amylase)
l	Litre
m	Metre
mg	Milligramme
ml	Millilitre
mm	Millimetre
mmol	Millimole
Mn	Manganese
µl	Microlitre
µm	Micrometre
µmol	Micromole
NADPH/$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NDF	Neutral-detergent fibre
NFE	Nitrogen-free extracts
ng	Nanogramme
NH_3-N	Ammonia-nitrogen
nm	Nanometre
OM	Organic matter
PCR	Polymerase chain reaction
Phe	Phenylalanine
pmol	Pikomole
Pro	Proline
PROT	Activity of protease
Prot	Protease treatment

qPCR	Quantitative polymerase chain reaction
rpm	revolutions per minute
rRNA	Ribosomal ribonucleic acid
SD	Standard deviation
Se	Selenium
SEM	Standard error of means
spp.	*Species pluralis*
Suc	Succinyl
t_0	Lag time
TiO_2	Titanium dioxide
TL	Total lipids
TMR	Total mixed ration
TRIS	Tris(hydroxymethyl)aminomethane
VFA	Volatile fatty acids
Zn	Zinc
°C	Degree Celsius
%	Percent
Σ	Sum

1 Summary

The objective of the current study was to evaluate the effects of exogenous amylase and protease on the rumen physiological parameters, the ruminal degradability, the rumen microbiota and the total tract digestibility of non-lactating dairy cows. Parameters were measured in a double 4 × 4 Latin square design with 40-day periods using eight rumen cannulated Holstein cows in four periods with following treatments: control (no enzyme supplementation), supplementation of amylase (300 KNU/kg DM), supplementation of protease (15000 PROT/kg DM), and supplementation of a combination of amylase and protease (150 KNU + 7500 PROT/kg DM). Diet was based on maize, containing 48.6 % maize silage and 19.9 % maize grain, respectively. Further ingredients were grass silage (14.9 %), hay (9.93 %), soybean meal (5.96 %), and a mineral and vitamin mix. Rumen degradability was determined by incubating feedstuffs in the rumen of cannulated cows up to 48 hours using the *in sacco*-method. Measured parameters were degradability of dry matter, starch, crude protein, and neutral-detergent fibre at different incubation times, parameters of degradation (soluble fraction, ruminal degradable fraction, rate of degradation, and lag-time), and the effective degradability assuming passage rates of 2, 5, and 8 %/h. Determination of total-tract digestibility was based on a marker study, using TiO_2 as an indigestible marker. Rumen fluid samples were taken prior to feeding and up to nine hours after feeding and analysed regarding rumen physiological parameters (pH-value, ammonia-nitrogen, and volatile fatty acids) and the rumen microbiota. Selected rumen microorganisms were: total bacteria, archaea, protozoa, anaerobic fungi, *Prevotella spp*., *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Streptococcus bovis*.

Effects of enzyme supplementation were observed on rumen degradability but not at the level of total tract digestibility. The combination of amylase and protease increased ruminal dry matter degradability of maize grain by 6.7 % and ruminal starch degradability by 10.6 % on average. Supplementation of protease and the combination of amylase and protease increased ruminal dry matter degradability of maize silage by 11.8 % on average, respectively. Protease supplementation also increased ruminal crude protein degradability of soybean meal, but only at short incubation times, thus dry matter degradability of soybean meal was not affected. Ruminal degradability of grass silage and hay was not affected by enzyme supplementation, neither dry matter nor neutral-detergent fibre degradation.

Rumen physiological parameters (pH-value, ammonia-nitrogen, volatile fatty acids) were not affected by exogenous enzymes. Only concentration of butyric acid was slightly decreased by enzyme supplementation prior to feeding times. No or only small changes

on selected fibrolytic and amylolytic bacteria and on the microbial community in general due to enzyme supplementation were observed.

Prior studies showed an increased fibre digestibility in dairy cows when exogenous amylase was supplemented. This could not be approved in the current study. Furthermore, no indication was found that fibrolytic rumen bacteria benefitted from amylase supplementation. Rumen degradation characteristics of maize grain lead us to the conclusion that exogenous enzymes may break down protein structures, which aggravate starch access, resulting in increased ruminal starch degradation of maize grain when amylase and protease were supplemented simultaneously.

2 Introduction

The rumen is an ecosystem in which feed is fermented to volatile fatty acids and microbial biomass that serve as sources of energy and protein to the ruminant. Structural carbohydrates from plants such as cellulose, hemicellulose and pectin are the major contributors to the energy requirements of the ruminant (Weimer, 1998). The insolubility, structural complexity and initial inaccessibility of cell wall components often limit the rate and extent of which they can be fermented inside the rumen (Nagaraja et al., 1997). Manipulation of ruminal metabolism to maximise rate and extent in which plant cell wall material is degraded in the rumen has become an important goal in modern livestock production (Weimer, 1998; Wang & McAllister, 2002). Exogenous enzymes may be one opportunity to increase utilisation of nutrients.

The principle rationale for the use of exogenous enzymes is to improve the nutritive value of feedstuffs. The main benefits of feed enzymes, mentioned by Sheppy (2001), are:

1. To break down anti-nutritional factors which are not susceptible to digestion by the animal's endogenous enzymes
2. To increase availability of starch, proteins, and minerals that are enclosed in fibre-rich cel walls or bound up in a chemical form that the animal is not able to digest
3. To break down specific chemical bonds in raw materials that are usually not broken down by endogenous enzymes, thus releasing more nutrients
4. To supplement the enzymes produced by young animals where, because of the immaturity of their own digestive system, endogenous enzyme production may be inadequate

Exogenous enzymes are mainly used in swine and poultry production. They allow pigs and poultry to extract more nutrients from feed, thus improving feed efficiency and reducing the negative impact of animal production on the environment (Barletta, 2010). This effect is resulting in reduction of manure volume up to 20 % and nitrogen excretion up to 15 % in pigs and 20 % in poultry (Sheppy, 2001).

The use of exogenous enzymes in monogastric animal production has increased dramatically in recent years, where mechanism and mode of action have been defined. In contrast, preparations of exogenous enzymes for ruminants showed inconsistent results and characteristics of the digestive tract complicate elucidation of mechanism and mode

of action (McAllister et al., 2001). As long ago as the 1960s several studies were carried out to investigate the effects of treating feed with exogenous enzymes on digestibility and animal performance of ruminants (Burroughs et al., 1960; Clark et al., 1961; Rovics & Ely, 1962; Van Walleghem et al., 1964; Rust et al., 1965), but response was inconsistent and authors gave no information on enzyme activity and mode of action. At this time production costs of enzymes were very high, preventing an economically reasonable use of exogenous enzymes. Another reason for the lack of the use of exogenous enzymes in ruminant nutrition was the opinion that these enzymes are rapidly deactivated inside the rumen due to proteolysis. That this is not the case and that some exogenous enzymes are stable inside the rumen was reported by Hristov et al. (1998a,b) and Morgavi et al. (2000, 2001).

Recent increase in feed costs and decrease in production costs of enzymes and better defined enzyme preparations led to a re-examination of enzyme products in ruminants, where commercial use of feed enzymes in beef and dairy cattle is still limited. A challenge for researches is to determine the mode of action that enables exogenous enzymes to improve feed efficiency and increase in animal performance (McAllister et al., 2001).

The primary objective of the use of exogenous enzymes in ruminants is the decrease of producing costs of meat and milk. Beef and dairy producers are seeking ways of improving feed conversion efficiency (reducing amount of feed required per kg of weight gain or milk yield) and animal performance (increased weight gain or milk production per day) (Beauchemin & Holtshausen, 2010).

Most research has been focused on fibrolytic enzymes, as reported in reviews of Wang & McAllister (2002) and Beauchemin et al. (2004), because increasing fibre digestibility can increase the intake of digestible energy by reducing physical fill in the rumen and also stimulates rumen microbial nitrogen synthesis (Oba & Allen, 2000). A one percentage unit increase in NDF (neutral-detergent fibre) degradability inside the rumen has the potential to increase dry matter intake by 0.17 kg per day and fat corrected milk yield by 0.25 kg per day (Oba & Allen, 1999). Thus, exogenous enzymes which increase NDF degradability in the rumen may improve feed conversion ratio and animal production in ruminants.

Recent investigations of Klingerman et al. (2009), Gencoglu et al. (2010), Weiss et al. (2011), and McCarthy et al. (2013) reported an increase in total tract NDF digestibility when diets of dairy cows were supplemented with α-amylase. These findings are relatively new and the exact mode of action in which exogenous α-amylase is able to increase fibre digestibility is still unknown. One hypothesis is that α-amylase provides substrate from starch for non-amylolytic, fibre-degrading bacteria (Tricarico et al., 2008).

The use of exogenous protease in ruminant nutrition was widely ignored, assuming an excessive degradation of protein in the rumen and thus inefficient utilisation of nitrogen (Eun & Beauchemin, 2005). An increase in ruminal crude protein degradation may be disadvantageous to dairy cows (Colombatto & Beauchemin, 2009), but exogenous protease also showed potential to increase NDF digestibility *in vitro* (Colombatto et al., 2003a,b; Eun et al., 2007) and *in vivo* (Eun & Beauchemin, 2005), especially of alfalfa. One hypothesis is that exogenous proteases may cleave specific bonds in structures of fibre and protein (Kopecny & Wallace, 1982) that serve as structural barriers to digestion.

Also in grains efforts were made to increase utilisation of nutrients. Ruminal fermentation is not limited by population or activity of rumen microorganisms, but by the amount of substrate available for microbial attack (Weimer, 1998). To improve digestibility of nutrients, in grains mainly starch, several grain processing techniques were developed, for example dry-rolling or steam-flaking (Theurer, 1986; Owens et al., 1997). These processing techniques are time-consuming and cost-intensive, thus also in this case exogenous enzymes may contribute to an increased digestibility of nutrients. Especially in maize grain, where access to starch granules is aggravated due to a hardly hydrolysable protein matrix (Tamminga, 1979; Larson & Hoffman, 2008).

The aim of the current study was to investigate the effects of amylase and protease supplementation on rumen fermentation characteristics. Thereto, an experiment was carried out using eight non-lactating, cannulated Holstein cows fed a maize based diet. It should be verified, whether α-amylase supplementation is able to improve ruminal and/or total tract digestibility of fibre and whether fibrolytic bacterial populations can benefit from exogenous amylase. Furthermore, our hypothesis was that protease will break down protein structures in maize grain, resulting in an increased ruminal starch degradation which may contribute to an increase of fibre digestibility.

3 Material and methods

3.1 Experimental design

The effects of amylase and protease supplementation on rumen degradation characteristics of commonly used ruminant feedstuffs were measured using the *in sacco*-method based on rumen cannulated cows. Altogether, four treatments (three enzyme treatments and a control) were tested on eight cows. The three enzyme treatments were: supplementation with an amylase preparation (Amy), supplementation with a protease preparation (Prot) and a combination of both enzyme formulations (Amy+Prot). The control (Con) received no enzyme supplementation. Animals were used as a double 4 × 4 Latin square arrangement of treatments in four periods (Table 1), so that every cow received each treatment once. The test duration was 160 days. Each period lasted 40 days (21 days of adaptation, 11 days measuring period and 8 days wash-out phase without any enzyme supplementation).

Table 1: Experimental design of the present study

	Treatment				
	Con	**Amy**	**Prot**	**Amy+Prot**	**Σ**
Period 1	n=2	n=2	n=2	n=2	n=8
Period 2	n=2	n=2	n=2	n=2	n=8
Period 3	n=2	n=2	n=2	n=2	n=8
Period 4	n=2	n=2	n=2	n=2	n=8

3.2 Enzyme specification

The two enzyme preparations RONOZYME® RumiStar and RONOZYME® ProAct (Table 2) were provided by DSM (Heerlen, the Netherlands). The active agent of RumiStar is α-amylase, produced by submerged fermentation of a genetically modified strain of *Bacillus licheniformis*. The α-amylase activity was expressed in KNU (kilo novozymes units) and was 600 KNU/g for the solid variant and 240 KNU/g for the liquid variant, respectively (one KNU is defined as the amount of enzyme that releases 6 µmol p-nitrophenol per minute from ethylidene-G_7-p-nitrophenyl-maltoheptaoside (1.86 mmol/l) at pH 7.0 and 37 °C). The active agent of ProAct is serine protease, produced also by submerged fermentation of a genetically modified strain of *Bacillus licheniformis*. The

enzymatic activity is expressed in protease units (PROT) and was 75000 PROT/g for the solid and the liquid variant, respectively (one PROT is defined as the amount of serine protease that liberates 1 µmol para-nitroaniline (pNA) per minute from Suc-Ala-Ala-Pro-Phe-pNA ($C_{30}H_{36}N_6O_9$, 1 mmol/l) at pH 9.0 and 37 °C). The solid variants were coated granulates and thermo-tolerant preparations. RumiStar had an average particle size of 450-460 µm and consisted of kaolin (10 % - 30 %), cellulose (10 % - 30 %), and sucrose (5 % - 10 %), additionally. ProAct had an average particle size of 500 µm and contained furthermore cellulose (5 % - 10 %) and calcium carbonate (5 % - 10 %). The granulated variants were used during the feeding trial for supplementing the feed with a defined amount of enzyme activity. The liquid forms were only used for mixing the enzymes to bag contents of the *in sacco*-method.

Table 2: Enzyme preparations applied in the present study

	RONOZYME® RumiStar		**RONOZYME® ProAct**	
	solid	**liquid**	**solid**	**liquid**
Activity (units/g)	600 KNU	240 KNU	75000 PROT	75000 PROT
Contained enzymes	α-amylase	α-amylase	serine protease	serine protease

3.3 Animals and diet

The feeding trial was carried out at the experimental facility of animal nutrition at the Department of Animal Science of the Technical University of Munich in Freising-Weihenstephan. In this trial, eight non-lactating Holstein cows weighing 624 ± 14 kg (mean ± SD) were used to study the effects of amylase and protease supplementation on rumen degradation characteristics. Each cow was fitted with a rumen cannula (Bar Diamond, Parma, Idaho, USA), with an internal diameter of 10 cm, at the dorsal rumen sac. Animals were kept in tie-stalls, with individual feeding, on rubber mats without litter in an air conditioned stable (20 °C). All animals had free access to drinking water and salt blocks.

Animals were fed twice a day in equal portions at 07:00 and 16:00 h. Daily intake of dry matter (DM) was 7.0 kg. The ration consisted of maize silage, grass silage, maize grain, soybean meal, hay, and a commercial mineral and vitamin mix and was administered as total mixed ration (TMR). The composition of the TMR is shown in Table 3.

Table 3: Composition and analysed nutrient content of the experimental diet

Item	% of the diet DM
Ingredient	
Maize silage	48.6
Grass silage	14.9
Hay	9.93
Maize grain	19.9
Soybean meal	5.96
Mineral/vitamin mix	0.71
Nutrient composition of TMR	
CP	10.7
TL	3.23
Starch	39.3
NDF	35.7

CP: crude protein, TL: total lipids, NDF: neutral-detergent fibre; Mineral/vitamin mix consisted of: 27 % limestone, 27 % sodium chloride, 23.5 % calcium phosphate, 13.7 % magnesium phosphate, 3.3 % magnesium oxide, 2.5 % beet molasses, 0.8 % Zn (from zinc oxide), 0.4 % Mn (from manganese oxide), 0.1 % Cu (from copper sulfate), 0.01 % I (from calcium iodate), 0.004 % Se (from sodium selenite), 0.003 % Co (from cobalt carbonate), 0.02 % antioxidant, 800 IU of vitamin A/g, 100 IU of vitamin D3/g, 0.3 % of vitamin E, 0.053 % of vitamin B premix

Table 4 shows the dry matter, crude protein, total lipids, NDF, starch, and crude ash contents of the single components of the TMR. Samples of maize and grass silage were taken before the start of each period and analysed, thus the amount of silage was adjusted for every period.

Table 4: Dry matter and nutrient content of the single feedstuffs of the experimental diet

Feedstuff	DM content (%)	Average nutrient content (% of DM)				
		CP	TL	NDF	Starch	CA
Maize silage	36.9	6.28	3.87	37.1	45.1	3.20
Grass silage	38.5	15.5	3.34	51.4	5.41	9.11
Maize grain	89.1	8.50	2.68	13.0	78.3	1.38
Soybean meal	88.8	50.6	1.25	18.7	5.80	7.65
Hay	92.7	8.34	2.60	63.2	7.01	5.37

CP: crude protein, TL: total lipids, NDF: neutral-detergent fibre, CA: crude ash

The TMR was mixed daily individually for all animals. Therefore, maize silage, grass silage, hay, and the concentrate mix were weighed separately into tubs and were

subsequently mixed by hand. The concentrate mix contained maize grain, soybean meal, the mineral/vitamin mix and titanium dioxide (TiO_2). The concentrate mix was not pelletised but maize grain and soybean meal were ground coarsely (3.0 mm). To avoid interactions between the enzymes and the feed, the granulated enzyme preparations were admixed to the TMR just before feeding. The supplemented amounts of enzyme preparations (g/day) and enzyme activities (units/kg DM) to the diet are represented in Table 5.

Table 5: Supplemented enzyme amount and activity of different treatments

	Treatment			
	Con	**Amy**	**Prot**	**Amy+Prot**
Amount (g/day)	-	3.50	1.40	1.75 + 0.70
Activity (units/kg DM)	-	300 KNU	15000 PROT	150 KNU + 7500 PROT

3.4 *In sacco*-method

The ruminal degradation of the different feedstuffs was measured using the *in sacco*-method according to Ørskov & McDonald (1979) and Madsen & Hvelplund (1994). Thereto, white nitrogen-free polyester monofilament bags with a dimension of 10 × 20 cm and a pore size of 53 µm (± 10 µm) (Bar Diamond, Parma, Idaho, USA) were used. The empty bags were dried in a forced-air dry oven at 60 °C for 48 h, afterwards cooled to room temperature in a desiccator and then weighed. Following this, 4.0 g DM of the different feedstuffs were weighed into the bags. The tested feedstuffs were maize silage, grass silage, maize grain, soybean meal, hay, and the TMR. The dry matter and nutrient contents of the tested feeds are presented in Table 6. In preparation of the TMR, the components were weighed separately into the bags, in the same proportion as they were presented in the TMR. For measuring the effects of amylase and protease supplementation under conditions of practice, the tested feedstuffs were weighed into the bags as fresh material. To simplify that, grass silage and hay were slightly chopped once using a chaff cutter. Maize silage, maize grain and soybean meal were weighed into the bags in the same particle size as they were presented in the TMR. After filling the bags, they were sealed with a commercial cable tie and stored until incubation at -20 °C. The day before the incubation started, bags were thawed overnight (12 h) in a cooling chamber at 6 °C. Four bags of each feed (maize silage, grass silage, maize grain, soybean meal, hay, and TMR) were used per cow and incubation time. The 24 bags were

fixed to a cylindrical anchor weight (800 g). The four bags of each feedstuff were evenly distributed on both ends of the cylinder to avoid position effects inside the rumen. Incubation of the bags started right before the morning feeding at 07:00 h. The cylinder was set in the ventral sac of the rumen and fixed with a line at the inside of the cannula lid. The incubation times were 1, 2, 3, 4, 5, 6, 9, 12, 24, and 48 h. All bags were removed from the rumen at the same time and were immersed into ice water immediately to prevent further microbial activity inside the bags. After that, bags were removed from the cylinder and washed by hand in a sink with tap water until the water ran clear. Thereafter, bags were washed in a customary washing machine with cold water for 19 minutes without a spin cycle. After the washing procedure bags were lyophilised for 72 h, stored in a desiccator and weighed again with contents, instantly. For determination of dry matter disappearance caused by the washing process, one bag of each treatment and feedstuff was washed and dried, like described above, without prior rumen incubation, at all incubation times.

In order to guarantee homogeneous presence of the enzyme preparations in all tested material, the enzymes were admixed to the bag contents with the same enzyme activity per kg dry matter as they were presented in the TMR. Hereto, the liquid form of the enzyme preparation was used. Approximately 5.0 kg DM of each feedstuff was mixed with the different enzyme solutions for preparing the bags. Enzyme solutions consisted of enzyme preparations and 60 ml distilled water per kg feed dry matter. Adding the combination, two separate enzyme solutions were admixed to the feedstuffs. The amount of distilled water was divided in two equal portions, added with enzyme preparations and then mixed to the feed successive, so that the amount of supplemented water remained constant for all treatments. Control feedstuffs were admixed with 60 ml distilled water per kg DM without any enzyme addition. The enzyme solutions were admixed to maize grain and soybean meal using a blender for eight minutes, without cutting function. For maize silage, grass silage and hay a commercial hand sprayer was used. Enzyme solutions were sprayed onto the feed, while thoroughly being mixed by hand in a tub. After mixing the enzyme solutions to the feed, the mixtures were divided in several portions and frozen at -20 °C instantly. Additionally, dry matter contents of the mixtures were determined. For filling the bags, one portion was thawed in a cooling chamber at 6 °C, weighed into the bags and afterwards immediately frozen again at -20 °C, until incubation.

Table 6: Dry matter and nutrient content of incubated feedstuffs

Feedstuff	DM content (%)	Nutrient content (% of DM)				
		CP	TL	NDF	Starch	CA
Maize silage	33.3	5.92	3.51	37.3	39.2	3.48
Grass silage	38.3	15.2	3.54	50.2	5.97	8.95
Maize grain	89.1	8.50	2.68	13.0	78.3	1.38
Soybean meal	88.8	50.6	1.25	18.7	5.80	7.65
Hay	92.7	8.34	2.60	63.2	7.01	5.37

CP: crude protein, TL: total lipids, NDF: neutral-detergent fibre, CA: crude ash

3.5 Sampling and sample preparation

3.5.1 Feed samples

Dry matter content of maize silage, grass silage, and hay was determined by drying the samples at 60 °C for 48 h in a forced-air dry oven. Dry matter content of maize grain, soybean meal and pre-dried samples was determined by oven-drying at 103 °C (4 h).

Prior to nutrient analysis samples of feed and faeces were ground through a 1.0 mm screen using a hammer mill (SR3, Retsch, Haan, Germany) for maize grain and soybean meal and a cutting mill (type 880800, Brabender, Duisburg, Germany) for maize silage, grass silage, hay, and faeces. For the determination of starch, feed and faeces samples were ground through a 0.5 mm screen.

For measuring the disappearance of selected crude nutrients of some feedstuffs, sample preparation was different because of restricted quantity of bag residues. Maize silage and grass silage were ground in a cutting mill (type 880800, Brabender, Duisburg, Germany) using a 0.5 mm screen for maize silage and 1.0 mm for grass silage. Bag residues of maize grain and soybean meal were ground in an analytical mill (A10, IKA, Staufen im Breisgau, Germany) with a star shaped cutter for 30 seconds.

3.5.2 Rumen physiological parameters

The rumen physiological parameters pH-value, ammonia-nitrogen (NH_3-N), and volatile fatty acids (VFA) were determined in the rumen fluid. Samples were collected right before the morning feeding (0 h) and 1, 2, 3, 4, 5, 6, and 9 h after feeding. Approximately 500 ml per animal and sampling time were obtained from the ventral sac of the rumen through the

cannula using a mouth-to-rumen tube and vacuum. The sampling occurred once a day in association with removing the bags from the rumen, so that the cannula did not have to be opened more times than necessary.

Rumen pH-value was measured directly after sampling. The remaining sample was divided into two portions. The first part was centrifuged for 15 minutes at 5000 rpm (revolutions per minute) using the centrifuge Z323 (Hermle Labortechnik, Wehingen, Germany). Supernatant was removed and stored at -20 °C for following determination of ammonia concentration. The second part was centrifuged for 5 minutes at 5000 rpm (Z323, Hermle Labortechnik, Wehingen, Germany). An aliquot of 10 ml of the supernatant was removed and 1.5 ml metaphosphoric acid (25 %) and 0.5 ml formic acid were added. After that, samples were centrifuged again at 5000 rpm for 20 minutes and stored at -20 °C for subsequent determination of VFA.

3.5.3 Rumen microbiota

For studying the effects of amylase and protease supplementation on rumen microbiota, selected microorganisms were measured in the rumen fluid by using qPCR (quantitative polymerase chain reaction). Samples were collected right before the morning feeding (0 h) and one and three hours after feeding. Sampling procedure was the same as described above. Approximately 200 ml of rumen fluid were frozen immediately at -20 °C. Samples were lyophilised for 72 h afterwards, pestled and then DNA was extracted. Following microorganisms were determined in the rumen fluid: total bacteria, archaea, protozoa, anaerobic fungi, *Prevotella spp*., *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Streptococcus bovis*.

3.6 Analytical procedures

3.6.1 Crude nutrient analysis

Analysis of crude nutrients (crude protein, crude fibre, total lipids, crude ash) of feedstuffs and faeces were carried out according to “Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten” (VDLUFA; Naumann & Bassler, 1976, 2012).

Analysis of crude protein (CP) was carried out based on a standard Kjeldahl procedure. Sample material (1.0 g from feed or faeces samples, 0.3 g of bag residues) was solubilised by 20 ml of concentrated sulphuric acid and a catalyst (Kjelcat CuTi, C. Gerhardt, Königswinter, Germany). The fusion was implemented with the Turbotherm

(C. Gerhardt, Königswinter, Germany) and lasted for 115 minutes. Afterwards, samples were alkalised by sodium hydroxide solution (30 %), followed by a steam distillation. Ammonia was collected in boric acid (2.0 %) and was determined by titration of hydrochloric acid (0.1 %). Steam distillation and titration were carried out with the Vapodest (C. Gerhardt, Königswinter, Germany).

Crude fibre (CF) was analysed by treating the samples (1.0 g) with simmering sulphuric acid (0.128 mol/l) and simmering potassium hydroxide solution (0.223 mol/l) (Fibretherm, C. Gerhardt, Königswinter, Germany). Residues were separated by filtration with following washing, drying and weighing. Crude ash amount was determined subsequently and CF proportion was calculated by the difference between weight before and after determination of crude ash.

Total lipids (TL) were analysed by solubilising the samples (1.5 g) with hot hydrochloric acid (15 %) for 75 minutes with following filtering (Hydrotherm, C. Gerhardt, Königswinter, Germany). After that, samples were extracted for 105 minutes with 140 ml of petroleum ether, the solvent was removed by distillation (Soxtherm, C. Gerhardt, Königswinter, Germany) and residues were dried and weighed.

Content of crude ash (CA) was determined by incinerating the samples (3.0 g) in a muffle kiln at 550 °C overnight.

3.6.2 Fibre analysis

Determination of the cell wall components neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) were carried out according to VDLUFA (Naumann & Bassler, 1976, 2012) based on Van Soest (1991). The component NDF consists of hemicellulose, cellulose and lignin. ADF consists of cellulose and lignin (lignocellulose). For determination of NDF, 1.0 g of sample material (0.3 g of bag residues) was weighed into FibreBags (C. Gerhardt, Königswinter, Germany) and boiled with the addition of thermostable α-amylase (Termamyl 120L, Univar, Essen, Germany) in neutral detergent solution for 165 minutes, using the Fibretherm (C. Gerhardt, Königswinter, Germany). For determination of ADF, samples (1.0 g) were boiled in acid detergent solution without addition of α-amylase for 120 minutes (Fibretherm, C. Gerhardt, Königswinter, Germany). After the boiling procedure, samples were washed with distilled water, dried and weighed. Crude ash content of the samples was determined subsequently and NDF proportion, respectively ADF proportion, was calculated by the difference between weight before and after determination of crude ash.

3.6.3 Determination of starch

Determination of starch content in feed and faeces samples was carried out according to Batey (1982) and Brandt et al. (1987). The method is based on the solubilisation of starch by a thermostable α-amylase followed by the complete hydrolysis by an amyloglucosidase. Glucose content was determined afterwards by the hexokinase/glucose-6-phosphate dehydrogenase procedure.

Samples (0.3 g) were weighed in a volumetric flask, suspended in 25 ml sodium citrate buffer (0.3 mol/l, pH 5.8) and heated in a water bath with shaking for 10 minutes. Then, 1 ml of a thermostable α-amylase (Termamyl 120L, Univar, Essen, Germany) was added to the mixture and samples were shaken again in the water bath for one hour at 90 - 95 °C. Afterwards, volumetric flasks were quenched, filled up with distilled water to a defined volume and the solution was filtered (595 1/2, Whatman, Dassel, Germany). Filtered samples (200 µl) were mixed 1:1 with amyloglucosidase (10113-1G, Sigma-Aldrich, St. Louis, Missouri, USA) solution and sat for 20 minutes at room temperature. Amyloglucosidase was diluted to 0.1 % with sodium acetate buffer (0.5 mol/l, pH 4.6). The amount of glucose liberated in the degradation was measured using the hexokinase/glucose-6-phosphate dehydrogenase procedure with a commercial enzymatic UV-method (D-Glucose, R-Biopharm, Darmstadt, Germany). Glucose molecules were cleaved by the enzymes hexokinase and glucose-6-phosphate dehydrogenase with the formation of NADPH. The amount of formed NADPH is equivalent to that of glucose and was measured in a spectral photometer (UVmc2, Safas, Monaco, Monaco) at a wavelength of 340 nm. The reaction and calculation were carried out according to the instruction manual of the manufacturer.

3.6.4 Determination of TiO_2

Determination of titanium dioxide (TiO_2) in feed and faeces samples was carried out according to Brandt & Allam (1987). TiO_2 was solubilised by concentrated sulphuric acid and formed with hydrogen peroxide a stabile yellow product. This product could be measured at 405 nm.

Samples (0.5 g) were weighed into Kjeldahl-flasks with the addition of 10 g K_2SO_4, 2.5 ml $CuSO_4$ (10 %) and 20 ml concentrated H_2SO_4 (K_2SO_4 and $CuSO_4$ served as catalysts). The mixture was boiled for 160 minutes (Turbotherm. C. Gerhardt, Königswinter, Germany), filled up to 250 ml with distilled water and then filtered (595 1/2, Whatman, Dassel, Germany). For measuring TiO_2 content, 1.0 ml of filtered sample was mixed in a cuvette with 100 µl of a mixture consisting of 40 ml H_2O_2 (35 %), 120 ml H_3PO_4 (85 %),

200 ml concentrated H_2SO_4 and 360 ml double distilled water. Due to reaction with H_2O_2 the yellow product was formed and samples were measured after 30 minutes at 405 nm using a spectral photometer (UVmc2, Safas, Monaco, Monaco). Sample blanks were measured following the same procedure as described above, only H_2O_2 in the mixture was replaced by distilled water. Sulphate solutions with known TiO_2 contents were used to create a calibration curve.

3.6.5 Determination of rumen physiological parameters

3.6.5.1 Rumen fluid pH-value

Determination of rumen fluid pH-value followed straight after withdrawal of rumen fluid using a calibrated pH meter (CG 842, Schott, Mainz, Germany).

3.6.5.2 Ammonia-nitrogen in rumen fluid

For determination of ammonia nitrogen, rumen fluid samples were prepared as described earlier. Frozen samples were thawed and diluted 1:10 with distilled water. The amount of ammonia was measured using a commercial enzymatic UV-method (Ammonia, Randox Laboratories, Crumlin, County Antrim, UK) following the principle that ammonia combines with α-ketoglutarate and NADPH in the presence of glutamate dehydrogenase yielding glutamate and $NADP^+$. The corresponding decrease in absorbance at 340 nm is proportional to ammonia concentration and was measured using a spectral photometer (UVmc2, Safas, Monaco, Monaco). Reaction and calculation were carried out according to the instruction manual of the manufacturer.

3.6.5.3 Volatile fatty acids in rumen fluid

For determination of volatile fatty acids, rumen fluid samples were prepared as described earlier. Detected volatile fatty acids were acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid. Centrifuged, frozen rumen fluid samples were thawed and 250 µl were mixed with 850 µl internal standard. The internal standard consisted of 100 µl 2-methyl valeric acid diluted with meta-phosphoric acid (2.0 %) to a volume of 250 ml. The mixture was filtered through a syringe filter with a 0.2 µm membrane and analysed in a gas chromatograph (Clarus 580, PerkinElmer, Waltham, Massachusetts, USA) with a flame ionisation detector. If measured values were very high, the internal standard was mixed with 125 µl sample and 125 µl meta-phosphoric acid. The free fatty acid test standard (Restek, Bellefonte, Pennsylvania, USA), consisting of the six

analysed volatile fatty acids, served as external standard and was used in the same way as the samples. A capillary column with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 µm was used (PerkinElmer, Waltham, Massachsusetts, USA). Nitrogen served as carrier gas, the injector temperature was 220 °C, the detector temperature 275 °C and temperature in the oven raised from 100 °C to 235 °C.

3.6.6 Determination of rumen microbiota

Rumen fluid samples for determination of rumen microbiota were prepared as described earlier. Total genomic DNA was extracted from samples using the FastDNA SPIN Kit for faeces (MP Biomedicals, Santa Ana, California, USA). The extraction was carried out according to the instruction manual of the manufacturer, with the exception that 100 mg of lyophilised rumen fluid was weighed directly into the lysing tubes containing silica beads and lysis buffer. Cells were lysed using the FastPrep system (FastPrep 24, MP Biomedicals, Santa Ana, California, USA). Purified DNA was eluted with 100 µl of TES buffer. DNA amount and purity was assessed after extraction by spectrophotometry using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and the elution buffer as blank. DNA extracts were stored at -20 °C until further analyses.

Primers were obtained from the following literature: total bacteria (Edwards et al., 2007), *Prevotella spp*. and *Streptococcus bovis* (Stevenson & Weimer, 2007), *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, anaerobic fungi (Denman & McSweeney, 2006), archaea (Stahl & Amann, 1991; Großkopf et al., 1998), protozoa (Sylvester et al., 2004) and synthesised by Eurofins (Ebersberg, Germany). Primers were designed to 16S rRNA gene sequences for bacteria and for 18S rRNA gene sequences for protozoa and anaerobic fungi, respectively. In order to determine optimal reaction conditions a gradient PCR was carried out for each primer system with different annealing temperatures of 53.5 - 63.6 °C and different samples dilutions (1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280) using the Bio-Rad CFX Connect Real-Time System and software (Bio-Rad Laboratories, Hercules, California, USA). Detection was based on the SYBR green chemistry. Reaction for primer test was run in total volume of 15 µl in 96-well reaction plates (4titude, Wotton, Surrey, England). Reaction mix was composed of 7.5 µl SensiFAST SYBR No-ROX Mix (Bioline Reagents, London, England), 0.6 µl of each forward and reverse primer (100 pmol/µl), 4.8 µl nuclease-free water, and 1.5 µl DNA extract. Amplification programs consisted of an initial denaturation for 5 minutes at 95 °C followed by 30 cycles of 95 °C for 20 seconds, different annealing temperatures (53.5 - 63.6 °C) for 60 seconds and 72 °C for 30 seconds. A final melting curve analysis (from 60 °C to 95 °C) was performed for verification of specific amplification. Primer

systems used in the present study and corresponding annealing temperatures are presented in Table 7.

For generating standards for absolute quantification, PCR products from primer tests were purified (MinElute PCR Purification Kit, Quiagen, Hilden, Germany) and run for 60 minutes at 100 volt on a 2.0 % agarose gel according to manual instuctions (peqGold Universal Agarose, Peqlab, Erlangen, Germany) in a 1:50 dilution of a TAE buffer (pH: 8.0; composition TAE buffer: 242 g TRIS, 57,1 ml acetate, and 100 ml EDTA (0.5 mol/l) filled up to 1 l with double distilled water). PCR products were visualised by SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, California, USA), for verification of specific amplification and estimating the amplicon size using the peqGold 50 bp DNA ladder (Peqlab, Erlangen, Germany). PCR product bands were cut out from gel and extracted using the innuPREP Gel Extraction Kit (Analytik Jena, Jena, Germany) according to manual instructions and amplified with similar reaction conditions as used for the primer test and the specific annealing temperatures in the Bio-Rad T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, California, USA). Afterwards, PCR products were purified again (MinElute PCR Purification Kit, Quiagen, Hilden, Germany) and DNA amount of the eluate was measured using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Copy number of DNA fragments per µl was calculated according to Whelan et al. (2003) using following equation:

$$\text{Copy number per µl} = \frac{\text{DNA amount (ng/µl)} \times 6.022 \times 10^{23}\ (\text{mol}^{-1})}{\text{amplicon length (bp)} \times 660\ (\text{g/mol/bp}) \times 10^{9}}$$

Dilution standards ranging from 10^1 to 10^8 copy numbers per µl were generated and used to create calibration curves for absolute quantification of samples. The qPCR was carried out using the Bio-Rad CFX Connect Real-Time System and software (Bio-Rad Laboratories, Hercules, California, USA) with the same composition of reaction mix and amplification protocol as described for the primer test, only with a change to the specific annealing temperature of primers. Standards used for PCR efficiency calculations were run on the same plate as the samples. Standards, samples and a negative control were run in duplicates. Only assays with efficiencies between 90 % and 105 % and with coefficients of determination of the calibration curve (R^2) > 0.99 were consulted for calculation. A minimum range of 5 consecutive orders of magnitude $\log_{10}$ concentrations of dilution standards were used to create the calibration curve.

Number of copies in samples was calculated as follows:

$$GC = (SQ \times DV \times EV) / (W \times SV)$$

GC = gene copies (copy number / g dry matter)

SQ = starting quantity of the amplicon (copies/µl)

DV = dilution volume (µl)

EV = elution volume (µl)

W = sample weight subjected to DNA extraction (g)

SV = sample volume subjected to reaction (µl)

Copy numbers of measured microorganisms are expressed as $\log_{10}$ counts of detected target genes per g dry matter of rumen fluid.

Table 7: Primers used in this study for qPCR analysis

Target species	**Primer sequences (5' - 3')**	**Amplicon length (bp)**	**Annealing temperature (°C)**
Total bacteria	AGCAGCCGCGGTAAT CAGGGTATCTAATCCTGTT	280	61.9
Prevotella spp.	GGTTCTGAGAGGAAGGTCCCC TCCTGCACGCTACTTGGCTG	121	60.0
Fibrobacter succinogenes	GTTCGGAATTACTGGGCGTAAA CGCCTGCCCCTGAACTATC	121	57.5
Ruminococcus flavefaciens	CGAACGGAGATAATTTGAGTTTACTTAGG CGGTCTCTGTATGTTATGAGGTATTACC	132	60.0
Streptococcus bovis	TTCCTAGAGATAGGAAGTTTCTTCGG ATGATGGCAACTAACAATAGGGGT	127	54.3
Archaea	ACKGCTCAGTAACACGT GTGCTCCCCCGCCAATTCCT	826	63.6
Protozoa	GCTTTCGWTGGTAGTGTATT CTTGCCCTCYAATCGTWCT	223	55.6
Anaerobic fungi	GAGGAAGTAAAAGTCGTAACAAGGTTTC CAAATTCACAAAGGGTAGGATGATT	120	61.9

3.7 Calculations

3.7.1 Calculation of dry matter and crude nutrient disappearance

Disappearance of dry matter was calculated for all feedstuffs and treatments at each incubation time. The disappearance of selected crude nutrients was calculated at 1, 3, 6, 9, 12, and 24 h of incubation. Crude protein disappearance was determined for maize silage, maize grain, and soybean meal. Starch disappearance was determined for maize silage and maize grain and disappearance of NDF was determined based on grass silage. The disappearance was calculated using following equation:

$$\text{Disappearance (\%)} = \frac{\text{weighed-in quantity (g)} - \text{weighed-out quantity (g)}}{\text{weighed-in quantity (g)}} \times 100$$

Further calculations, rested upon the dry matter or crude nutrient disappearance, of parameters of degradability and effective degradability based on the exponential model of Ørskov & McDonald (1979).

3.7.2 Estimation of parameters of degradability

The parameters of degradation were estimated using the equation of McDonald (1981):

$$p = a + b\,(1 - e^{-c(t - t0)}) \quad \text{for } t > t_0,$$

where p is the disappearance at time t, a is the soluble fraction, b is the fraction not soluble but ruminal degradable, c is the constant rate of degradation of b, t is the time of incubation and t_0 the lag-time (time from start of incubation to the beginning of degradation of fraction b). Sum of a + b gives the degradable fraction d.

The parameters of degradability were estimated by an iterative NLIN-procedure of SAS (SAS 9.4, SAS Institute, Cary, USA) based on the standard algorithm of Marquardt (1963).

3.7.3 Calculation of effective degradability

The effective degradability (ED) was calculated using following equation of Wulf & Südekum (2005), which is a modification of the one by McDonald (1981):

$$ED = a + [(b \times c) / (c + k)]\ e^{-kt0},$$

where k is the estimated solid rumen outflow rate (rate of passage) and a, b, c, and t_0 are the same parameters as described above.

To evaluate the enzyme supplementation on different feed intake levels, the ED was calculated for different rates of passage. The assumed solid rumen outflow rates were 2, 5, and 8 %/h. Whereby, a passage rate of 2 %/h represents a low, 5 %/h a medium and 8 %/h a high level of feed intake (Agricultural Research Council, 1984). It is important to assume different feed intake levels, because with increasing feed intake the retention time of feed inside the rumen is reduced and this leads to a decrease in ruminal degradation.

3.7.4 Calculation of total tract digestibility

Apparent total tract digestibility of the single ingredients was calculated based on a marker study to investigate the effects of amylase and protease supplementation on the digestibility of the entire ration. Therefor a non-digestible and non-absorbable marker, in this case TiO_2, was mixed to the TMR in a proportion of 0.1 % (on DM basis). Sampling of faeces was carried out during the last seven days of the measuring period. Approximately 200 g per animal and day were collected between 07:00 h and 09:00 h and were directly frozen at -20 °C. Prior to analysis faeces samples were lyophilised, pooled per animal and ground. The apparent total tract digestibility was determined for dry matter, organic matter, crude fibre, crude protein, total lipids, nitrogen-free extracts, crude ash, neutral detergent fibre, acid detergent fibre, and starch using the following equation:

$$\text{Digestibility (\%)} = 100 - \frac{\text{marker in feed (\%)} \times \text{ingredient in faeces (\%)}}{\text{marker in faeces (\%)} \times \text{ingredient in feed (\%)}} \times 100$$

3.8 Statistics

Data were statistically analysed by 2-way analysis of variance with the effects of treatment and animal using the GLM-procedure of SAS software (SAS 9.4, SAS Institute, Cary, North Carolina, USA), followed by a Duncan-test (Duncan, 1955). Significance level was $p < 0.05$. Following statistical model was used:

$$y_{ij} = \mu + treatment_i + animal_j + e_{ij}$$

y_{ij}	= observation
μ	= overall mean
$treatment_i$	= effect of treatment
$animal_j$	= effect of animal
e_{ij}	= residual error
i	= index of treatment (1 - 4)
j	= index of animal (1 - 8)

The following Tables show the means of all animals with the appropriate standard deviations (SD). Furthermore, p-values and the standard error of means (SEM), representing the pooled standard error of the respective general linear model, are depicted. Different superscripts indicate significant differences between treatment means.

4 Results

The following passage shows the results of the present study. First of all, the rumen physiological parameters in the rumen fluid (pH-value, ammonia-nitrogen, and volatile fatty acids) are elucidated. After that, the ruminal degradation characteristics of the different feedstuffs (maize grain, soybean meal, maize silage, grass silage, hay, and the TMR) are presented. Afterwards, results concerning the rumen microbiota and finally total tract digestibility are shown. The following Tables show the means of the eight experimental animals.

4.1 Effects of amylase and protease supplementation on rumen physiological parameters

Rumen fluid samples were collected at the time of the morning feeding (0 h) and 1, 2, 3, 4, 5, 6, and 9 h after feeding for determination of rumen pH-value, ammonia-nitrogen and volatile fatty acids. Figure 1 shows the time course of ammonia-nitrogen and volatile fatty acid concentrations in the rumen fluid (means across treatments). Ammonia-nitrogen concentration showed a strong and significant increase in rumen fluid already 1 h after feeding due to microbial protein degradation inside the rumen. This effect could also be observed for volatile fatty acids. Concentrations of acetic acid, propionic acid, butyric acid, and valeric acid in the rumen fluid increased significantly after feeding. During the time course the concentrations in the rumen fluid decreased and after 9 h (right before the second feeding) the concentrations of volatile fatty acids and ammonia-nitrogen in the rumen fluid reached almost the basal level of 0 h. These courses are typical for rumen physiological parameters in the rumen fluid after feeding. However, the subject of the investigation was to examine whether enzyme treatments led to significant differences in rumen physiological parameters at defined times after feeding. These findings are shown in the following passage.

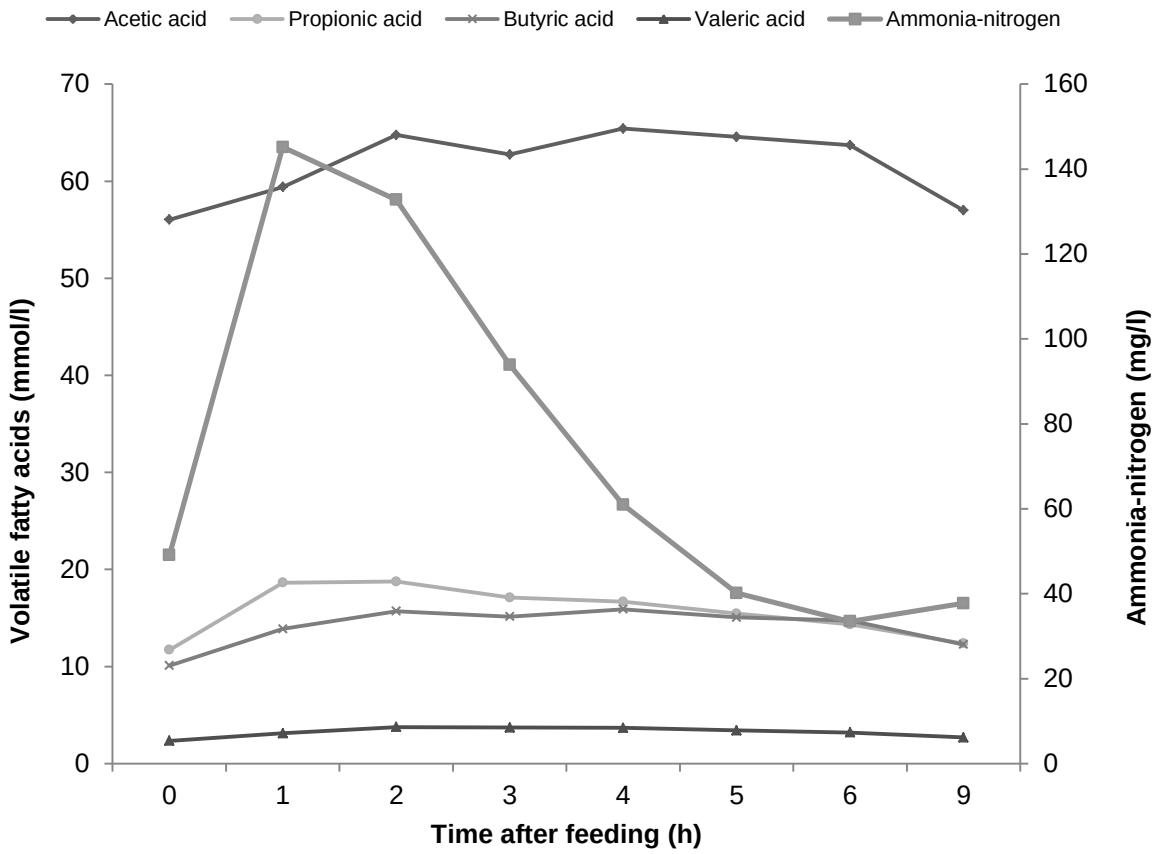

Figure 1: Time course of ammonia-nitrogen and volatile fatty acid concentrations in the rumen fluid (means across treatments)

Following p-values deriving from 2-way ANOVA indicating significant differences between sampling time (0 h: time of morning feeding): acetic acid ($p < 0.0001$, SEM 1.13), propionic acid ($p < 0.0001$, SEM 0.35), butyric acid ($p < 0.0001$, SEM 0.33), valeric acid ($p < 0.0001$, SEM 0.08), ammonia-nitrogen ($p < 0.0001$, SEM 3.86)

4.1.1 pH-value

The rumen fluid pH-values are represented in Table 8. The enzyme supplementation had no significant effects on the pH-value of the rumen fluid. The measurements showed a typical time course of rumen pH-values. After feeding a decrease of the rumen pH was observed. The minimum for the different treatments was between two and five hours after feeding. After that, the rumen pH increased and reached nine hours after feeding almost the base level of the morning feeding. No critical values, concerning rumen acidosis, were observed.

The supplementation with amylase and protease showed no significant effects on the rumen pH-value.

Table 8: pH-value in the rumen fluid (± SD) dependent on treatment and sampling time

Time after feeding	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
0 h	6.90 ±0.1	6.92 ±0.3	6.93 ±0.1	6.90 ±0.2	0.05	0.966
1 h	6.73 ±0.4	6.69 ±0.2	6.74 ±0.3	6.63 ±0.3	0.11	0.851
2 h	6.59 ±0.4	6.60 ±0.3	6.79 ±0.2	6.63 ±0.2	0.09	0.353
3 h	6.68 ±0.2	6.51 ±0.3	6.70 ±0.2	6.63 ±0.3	0.07	0.212
4 h	6.67 ±0.5	6.63 ±0.7	6.74 ±0.4	6.58 ±0.4	0.16	0.894
5 h	6.64 ±0.3	6.58 ±0.5	6.68 ±0.3	6.61 ±0.4	0.06	0.623
6 h	6.64 ±0.4	6.70 ±0.4	6.76 ±0.3	6.59 ±0.5	0.10	0.556
9 h	6.81 ±0.2	6.82 ±0.2	6.90 ±0.2	6.90 ±0.1	0.05	0.328

0 h: time of morning feeding

4.1.2 Ammonia-nitrogen

Table 9 shows the ammonia-nitrogen (NH_3-N) concentrations in the rumen fluid. No effect of the enzyme supplementation on the NH_3-N concentrations could be observed. During the first two hours after feeding an increase of the NH_3-N concentration was determined. After that, a decline of the NH_3-N content in the rumen fluid took place and resulted, nine hours after feeding, even in lower values than the base level of the morning feeding.

The concentration of NH_3-N in the rumen fluid was not significantly affected by the supplementation of amylase and protease.

Table 9: Ammonia-nitrogen concentration (mg/l) in the rumen fluid (± SD) dependent on treatment and sampling time

Time after feeding	Treatment				SEM	p-value
	Con - -	**Amy** 300 KNU -	**Prot** - 15000 PROT	**Amy+Prot** 150 KNU 7500 PROT		
0 h	48.3 ±16.1	48.1 ±14.8	54.5 ±17.6	45.4 ±12.0	4.97	0.576
1 h	143 ±24.9	141 ±35.5	150 ±13.6	145 ±16.2	8.98	0.894
2 h	145 ±46.7	123 ±21.6	132 ±17.0	129 ±31.3	11.9	0.579
3 h	100 ±16.6	86.9 ±38.5	89.4 ±17.5	99.2 ±27.6	8.82	0.585
4 h	54.2 ±20.1	65.8 ±23.5	66.2 ±28.7	57.7 ±31.7	8.80	0.664
5 h	36.7 ±26.9	42.8 ±21.4	41.4 ±19.9	39.6 ±19.9	6.14	0.890
6 h	31.4 ±15.5	38.8 ±23.0	32.6 ±24.3	31.0 ±14.3	6.60	0.796
9 h	39.3 ±15.4	34.4 ±15.6	43.1 ±17.5	34.1 ±13.2	4.63	0.418

0 h: time of morning feeding

4.1.3 Volatile fatty acids

The rumen fluid samples also served for the determination of the rumen volatile fatty acid concentration. The amounts of acetic acid, propionic acid, butyric acid, and valeric acid were measured. Additionally, the quantity of total rumen volatile fatty acids and the ratio of acetic to propionic acid are depicted in the following Tables.

4.1.3.1 Acetic acid

Table 10 shows the acetic acid concentration in the rumen fluid. The enzyme supplementation had no significant effect on the concentration of acetic acid in the rumen. At the point of feeding (0 h), the different treatments showed almost identical values of acetic acid concentration. The basal level was 56.0 mmol/l (overall mean). After feeding, an increase of acetic acid concentration was observed. The concentration reached peaks between two and five hours after feeding. During this time frame, the values stayed relatively constant and ranged from 60.1 to 68.0 mmol/l. After five hours the acetic acid concentration decreased and achieved after nine hours a concentration, slightly above the basal level, of 57.0 mmol/l (overall mean).

The supplementation with amylase and protease showed no significant effects on the concentration of acetic acid in the rumen fluid.

Table 10: Acetic acid concentration (mmol/l) in the rumen fluid (± SD) dependent on treatment and sampling time

Time after feeding	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
0 h	56.2 ±7.0	55.7 ±7.8	56.0 ±6.1	56.2 ±4.3	1.59	0.995
1 h	59.7 ±8.1	59.4 ±5.8	58.0 ±8.2	60.5 ±7.1	1.64	0.698
2 h	66.1 ±9.2	65.3 ±10.4	61.2 ±6.2	66.5 ±7.9	1.99	0.197
3 h	63.4 ±8.4	65.8 ±5.6	61.6 ±11.5	60.1 ±4.5	2.62	0.418
4 h	64.9 ±13.1	64.3 ±9.7	64.4 ±7.9	68.0 ±13.3	2.93	0.804
5 h	65.8 ±10.5	66.6 ±13.8	64.1 ±10.8	61.8 ±12.3	2.36	0.458
6 h	65.8 ±10.4	62.8 ±10.6	60.1 ±7.1	66.2 ±10.8	2.32	0.186
9 h	58.6 ±5.1	57.8 ±6.8	56.7 ±7.4	54.9 ±3.9	1.58	0.352

0 h: time of morning feeding

4.1.3.2 Propionic acid

The concentration of propionic acid is shown in Table 11. The enzyme supplementation had no significant effects on the propionic acid concentration in the rumen fluid. At the moment of feeding (0 h), only nominal differences between the treatments existed and the overall mean was 11.7 mmol/l. After that, a rapid and strong rise about 60.1 % up to 18.7 mmol/l (overall mean) within the next two hours was observed. A slow decrease in the propionic acid concentration took place, subsequently, and ended in an overall mean of 12.4 mmol/l nine hours after feeding.

The supplementation with amylase and protease showed no significant effects on the concentration of propionic acid in the rumen fluid.

Table 11: Propionic acid concentration (mmol/l) in the rumen fluid (± SD) dependent on treatment and sampling time

Time after feeding	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
0 h	11.6 ±1.8	11.9 ±2.5	11.8 ±2.5	11.5 ±1.7	0.42	0.921
1 h	18.7 ±2.1	18.2 ±1.2	18.5 ±3.1	19.2 ±2.7	0.71	0.784
2 h	18.9 ±3.9	18.6 ±2.7	18.1 ±2.2	19.3 ±3.3	0.77	0.682
3 h	16.9 ±2.2	17.8 ±3.1	16.6 ±3.1	17.1 ±2.9	0.54	0.397
4 h	15.8 ±3.4	18.0 ±6.2	16.4 ±2.9	16.5 ±3.1	1.15	0.538
5 h	15.9 ±3.1	15.6 ±4.3	15.4 ±2.9	15.0 ±3.6	0.60	0.729
6 h	14.7 ±2.8	14.0 ±2.9	13.8 ±2.1	14.8 ±3.3	0.53	0.454
9 h	12.9 ±1.7	12.4 ±2.0	12.5 ±2.1	11.7 ±1.8	0.45	0.284

0 h: time of morning feeding

4.1.3.3 Butyric acid

Table 12 represents the concentration of butyric acid in the rumen fluid. Statistical significant differences between treatments were observed right before the morning feeding (0 h) and nine hours after feeding (right before the second feeding). At the point of feeding, the control showed a significant higher concentration of butyric acid in the rumen fluid than the supplementation with amylase and the combination of both enzymes. Butyric acid concentration of all enzyme treatments was significantly decreased compared to the control nine hours after feeding. Regarding the remaining times no significant differences between treatments were observed. The concentration of butyric acid increased after feeding and reached a maximum of 15.9 mmol/l (overall mean) four hours after feeding.

The concentration of butyric acid was only affected by the enzyme treatments straight before the morning feeding and 9 h after feeding. Right before the morning feeding butyric acid concentration was significantly decreased by the supplementation with amylase and the combination of both enzymes. Butyric acid concentration of even all enzyme treatments was significantly decreased compared to the control, 9 h after feeding.

Table 12: Butyric acid concentration (mmol/l) in the rumen fluid (± SD) dependent on treatment and sampling time

Time after feeding	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
0 h	10.7[a] ±1.9	9.58[b] ±1.4	10.4[ab] ±2.1	9.72[b] ±1.0	0.32	0.042
1 h	14.2 ±1.6	13.6 ±1.6	13.7 ±2.3	13.9 ±2.0	0.43	0.756
2 h	16.3 ±3.7	15.6 ±2.5	15.2 ±2.0	15.8 ±2.5	0.64	0.577
3 h	15.7 ±2.7	16.0 ±2.3	14.6 ±3.0	14.2 ±1.3	0.53	0.055
4 h	15.9 ±3.5	17.2 ±6.5	15.6 ±2.5	15.0 ±3.3	1.17	0.550
5 h	16.3 ±4.2	15.3 ±4.0	15.1 ±2.6	13.5 ±2.7	0.79	0.092
6 h	15.6 ±3.1	14.5 ±3.2	14.0 ±2.2	14.9 ±4.0	0.70	0.400
9 h	13.3[a] ±1.8	12.0[b] ±1.4	12.2[b] ±1.8	11.6[b] ±1.7	0.32	0.010

0 h: time of morning feeding; different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective sampling time

4.1.3.4 Valeric acid

The valeric acid concentration in the rumen fluid is shown in Table 13. Supplementation of amylase and protease had no significant effects on the concentration of valeric acid. The concentration started with 2.35 mmol/l, right before the morning feeding and increased quickly about 60.0 %, to a maximum of 3.76 mmol/l two hours after feeding (overall means). Subsequently, the concentration of valeric acid slightly declined and reached an overall mean of 2.70 mmol/l nine hours after feeding.

The supplementation with amylase and protease showed no significant effects on the concentration of valeric acid in the rumen fluid.

Table 13: Valeric acid concentration (mmol/l) in the rumen fluid (± SD) dependent on treatment and sampling time

Time after feeding	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
0 h	2.42 ±0.4	2.29 ±0.4	2.36 ±0.6	2.31 ±0.4	0.08	0.679
1 h	3.06 ±0.6	3.16 ±0.5	3.08 ±0.7	3.17 ±0.6	0.19	0.965
2 h	3.81 ±0.9	3.70 ±0.6	3.67 ±0.5	3.87 ±0.7	0.18	0.803
3 h	3.75 ±0.6	3.69 ±0.6	3.71 ±0.9	3.68 ±0.6	0.19	0.994
4 h	3.52 ±1.0	3.78 ±1.1	3.77 ±0.8	3.64 ±0.7	0.20	0.748
5 h	3.54 ±0.8	3.52 ±0.9	3.49 ±0.9	3.14 ±0.8	0.13	0.112
6 h	3.35 ±1.0	3.16 ±0.6	2.96 ±0.5	3.37 ±1.1	0.18	0.291
9 h	2.80 ±0.5	2.77 ±0.6	2.65 ±0.6	2.56 ±0.5	0.12	0.444

0 h: time of morning feeding

4.1.3.5 Total volatile fatty acids

Table 14 shows the total volatile fatty acid (VFA) concentration (sum of all measured VFAs) in the rumen fluid. No statistical significant effect of the enzyme supplementation on the total VFA concentration was observed. The amount of VFA in the rumen fluid started with a base level of 80.2 mmol/l, right before the morning feeding and increased about 28.4 % to a maximum of 103.0 mmol/l two hours after feeding (overall means). After that, the concentration of total VFA slowly decreased to 84.3 mmol/l, slightly above the base level.

The supplementation with amylase and protease showed no significant effects on the amount of VFA in the rumen fluid.

Table 14: Total volatile fatty acid concentration (mmol/l) in the rumen fluid (± SD) dependent on treatment and sampling time

Time after feeding	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
0 h	81.0 ±10.3	79.5 ±11.3	80.5 ±10.9	79.7 ±6.5	2.05	0.940
1 h	95.7 ±11.7	94.4 ±8.0	93.3 ±12.8	96.7 ±11.5	2.63	0.768
2 h	105 ±16.9	103 ±15.4	98.2 ±9.4	105 ±13.5	3.16	0.300
3 h	99.9 ±13.0	103 ±10.4	96.5 ±17.4	95.1 ±6.9	3.39	0.295
4 h	100 ±20.4	99.1 ±17.0	100 ±13.3	103 ±19.9	4.36	0.953
5 h	101 ±17.6	101 ±22.4	98.2 ±16.4	93.4 ±18.2	3.17	0.232
6 h	99.5 ±16.8	94.4 ±17.2	90.9 ±11.3	99.2 ±18.7	3.45	0.212
9 h	87.5 ±8.2	85.0 ±10.4	84.1 ±11.4	80.7 ±6.1	2.07	0.128

0 h: time of morning feeding

4.1.3.6 Acetic to propionic acid ratio

Table 15 shows the acetic to propionic acid ratio. The supplementation with amylase and protease had no statistical significant effects on the ratio between acetic and propionic acid. The acetic to propionic acid ratio showed almost identical curves over the course of time. The ratio started with a base level of 4.87 and then dropped to minimum of 3.20 within one hour (overall means). Subsequently, the ratio between acid and propionic acid constantly increased up to the time of the second feeding (4.66).

The acetic to propionic acid ratio was not significantly affected by one of the enzyme treatments.

Table 15: Acetic to propionic acid ratio in the rumen fluid (± SD) dependent on treatment and sampling time

Time after feeding	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
0 h	4.87 ±0.4	4.77 ±0.6	4.89 ±0.8	4.94 ±0.6	0.14	0.828
1 h	3.19 ±0.3	3.27 ±0.3	3.17 ±0.4	3.18 ±0.3	0.09	0.845
2 h	3.55 ±0.5	3.52 ±0.5	3.41 ±0.4	3.47 ±0.4	0.13	0.834
3 h	3.76 ±0.4	3.76 ±0.5	3.76 ±0.6	3.59 ±0.6	0.15	0.781
4 h	4.13 ±0.4	4.15 ±0.5	3.98 ±0.5	4.15 ±0.3	0.12	0.703
5 h	4.20 ±0.5	4.36 ±0.5	4.19 ±0.5	4.22 ±0.7	0.17	0.860
6 h	4.52 ±0.4	4.51 ±0.4	4.39 ±0.5	4.55 ±0.5	0.13	0.791
9 h	4.60 ±0.5	4.68 ±0.4	4.60 ±0.6	4.78 ±0.6	0.14	0.719

0 h: time of morning feeding

4.2 Effects of amylase and protease supplementation on ruminal degradation characteristics

During the *in situ* trial the effects of amylase and protease supplementation to ruminant diet were verified by the examination of all components of the TMR regarding their ruminal degradability of dry matter. Some selected feedstuffs (maize grain, soybean meal, maize silage, grass silage) were examined regarding the ruminal degradability of their typical ingredients (starch, crude protein, NDF), additionally. Thereto, nutrient disappearance was measured based on a subset of incubation times of dry matter disappearance.

Figure 2 shows the ruminal dry matter disappearance of incubated feedstuffs (means across treatments). Noticeable are the differences between ruminal degradation characteristics of the single feedstuffs, for example slow degradation of fibre-rich material such as hay, or rapid degradation for example of soybean meal. The following passage shows the results of enzyme supplementation on ruminal degradation characteristics of the different feedstuffs.

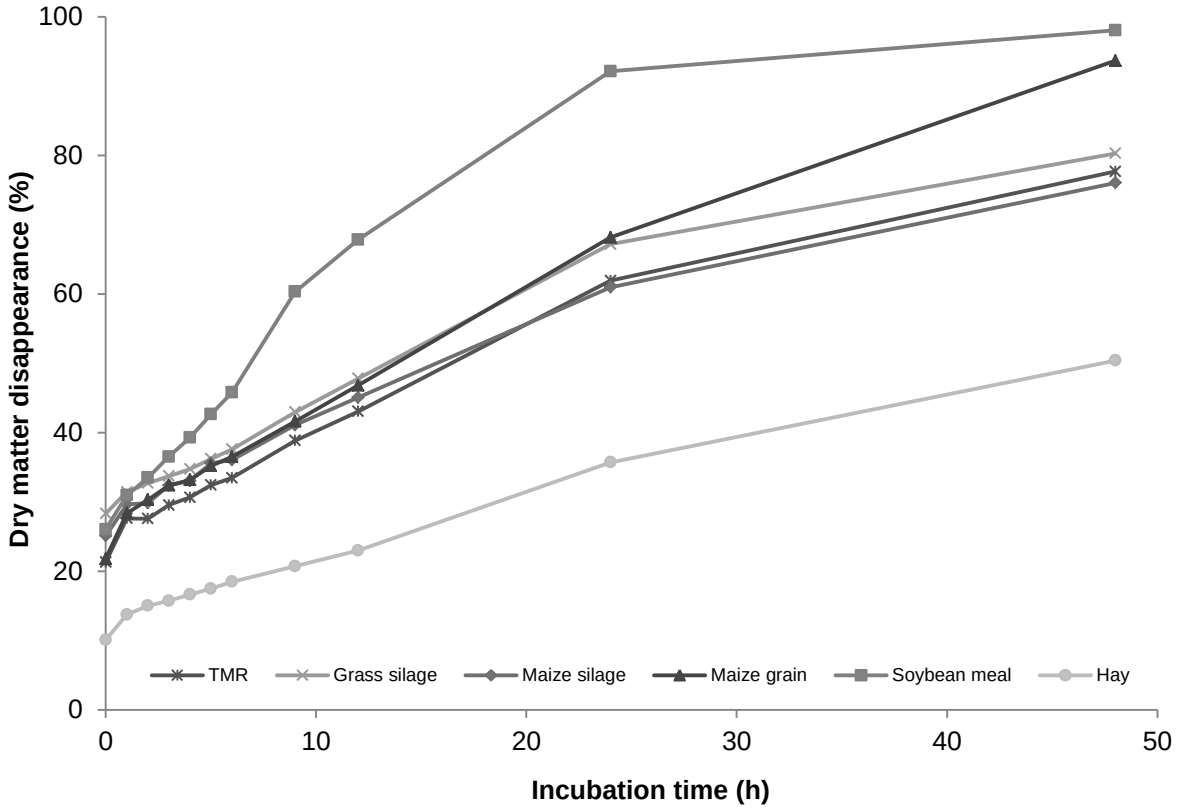

Figure 2: Ruminal dry matter disappearance of incubated feedstuffs (means across treatments)

Following p-values deriving from 2-way ANOVA indicating significant differences between incubation times: TMR ($p < 0.0001$, SEM 0.68), grass silage ($p < 0.0001$, SEM 0.59), maize silage ($p < 0.0001$, SEM 0.83), maize grain ($p < 0.0001$, SEM 0.59), soybean meal ($p < 0.0001$, SEM 0.95), hay ($p < 0.0001$, SEM 0.33)

4.2.1 Effects of amylase and protease supplementation on ruminal degradability of maize grain

Maize grain was studied concerning the effects of the different enzyme treatments on ruminal degradability of dry matter, starch, and crude protein.

4.2.1.1 Degradability of dry matter

4.2.1.1.1 *In sacco* dry matter disappearance

Table 16 shows the dry matter disappearance (DMD) of maize grain. Compared to the control, the combination of both enzyme preparations showed a significant higher dry matter disappearance from one up to 24 hours of incubation. During this period, an average increase by 6.7 % caused by the combination of amylase and protease was observed. After 48 h, no significant differences between the control and the combination of both enzymes could be noticed. Supplementation with amylase had no significant influence on the DMD of maize grain. As well as the supplementation with protease, whereas this treatment showed even a significant lower dry matter disappearance between two and six hours, compared to the control. Thus, the combination of both enzyme preparations led to a significant higher DMD of maize grain than the amylase treatment from one up to 12 hours and to a higher DMD than the supplementation with protease from one up to 24 hours.

The supplementation with the combination of amylase and protease showed a significant higher ruminal dry matter degradation of maize grain than the control. The supplementation of amylase and protease exclusively showed no significant influence on the dry matter degradability of maize grain.

Table 16: Ruminal dry matter disappearance (%) of maize grain (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	28.2^{b} ±1.2	27.7^{b} ±1.0	27.4^{b} ±2.0	30.2^{a} ±2.0	0.28	< 0.0001
2 h	30.2^{b} ±1.4	29.9^{b} ±1.2	28.9^{c} ±1.2	32.3^{a} ±1.1	0.20	< 0.0001
3 h	31.9^{b} ±2.4	31.6^{bc} ±1.6	30.9^{c} ±1.5	35.1^{a} ±1.9	0.33	< 0.0001
4 h	33.3^{b} ±2.3	32.7^{b} ±2.1	31.6^{c} ±3.1	35.1^{a} ±1.9	0.37	< 0.0001
5 h	35.2^{b} ±2.0	34.9^{b} ±1.6	33.6^{c} ±1.6	37.2^{a} ±2.5	0.30	< 0.0001
6 h	36.8^{b} ±2.5	36.0^{b} ±2.7	34.5^{c} ±2.0	38.8^{a} ±2.1	0.35	< 0.0001
9 h	41.3^{b} ±3.4	39.8^{c} ±2.9	41.5^{b} ±3.2	43.8^{a} ±3.5	0.37	< 0.0001
12 h	46.3^{b} ±4.5	45.1^{b} ±4.3	46.0^{b} ±4.4	49.8^{a} ±5.2	0.57	< 0.0001
24 h	67.4^{bc} ±4.7	69.0^{ab} ±8.0	65.1^{c} ±5.6	71.2^{a} ±4.8	0.93	< 0.0001
48 h	94.5^{a} ±2.8	92.0^{b} ±6.2	94.5^{a} ±2.1	93.6^{ab} ±3.5	0.62	0.011

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.1.1.2 Parameters of degradability and effective degradability

Table 17 shows the parameters of degradability (a, b, c, d, t_0) and the effective degradability (ED) of dry matter of maize grain. The soluble fraction (a) was significantly increased by the combination of amylase and protease (24.4 %) compared to the control (22.1 %), without a concomitant decrease of the ruminal degradable fraction (b). Both, supplementation with amylase and supplementation with protease did not affect the soluble fraction of the dry matter of maize grain. There was no further parameter which was significantly influenced by one of the treatments.

The parameters of degradability predominantly clarify that supplementation of the combination of amylase and protease led to a significant increase of the rapid degradable or soluble fraction (a) in maize grain.

Table 17: Parameters of degradability and effective degradability of dry matter of maize grain (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	22.1[b] ±0.8	21.9[b] ±0.9	21.6[b] ±0.3	24.4[a] ±1.0	0.27	< 0.0001
b (%)	75.9 ±4.9	77.2 ±3.0	78.4 ±0.3	75.6 ±1.0	1.13	0.241
c (%/h)	4.02 ±0.6	3.78 ±0.5	3.69 ±0.3	3.99 ±0.6	0.17	0.381
d (%)	98.0 ±5.6	99.1 ±2.5	100 ±0.0	100 ±0.0	1.18	0.549
t_0 (h)	0.06 ±0.1	0.04 ±0.1	0.12 ±0.2	0.00 ±0.0	0.05	0.444
Effective degradability						
ED2 (%)	72.5[b] ±2.5	72.1[b] ±2.9	72.2[b] ±1.3	74.6[a] ±2.0	0.67	0.036
ED5 (%)	55.6[b] ±1.6	54.9[b] ±2.7	54.6[b] ±1.5	57.8[a] ±2.2	0.51	0.0004
ED8 (%)	47.2[b] ±1.3	46.5[b] ±2.3	46.0[b] ±1.4	49.4[a] ±2.0	0.40	< 0.0001

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

The effective degradability was calculated for rumen passage rates of 2, 5, and 8 %/h (ED2, ED5, ED8), respectively. The ED of dry matter of maize grain was increased by the combination of amylase and protease for all different feed intake levels. The other treatments (Amy, Prot) did not affect the ED of the dry matter of maize grain. At a low rate of passage (ED2), a significant increase by 2.9 % towards the control could be achieved by the combination of amylase and protease. This effect is becoming clearer with a raise of feed intake and resulted in an increase by 4.7 % of the combination of both enzyme products compared to the control for ED8.

The calculated effective degradability of dry matter of maize grain was significantly increased by the combination of both enzyme preparations for passage rates of 2, 5, and 8 %/h, representing low, medium, and high feed intake levels.

4.2.1.2 Degradability of starch

4.2.1.2.1 *In sacco* starch disappearance

Table 18 represents the starch disappearance of maize grain. Also in this case, the combination of amylase and protease showed a significant higher disappearance of starch than the control from six up to 24 hours of incubation. During this time frame the combination of amylase and protease increased starch disappearance by 10.6 % on average, compared to the control. After one and three hours there was a numeric, but not significant, increase caused by the combination of amylase and protease. Amylase supplementation showed no effect on the starch disappearance of maize grain, as well as the protease treatment.

The combination of amylase and protease had also significant increasing effects on the ruminal starch degradation of maize grain. Neither amylase nor protease supplementation affected starch disappearance of maize grain.

Table 18: Ruminal starch disappearance (%) of maize grain (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	28.9 ±3.2	27.9 ±3.2	27.8 ±4.9	29.7 ±3.5	1.59	0.772
3 h	30.8 ±2.4	30.4 ±1.9	30.2 ±4.2	33.8 ±3.5	1.21	0.110
6 h	34.7^{bc} ±2.2	35.5^{ab} ±2.5	33.1^{c} ±2.3	37.4^{a} ±2.1	0.79	0.005
9 h	37.8^{b} ±2.7	38.7^{b} ±2.4	39.4^{b} ±3.5	42.1^{a} ±4.1	0.95	0.016
12 h	43.5^{b} ±4.1	44.0^{b} ±3.8	44.9^{b} ±5.3	49.5^{a} ±6.9	1.38	0.014
24 h	67.6^{b} ±5.5	71.8^{ab} ±9.0	66.4^{b} ±5.2	73.9^{a} ±5.0	2.14	0.047

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.1.2.2 Parameters of degradability and effective degradability

In Table 19 the parameters of degradability and the effective degradability of starch of maize grain are shown. The soluble fraction (a) and the ruminal degradable fraction (b) were not affected by one of the treatments. The lag-time (t_0) and the degradable fraction (d) showed that the degradation of starch occurred very quickly and that the starch of maize grain is approximately completely degradable, where these parameters showed no significant differences between treatments either. The only parameter, which was affected by treatments, was the constant rate of degradation (c) of fraction b. The combination of amylase and protease showed a significant higher rate of degradation than the other enzyme treatments. Additionally, there was a considerable difference between the combination of the both enzyme preparations and the control (4.97 % vs. 3.97 %), whereas a significant effect could not be ensured.

Only the constant rate of degradation (c) of the fraction b was affected by treatments. The combination of both enzymes led to a significant higher rate of degradation than Amy and Prot, and to a numeric increase compared to the control.

Table 19: Parameters of degradability and effective degradability of starch of maize grain (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	20.5 ±1.9	20.9 ±1.5	20.9 ±2.9	20.6 ±2.2	0.88	0.983
b (%)	72.6 ±12.1	76.5 ±8.1	76.0 ±9.5	74.3 ±7.7	3.63	0.839
c (%/h)	3.97^{ab} ±1.0	3.74^{b} ±0.5	3.49^{b} ±0.3	4.97^{a} ±1.6	0.38	0.041
d (%)	93.1 ±12.8	97.4 ±7.3	96.9 ±8.8	94.9 ±9.5	3.79	0.815
t_0 (h)	0.00 ±0.0	0.03 ±0.0	0.00 ±0.0	0.00 ±0.0	0.02	0.412
Effective degradability						
ED2 (%)	67.7 ±6.0	70.6 ±5.4	69.0 ±5.0	72.4 ±4.8	1.86	0.285
ED5 (%)	51.6^{b} ±3.1	53.5^{ab} ±4.0	52.0^{b} ±3.6	56.5^{a} ±3.4	1.11	0.012
ED8 (%)	43.8^{b} ±2.1	45.2^{b} ±3.2	43.8^{b} ±3.2	48.2^{a} ±2.8	0.88	0.003

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

The highest effective degradability of all treatments was observed for the combination of amylase and protease at all different assumed passage rates. However, this observation was not significant for ED2, but ED5 and ED8 showed a significant increase of effective degradability by the combination of both enzymes compared to the control. Effective degradability at a medium passage rate (ED5) was significantly increased by 9.5 % and at a high passage rate (which is typical for a high feed intake level) by 10.1 %, related to the control. Amylase and Protease exclusively showed no effects on effective degradability.

The effective degradability of starch of maize grain was significantly increased by supplementation of amylase and protease for medium and high passage rates.

Supplementation of amylase and protease exclusively showed no effects on effective degradability of starch of maize grain.

4.2.1.3 Degradability of crude protein

4.2.1.3.1 *In sacco* crude protein disappearance

Crude protein disappearance of maize grain is represented in Table 20. Significant differences between treatments were observed at incubation times of three and nine hours. After three hours, crude protein disappearance of the combination of amylase and protease was significantly higher than crude protein disappearance of the control and of supplementation with amylase. After nine hours, the control, supplementation with protease, and the combination of amylase and protease showed a higher crude protein disappearance than amylase supplementation. Regarding the remaining times of incubation, no significant differences among treatments were observed. At short incubation times, the combination of amylase and protease showed the highest values of all treatments, after nine and 12 hours protease supplementation shows the highest values and after 24 hours the control. This indicates that no consistent effect of one of the treatments on crude protein losses of maize grain could be observed. However, a noticeable observation was that amylase supplementation showed the lowest crude protein losses of all treatments at every time of incubation.

There were only little significant differences between treatments, so no consistent effect concerning the ruminal crude protein degradation of maize grain could be observed.

Table 20: Ruminal crude protein disappearance (%) of maize grain (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	31.4 ±1.4	30.8 ±1.2	30.8 ±1.0	32.1 ±1.4	0.48	0.143
3 h	31.8^{b} ±1.7	31.4^{b} ±1.6	32.6^{ab} ±1.4	34.2^{a} ±1.0	0.59	0.008
6 h	34.4 ±1.8	32.4 ±1.3	34.5 ±2.3	35.3 ±2.7	0.82	0.088
9 h	37.3^{a} ±1.5	34.2^{b} ±0.8	38.6^{a} ±2.5	37.5^{a} ±2.0	0.66	0.0004
12 h	38.7 ±2.7	37.0 ±3.0	40.2 ±2.8	37.2 ±2.4	1.02	0.076
24 h	50.0 ±3.8	46.8 ±5.6	47.2 ±2.6	49.0 ±4.2	1.77	0.478

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.1.3.2 Parameters of degradability and effective degradability

Table 21 shows the parameters of degradability and the effective degradability of crude protein of maize grain. Regarding parameter a, the control and the combination of amylase and protease showed a significant higher soluble fraction than supplementation of amylase and protease exclusively. The control and the combination of both enzymes had also the highest ruminal degradable fraction (b), but no effects could be observed concerning the remaining parameters. The lag-time (t_0) indicates that the degradation of protein occurred straight after the incubation.

The different treatments showed no effects on the parameters of degradability of crude protein of maize grain. Admittedly, for the soluble fraction (a) some statistical differences could be observed, certainly no enzyme treatment showed positive effects rumen fermentation kinetics compared to the control.

Table 21: Parameters of degradability and effective degradability of crude protein of maize grain (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	23.7[a] ±1.3	20.6[b] ±2.7	21.0[b] ±1.8	25.0[a] ±2.2	0.69	0.0001
b (%)	38.2 ±18.9	33.5 ±20.8	26.6 ±4.6	34.9 ±19.3	6.75	0.625
c (%/h)	7.39 ±4.1	8.39 ±6.2	12.9 ±8.0	7.73 ±7.1	2.52	0.306
d (%)	61.9 ±19.5	54.1 ±21.7	47.6 ±6.1	60.0 ±20.3	7.04	0.430
t_0 (h)	0.00 ±0.0	0.00 ±0.0	0.00 ±0.0	0.00 ±0.0	0.00	-
Effective degradability						
ED2 (%)	49.9 ±7.4	44.0 ±10.1	43.3 ±4.0	48.2 ±8.8	3.20	0.361
ED5 (%)	42.3 ±2.7	37.5 ±6.1	39.1 ±2.8	41.2 ±4.8	1.77	0.213
ED8 (%)	38.4 ±1.3	34.1 ±4.8	36.3 ±2.4	37.7 ±3.7	1.34	0.111

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

Due to the parameters of degradability the control and the combination of amylase and protease showed the highest effective crude protein degradability of maize grain for all different rates of passage. However, no statistical significant effects of the enzyme supplementation on effective degradability of crude protein of maize grain could be observed.

4.2.2 Effects of amylase and protease supplementation on ruminal degradability of soybean meal

Soybean meal was studied concerning the effects of the different enzyme treatments on ruminal degradability of dry matter and crude protein.

4.2.2.1 Degradability of dry matter

4.2.2.1.1 *In sacco* dry matter disappearance

Table 22 represents the DMD of soybean meal. Regarding the amylase treatment, no significant differences could be observed compared to the control. This referred also to the combination of amylase and protease. Protease supplementation showed statistical significant differences at two times, towards the control. There was a significant increase in DMD of soybean meal by protease supplementation at three (36.1 % vs. 37.3 %) and nine hours (59.4 % vs. 63.2 %) of incubation.

Supplementation with amylase and the combination of amylase and protease showed no effects on the ruminal dry matter degradation of soybean meal. Protease supplementation showed a significant increase only at two points in time. Thus, no consistent effect of one of the enzyme treatments on dry matter degradability of soybean meal could be observed.

Table 22: Ruminal dry matter disappearance (%) of soybean meal (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
1 h	30.9 ±0.8	31.0 ±1.0	30.9 ±1.3	31.0 ±1.8	0.22	0.972
2 h	33.8 ±1.7	33.2 ±1.5	33.8 ±1.7	33.2 ±1.3	0.23	0.051
3 h	36.1^{bc} ±1.7	35.6^{c} ±2.4	37.3^{a} ±2.9	37.0^{ab} ±2.2	0.37	0.004
4 h	39.2 ±2.8	39.1 ±3.6	39.8 ±3.4	39.0 ±3.1	0.43	0.593
5 h	42.0 ±4.2	42.2 ±5.0	43.5 ±5.1	42.8 ±4.6	0.61	0.243
6 h	45.0 ±4.7	46.1 ±7.7	45.8 ±3.9	46.3 ±6.2	0.78	0.696
9 h	59.4^{bc} ±11.1	56.9^{c} ±11.4	63.2^{a} ±10.7	61.8^{ab} ±10.4	1.21	0.003
12 h	69.0^{ab} ±10.9	64.7^{b} ±14.2	66.4^{b} ±13.6	71.2^{a} ±10.9	1.60	0.030
24 h	93.3 ±5.1	91.6 ±6.8	91.2 ±4.8	92.4 ±4.8	0.76	0.195
48 h	98.1 ±1.1	97.9 ±1.0	98.1 ±0.3	98.1 ±0.4	0.13	0.899

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.2.1.2 Parameters of degradability and effective degradability

Table 23 represents the parameters of degradability and the effective degradability of dry matter of soybean meal. Regarding the soluble fraction (a), the control showed a significant higher soluble fraction than supplementation with protease. This effect turns at the ruminal degradable fraction (b), so protease supplementation had a higher ruminal degradable fraction than the control. Supplementation with amylase and the combination of amylase and protease showed no significant differences, neither on the soluble fraction, nor on the ruminal degradable fraction, compared to the control. Effects of protease supplementation on the soluble fraction (a) and the ruminal degradable fraction (b) annulled each other in the degradable fraction (d). This fraction showed that dry matter of soybean meal is almost completely degradable inside the rumen and the constant rate of

degradation (c) indicated that the ruminal degradation of dry matter of soybean meal occurred very quickly.

The control showed a higher soluble fraction (a) than protease supplementation. This effect was associated with a significant decrease of the control towards supplementation with protease for the ruminal degradable fraction (b). The remaining parameters of degradability were not affected by one of the treatments.

Table 23: Parameters of degradability and effective degradability of dry matter of soybean meal (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	29.6^{a} ±2.2	28.4^{ab} ±1.9	26.3^{b} ±1.7	27.4^{ab} ±2.3	0.77	0.028
b (%)	70.3^{b} ±2.4	71.6^{ab} ±1.9	73.6^{a} ±1.9	72.5^{ab} ±2.3	0.81	0.036
c (%/h)	9.28 ±3.8	8.07 ±2.5	8.08 ±2.5	8.86 ±2.3	0.62	0.378
d (%)	99.9 ±0.3	100 ±0.0	99.9 ±0.2	99.9 ±0.1	0.08	0.698
t_0 (h)	2.35 ±1.2	2.01 ±1.4	1.00 ±1.0	1.73 ±1.3	0.46	0.180
Effective degradability						
ED2 (%)	83.8 ±2.8	82.8 ±3.7	83.4 ±3.0	84.2 ±2.6	0.52	0.246
ED5 (%)	69.0 ±4.0	67.7 ±5.2	68.7 ±4.2	69.5 ±3.9	0.69	0.250
ED8 (%)	59.8 ±4.1	58.5 ±5.3	59.7 ±4.1	60.4 ±4.1	0.69	0.266

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

The highest effective degradability for all different passage rates was observed for the combination of amylase and protease. The control and supplementation with protease were closed together scarce below the combination of both enzymes. Supplementation

with amylase showed the lowest effective degradability for all different rates of passage. However, these observations were statistically not significant.

The enzyme treatments showed no statistically significant effects on the effective degradability of dry matter of soybean meal.

4.2.2.2 Degradability of crude protein

4.2.2.2.1 *In sacco* crude protein disappearance

Table 24 shows the crude protein disappearance of soybean meal. Significant differences between treatments were observed up to nine hours of incubation. After 12 h and 24 h, no effects on crude protein losses could be detected. Supplementation with amylase led to an increased crude protein disappearance after one hour of incubation. Furthermore, no effects of amylase supplementation towards the control could be noticed. Regarding the combination of amylase and protease, crude protein disappearance of soybean meal was significantly increased by this treatment from one up to six hours of incubation, compared to the control. Supplementation with protease showed the clearest effects on crude protein disappearance. In this case, an increased crude protein disappearance from one up to nine hours by 29.9% on average was observed.

Ruminal crude protein degradability was increased by supplementation with protease and the combination of amylase and protease at short incubation times. Amylase supplementation showed, except after one hour, no statistically significant effect on the degradation of crude protein from soybean meal.

Table 24: Ruminal crude protein disappearance (%) of soybean meal (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	19.1^{d} ±1.1	20.8^{c} ±1.8	26.9^{a} ±2.0	24.1^{b} ±1.3	0.55	< 0.0001
3 h	25.1^{b} ±2.1	25.9^{b} ±3.9	34.0^{a} ±2.4	31.3^{a} ±3.3	1.02	< 0.0001
6 h	34.2^{b} ±5.0	36.9^{ab} ±9.9	41.3^{a} ±4.1	40.0^{a} ±6.7	1.78	0.030
9 h	51.5^{bc} ±12.8	49.5^{c} ±15.1	63.2^{a} ±13.0	58.4^{ab} ±13.8	2.69	0.003
12 h	61.9 ±13.8	58.7 ±17.4	65.9 ±16.0	70.0 ±13.5	3.50	0.113
24 h	93.8 ±6.4	92.1 ±8.6	93.8 ±4.1	93.9 ±5.5	1.83	0.863

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.2.2.2 Parameters of degradability and effective degradability

Table 25 represents the parameters of degradability and the effective degradability of crude protein of soybean meal. The soluble fraction (a) was significantly increased by supplementation with amylase and supplementation with protease, while the ruminal degradable fraction (b) was significantly decreased by these treatments. However, the total degradability was not affected by enzyme supplementation. This shows the degradable fraction (d), which was 100 % for all treatments. The highest rate of degradation was noticed for the combination of amylase and protease, and the degradation of crude protein started most rapidly by supplementation with protease. Nevertheless, these observations were not statistically significant.

In total, amylase supplementation led to a significantly increased soluble fraction (a) and to a significantly decreased ruminal degradable fraction (b), as well as protease supplementation. The remaining parameters of degradability of crude protein of soybean meal were not affected by one of the enzyme treatments.

Table 25: Parameters of degradability and effective degradability of crude protein of soybean meal (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	13.5^{b} ±2.3	16.5^{a} ±3.3	17.9^{a} ±0.8	16.0^{ab} ±3.4	0.97	0.020
b (%)	86.5^{a} ±2.3	83.5^{b} ±3.3	82.1^{b} ±0.8	84.0^{ab} ±3.4	0.97	0.020
c (%/h)	8.65 ±2.1	8.54 ±2.9	8.49 ±2.6	9.63 ±2.9	0.58	0.281
d (%)	100 ±0.0	100 ±0.0	100 ±0.0	100 ±0.0	0.00	-
t_0 (h)	1.79 ±1.7	1.78 ±1.5	0.27 ±0.3	1.21 ±2.6	0.60	0.247
Effective degradability						
ED2 (%)	80.7 ±3.7	80.8 ±5.3	83.1 ±3.8	83.2 ±3.4	0.91	0.073
ED5 (%)	63.1^{b} ±5.4	63.7^{b} ±7.5	67.8^{a} ±5.5	67.4^{a} ±5.3	1.27	0.018
ED8 (%)	52.2^{b} ±5.7	53.3^{b} ±7.8	58.3^{a} ±5.7	57.3^{a} ±5.9	1.34	0.005

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

At a passage rate of 2 %/h, which represents a low feed intake level, no significant differences among treatments could be observed. Supplementation with protease and the combination of amylase and protease showed a significant increase in effective degradability at medium (ED5) and high (ED8) rates of passage, where protease supplementation showed the highest effective degradability. Supplementation with protease showed an increase by 7.4 % (ED5) and 11.7 % (ED8) compared to the control and the combination of amylase and protease by 6.8 % (ED5) and 9.7 % (ED8). Regarding amylase supplementation, no significant differences were noticed.

Supplementation with protease and the combination of amylase and protease led to a significant increase of the effective degradability of crude protein of soybean meal

assuming passage rates representing medium and high feed intake levels. The effective degradability was not affected by amylase supplementation.

4.2.3 Effects of amylase and protease supplementation on ruminal degradability of maize silage

Maize silage was studied concerning the effects of the different enzyme treatments on ruminal degradability of dry matter, starch, and crude protein.

4.2.3.1 Degradability of dry matter

4.2.3.1.1 *In sacco* dry matter disappearance

Table 26 shows the DMD of maize silage. Amylase supplementation showed significant differences towards the control at 3, 4, 5, and 9 hours of incubation only, unlike the supplementation with protease and the combination of both enzymes. Protease supplementation led to a significant increase of DMD from two up to 24 hours and the combination of amylase and protease to an increase from one up to 24 hours of incubation, except for six hours. In this time frame, an average increase of 11.8 % of DMD was observed for protease supplementation and the combination of both enzymes, respectively.

The dry matter degradability of maize silage was affected by protease supplementation and the combination of both enzyme preparations. Both treatments led to a significant increased dry matter disappearance. Amylase supplementation showed no consistent effect on the dry matter degradability of maize silage.

Table 26: Ruminal dry matter disappearance (%) of maize silage (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	27.9^{b} ±3.5	28.9^{b} ±4.2	29.3^{b} ±3.8	32.5^{a} ±3.6	0.68	< 0.0001
2 h	27.9^{b} ±3.4	28.8^{b} ±3.9	31.0^{a} ±3.9	31.8^{a} ±3.9	0.64	< 0.0001
3 h	29.8^{c} ±3.5	32.1^{b} ±3.7	33.7^{a} ±3.4	33.9^{a} ±3.6	0.56	< 0.0001
4 h	30.4^{c} ±4.6	33.3^{b} ±4.2	35.3^{a} ±4.6	33.3^{b} ±4.9	0.66	< 0.0001
5 h	32.7^{b} ±5.3	35.3^{a} ±5.3	37.0^{a} ±5.5	37.2^{a} ±4.9	0.80	0.0003
6 h	34.6^{b} ±5.4	35.5^{b} ±6.4	38.5^{a} ±4.0	35.7^{b} ±6.1	0.88	0.011
9 h	37.8^{c} ±6.2	40.2^{b} ±6.1	44.7^{a} ±6.0	41.7^{b} ±5.6	0.79	< 0.0001
12 h	42.9^{c} ±4.9	43.9^{bc} ±6.2	45.8^{ab} ±6.1	47.7^{a} ±7.0	0.82	0.0002
24 h	59.4^{c} ±5.3	60.1^{bc} ±6.8	61.9^{ab} ±4.6	62.5^{a} ±5.4	0.80	0.021
48 h	75.6 ±4.9	75.9 ±5.1	76.8 ±4.3	75.8 ±3.7	0.73	0.647

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.3.1.2 Parameters of degradability and effective degradability

Table 27 represents the parameters of degradability and the effective degradability of dry matter of maize silage. The soluble fraction (a) was significantly increased by amylase supplementation, compared to the control. Protease supplementation and the combination of amylase and protease showed a significant higher soluble fraction (a) than supplementation with amylase and the control. Regarding the ruminal degradable fraction (b), the control showed a significant higher value than all of the enzyme treatments. This fact (decreased soluble fraction (a) and increased ruminal degradable fraction (b) of the control) led to no differences concerning the degradable fraction (d). The combination of both enzymes showed the highest constant rate of degradation (c) and the highest lag-time (t_0). However, no significant differences regarding these parameters were observed.

All enzyme treatments led to an increase of the soluble fraction (a) accompanied by a decrease of the ruminal degradable fraction (b). The other parameters of degradability of dry matter of maize silage were not affected by one of the treatments.

Table 27: Parameters of degradability and effective degradability of dry matter of maize silage (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	23.3^{c} ±1.6	25.5^{b} ±1.8	27.4^{a} ±0.8	28.7^{a} ±1.5	0.56	< 0.0001
b (%)	70.8^{a} ±7.5	63.0^{b} ±8.6	62.6^{b} ±8.4	60.5^{b} ±10.1	2.55	0.029
c (%/h)	2.97 ±0.9	3.70 ±1.3	3.69 ±1.5	4.16 ±2.0	0.44	0.266
d (%)	94.2 ±6.5	88.5 ±8.9	89.9 ±8.4	89.2 ±9.5	2.46	0.321
t_0 (h)	0.05 ±0.1	0.65 ±1.3	0.23 ±0.2	1.63 ±3.0	0.59	0.213
Effective degradability						
ED2 (%)	64.5 ±2.3	64.3 ±3.3	66.0 ±1.3	65.5 ±2.4	0.82	0.384
ED5 (%)	48.9^{c} ±2.9	50.2^{bc} ±3.7	52.2^{a} ±2.1	51.8^{ab} ±3.5	0.64	0.003
ED8 (%)	41.9^{c} ±2.8	43.6^{b} ±3.6	45.6^{a} ±2.2	45.3^{a} ±3.6	0.58	0.0002

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

Assuming a low rate of passage (ED2), no significant differences between treatments were observed. At a passage rate of 5 %/h, representing a medium feed intake level, protease supplementation and the combination of both enzymes showed a significant increase in effective degradability. This effect is becoming clearer at a high passage rate (ED8). In this case, amylase supplementation led to a significant higher effective degradability, compared to the control, whereby supplementation with protease and the

combination of amylase and protease showed a significant higher effective degradability than the control and amylase supplementation.

At a medium rate of passage (ED5), effective degradability was significantly increased by protease supplementation and the combination of amylase and protease. Assuming a passage rate of 8 %/h, representing a high feed intake level, effective degradability was significantly increased by amylase supplementation, additionally. The effective degradability of dry matter of maize silage was not affected by the enzyme treatments at a low rate of passage (ED2).

4.2.3.2 Degradability of starch

4.2.3.2.1 *In sacco* starch disappearance

Table 28 shows the starch disappearance of maize silage. In comparison to the control, the treatment which showed a higher numerical starch disappearance which commonly occurs in all enzyme treatments was the protease supplementation, but did not show significant differences regarding starch disappearance of maize silage.

The enzyme supplementation showed no statistically significant effects on the starch degradation of maize silage.

Table 28: Ruminal starch disappearance (%) of maize silage (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
1 h	42.9 ±13.3	36.0 ±14.5	36.9 ±13.8	41.5 ±10.2	4.99	0.673
3 h	43.7 ±9.3	46.1 ±16.7	52.8 ±11.3	42.4 ±11.3	3.62	0.164
6 h	52.8 ±17.3	51.9 ±20.1	57.5 ±10.5	45.3 ±16.5	5.53	0.440
9 h	51.7 ±9.6	55.8 ±16.6	63.5 ±14.0	53.7 ±9.1	3.36	0.071
12 h	64.9 ±12.3	64.4 ±12.8	65.5 ±5.8	67.2 ±7.8	3.50	0.939
24 h	84.0 ±6.4	84.1 ±9.2	84.5 ±3.6	82.9 ±10.4	2.44	0.964

4.2.3.2.2 Parameters of degradability and effective degradability

The parameters of degradability and the effective degradability of starch of maize silage are represented in Table 29. The soluble fraction (a) was significantly increased by protease supplementation. The remaining parameters were not affected by the enzyme treatments. The parameter c showed for all treatments high rates of degradability and the degradable fraction (d) was in the range of 87.2 % to 91.3 %, indicating that the majority of starch of maize silage is degradable in the rumen.

The soluble fraction (a) was significantly increased by protease supplementation, but no other parameter of degradability of starch of maize silage was affected by one of the enzyme treatments.

Table 29: Parameters of degradability and effective degradability of starch of maize silage (± SD) dependent on treatment

	Treatment				**SEM**	**p-value**
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	27.7^{b} ±4.3	28.8^{b} ±2.2	34.7^{a} ±5.8	26.5^{b} ±3.0	1.60	0.004
b (%)	61.1 ±13.6	58.4 ±11.6	56.5 ±8.6	63.1 ±14.9	4.52	0.709
c (%/h)	8.91 ±5.4	11.0 ±7.4	9.97 ±7.9	12.2 ±11.8	3.23	0.848
d (%)	88.8 ±12.7	87.2 ±11.2	91.3 ±10.4	89.6 ±15.6	4.44	0.917
t_0 (h)	0.33 ±0.7	1.65 ±2.2	0.52 ±0.4	0.67 ±1.9	0.60	0.418
Effective degradability						
ED2 (%)	75.0 ±7.0	74.8 ±8.9	78.3 ±5.8	75.4 ±10.2	2.90	0.776
ED5 (%)	63.3 ±5.5	63.5 ±8.4	67.5 ±5.1	63.5 ±8.6	2.63	0.576
ED8 (%)	56.4 ±5.8	56.4 ±8.1	61.1 ±5.5	56.5 ±8.6	2.72	0.487

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

Supplementation with protease showed the highest numeric effective degradability of all enzyme treatments. But neither assumption of a low (ED2), nor a medium (ED5), nor a high (ED8) rate of passage showed significant differences between treatments.

The enzyme supplementation had no statistically significant effects on the effective degradability of starch of maize silage.

4.2.3.3 Degradability of crude protein

4.2.3.3.1 *In sacco* crude protein disappearance

Table 30 shows the crude protein disappearance of maize silage. No effects of enzyme treatments on crude protein disappearance were observed. However, supplementation with protease showed the highest numeric crude protein disappearance of all treatments.

Ruminal crude protein degradation of maize silage was not affected by enzyme supplementation.

Table 30: Ruminal crude protein disappearance (%) of maize silage (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	38.5 ±7.4	44.1 ±9.1	44.6 ±8.0	43.4 ±11.7	3.87	0.620
3 h	36.7 ±3.4	41.4 ±9.8	45.0 ±5.8	35.1 ±10.3	3.18	0.106
6 h	36.7 ±7.8	44.4 ±9.0	46.7 ±7.4	40.1 ±11.8	3.81	0.229
9 h	38.0 ±4.7	41.7 ±8.8	43.2 ±12.4	37.5 ±13.3	4.24	0.692
12 h	40.6 ±7.2	45.0 ±8.8	47.4 ±6.7	46.3 ±7.2	2.98	0.351
24 h	52.6 ±8.9	54.2 ±5.9	56.1 ±4.6	55.8 ±7.9	2.51	0.219

4.2.3.3.2 Parameters of degradability and effective degradability

The parameters of degradability and the effective degradability of crude protein of maize silage are shown in Table 31. The soluble fraction (a) was significantly increased by the supplementation of protease. The combination of amylase and protease slightly increased the soluble fraction (a), but not statistically significant. The ruminal degradable fraction (b) and the degradable fraction (d) showed no differences between treatments. The rate of degradability (c) and the lag-time (t_0) were not affected by the enzyme treatments either.

A significant increase of the soluble fraction (a) was observed by protease supplementation. The remaining parameters of degradability of crude protein of maize silage were not affected by enzyme supplementation.

Table 31: Parameters of degradability and effective degradability of crude protein of maize silage (± SD) dependent on treatment

	Treatment				**SEM**	**p-value**
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	33.2[b] ±3.1	33.7[b] ±2.3	39.8[a] ±3.4	35.8[b] ±4.2	1.28	0.003
b (%)	51.5 ±23.5	30.9 ±22.8	30.0 ±20.3	39.8 ±20.6	8.36	0.213
c (%/h)	2.57 ±1.4	8.08 ±6.9	5.20 ±3.0	7.11 ±6.5	1.43	0.139
d (%)	84.7 ±22.6	64.7 ±22.7	69.8 ±19.0	75.7 ±21.3	8.26	0.322
t_0 (h)	1.44 ±3.8	2.62 ±4.8	1.67 ±3.9	7.53 ±5.5	1.81	0.062
Effective degradability						
ED2 (%)	56.8 ±9.7	52.0 ±7.6	57.0 ±5.5	59.1 ±10.6	3.14	0.402
ED5 (%)	46.3 ±6.6	45.5 ±4.0	50.6 ±3.9	48.8 ±7.2	1.93	0.216
ED8 (%)	42.2 ±5.7	42.5 ±3.9	47.6 ±4.5	44.1 ±6.7	1.88	0.142

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

Assuming a low passage rate (ED2), the combination of amylase and protease showed the highest effective degradability of all treatments. At medium (ED5) and high (ED8) rates of passage the supplementation with protease showed numerically the highest effective degradability of the different treatments. However, no statistically significant differences between treatments could be found.

The supplementation with enzymes used in the present study had no statistically significant effects on the effective degradability of crude protein of maize silage.

4.2.4 Effects of amylase and protease supplementation on ruminal degradability of grass silage

Grass silage was studied concerning the effects of the different enzyme treatments on ruminal degradability of dry matter and NDF.

4.2.4.1 Degradability of dry matter

4.2.4.1.1 *In sacco* dry matter disappearance

Table 32 shows the DMD of grass silage. Regarding all enzyme treatments, there was only one point in time with a significant increase towards the control. This was due to the combination of amylase and protease at nine hours of incubation (43.8 % vs. 42.1 %). The combination of both enzyme preparations showed no further significant differences. Supplementation with protease showed a significantly lower dry matter disappearance than the control at one and three hours of incubation. Amylase supplementation led even to a significantly lower dry matter disappearance than the control from one up to five hours and at 12 h and 48 h, additionally.

Supplementation with protease and the combination of both enzymes showed no statistically significant effects on the dry matter degradation of grass silage. The supplementation with amylase had rather a decelerating effect on the dry matter degradability of grass silage.

Table 32: Ruminal dry matter disappearance (%) of grass silage (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	31.8[a] ±1.1	31.0[b] ±0.9	31.1[b] ±0.9	31.9[a] ±0.9	0.15	< 0.0001
2 h	32.7[a] ±1.3	32.0[b] ±0.9	32.4[ab] ±1.1	32.8[a] ±0.7	0.18	0.016
3 h	34.1[a] ±1.1	32.9[b] ±0.9	33.3[b] ±1.0	34.0[a] ±1.1	0.18	< 0.0001
4 h	34.9[a] ±1.3	34.0[b] ±1.3	34.7[a] ±1.4	34.9[a] ±1.2	0.21	0.012
5 h	36.4[a] ±1.9	35.4[b] ±2.1	36.4[a] ±1.8	36.7[a] ±2.1	0.26	0.007
6 h	37.6 ±1.7	37.1 ±2.9	37.5 ±2.4	37.2 ±2.6	0.34	0.615
9 h	42.1[bc] ±3.2	41.5[c] ±3.8	42.9[ab] ±4.5	43.8[a] ±3.3	0.48	0.006
12 h	49.3[a] ±7.3	46.0[b] ±5.6	47.0[ab] ±6.1	49.0[a] ±5.8	0.88	0.021
24 h	68.0 ±4.1	67.4 ±8.1	66.4 ±4.9	66.9 ±4.6	0.88	0.596
48 h	81.1[a] ±3.9	79.3[b] ±4.4	81.0[a] ±1.6	79.7[ab] ±3.4	0.52	0.025

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.4.1.2 Parameters of degradability and effective degradability

Table 33 represents the parameters of degradability and the effective degradability of dry matter of grass silage. Supplementation with protease showed a significant decreased soluble fraction (a). In contrast, protease supplementation had the highest numeric ruminal degradable fraction (b) and this resulted in the highest numeric degradable fraction (d) with 96.5 %. Supplementation with amylase led to the lowest numeric degradable fraction (d) of the dry matter of grass silage. However, these observations were statistically not significant. The rate of degradability (c) and the lag-time (t_0) were not affected by the enzyme treatments either.

The soluble fraction (a) was significantly decreased by supplementation with protease. The enzyme treatments showed no statistically significant effects on the remaining parameters of degradability of dry matter of grass silage.

Table 33: Parameters of degradability and effective degradability of dry matter of grass silage (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	30.3[a] ±1.5	29.9[a] ±1.9	28.2[b] ±1.0	28.8[ab] ±1.7	0.53	0.023
b (%)	62.8 ±5.2	58.5 ±10.8	68.3 ±3.2	63.4 ±7.0	2.73	0.090
c (%/h)	4.04 ±1.4	5.66 ±3.4	3.25 ±0.6	3.91 ±1.2	0.79	0.159
d (%)	93.1 ±4.5	88.4 ±9.5	96.5 ±3.5	92.1 ±6.1	2.43	0.126
t_0 (h)	2.09 ±2.5	3.52 ±4.0	0.81 ±1.3	1.27 ±2.6	0.95	0.181
Effective degradability						
ED2 (%)	69.7 ±2.2	67.3 ±3.5	69.4 ±1.2	68.6 ±2.4	0.65	0.051
ED5 (%)	54.9 ±2.6	53.3 ±2.7	53.8 ±1.9	54.1 ±2.3	0.50	0.155
ED8 (%)	47.7 ±2.5	46.3 ±2.3	46.6 ±1.8	47.1 ±2.0	0.43	0.089

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

The different enzyme treatments showed no significant effects on the effective degradability. However, the supplementation with amylase led to the lowest effective degradability of all treatments assuming low, medium, and high rates of passage. At a passage rate of 2 %/h a strong trend of a decreased effective degradability by the amylase treatment ($p = 0.051$) could be observed.

Supplementation with the enzymes used in this study showed no statistically significant differences on the effective degradability of dry matter of grass silage.

4.2.4.2 Degradability of NDF

4.2.4.2.1 *In sacco* NDF disappearance

Table 34 shows the NDF disappearance of grass silage. Supplementation with amylase led to the highest numeric NDF disappearance of all enzyme treatments after 24 hours of incubation, though the other incubation times mostly showed the lowest NDF disappearance by the amylase treatment. Nevertheless, no significant differences between treatments regarding the NDF disappearance of grass silage could be observed.

The supplementation of the enzymes used in the present study had no statistically significant effects on the ruminal degradation of NDF of grass silage.

Table 34: Ruminal NDF disappearance (%) of grass silage (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
1 h	6.16 ±1.8	4.67 ±1.8	5.07 ±1.5	5.43 ±1.1	0.65	0.370
3 h	8.05 ±1.6	5.85 ±2.4	6.11 ±1.2	6.94 ±2.4	0.83	0.212
6 h	12.1 ±4.5	10.2 ±3.1	10.7 ±2.3	9.90 ±3.0	0.97	0.364
9 h	17.1 ±4.0	15.1 ±4.7	17.0 ±5.7	18.3 ±4.2	1.17	0.103
12 h	26.5 ±8.5	21.7 ±6.7	22.4 ±6.9	25.0 ±7.2	2.17	0.333
24 h	53.1 ±5.6	56.0 ±4.4	49.8 ±4.8	50.1 ±6.2	1.67	0.058

4.2.4.2.2 Parameters of degradability and effective degradability

Table 35 represents the parameters of degradability and the effective degradability of NDF of grass silage. No significant effects of the enzyme treatments on the parameters of degradability were observed. The combination of both enzymes showed the lowest soluble fraction (a). In contrast, it showed the highest ruminal degradable fraction (b). This resulted in the highest numeric degradable fraction (d) due to the combination of amylase and protease.

Supplementation with the enzymes used in this study had no statistically significant effects on the parameters of degradability of NDF of grass silage.

Table 35: Parameters of degradability and effective degradability of NDF of grass silage (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	7.75 ±4.4	6.60 ±4.6	5.37 ±1.7	3.28 ±1.4	1.27	0.078
b (%)	88.2 ±14.2	84.8 ±25.6	82.7 ±18.7	92.2 ±13.2	7.01	0.657
c (%/h)	4.64 ±3.3	5.43 ±4.0	4.85 ±2.5	3.67 ±1.7	1.14	0.712
d (%)	94.7 ±14.1	90.1 ±26.1	88.2 ±18.5	95.5 ±12.7	7.09	0.732
t_0 (h)	5.48 ±4.1	7.39 ±6.7	4.48 ±1.9	3.38 ±2.7	1.52	0.316
Effective degradability						
ED2 (%)	58.0 ±5.3	53.8 ±16.1	56.6 ±8.4	56.5 ±5.4	3.85	0.868
ED5 (%)	36.2 ±3.4	32.6 ±8.0	36.0 ±5.1	34.3 ±3.8	1.98	0.502
ED8 (%)	26.2 ±3.3	22.8 ±4.3	25.8 ±4.0	24.1 ±3.3	1.23	0.165

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$

The control showed the highest effective degradability assuming low (ED2), medium (ED5), and high (ED8) rates of passage. No statistically significant differences among treatments for the various levels of feed intake were observed.

No significant differences between the enzyme treatments, regarding the effective degradability of NDF of grass silage were observed.

4.2.5 Effects of amylase and protease supplementation on ruminal degradability of hay

Hay was studied concerning the effects of the different enzyme treatments on ruminal degradability of dry matter.

4.2.5.1 Degradability of dry matter

4.2.5.1.1 *In sacco* dry matter disappearance

Table 36 shows the DMD of hay. There were only two significant points of enzyme treatments, compared to the control. Supplementation with amylase significantly increased DMD at one hour of incubation (14.2 % vs. 13.5 %) and supplementation with protease significantly decreased DMD of hay at 24 hours of incubation (34.4 % vs. 36.0 %). Apart from that, no other significant differences between treatments regarding the dry matter disappearance of hay were observed.

The supplementation with amylase and protease showed no consistent statistically significant effects on the dry matter degradability of hay.

Table 36: Ruminal dry matter disappearance (%) of hay (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	13.5[b] ±1.1	14.2[a] ±0.9	13.7[ab] ±1.0	13.6[b] ±0.7	0.17	0.021
2 h	15.0 ±0.8	15.1 ±0.6	15.1 ±0.8	15.0 ±0.6	0.13	0.902
3 h	15.9 ±0.8	15.8 ±0.8	15.7 ±0.9	15.6 ±0.9	0.14	0.406
4 h	16.6 ±0.8	16.6 ±0.7	16.5 ±0.7	16.8 ±0.7	0.12	0.565
5 h	17.6 ±1.0	17.7 ±1.0	17.5 ±0.9	17.2 ±1.0	0.15	0.067
6 h	18.5 ±0.9	18.7 ±1.3	18.6 ±1.1	18.2 ±1.0	0.17	0.186
9 h	20.9 ±1.9	21.0 ±1.8	20.7 ±1.9	20.3 ±1.4	0.22	0.104
12 h	23.0 ±2.1	22.8 ±2.5	23.1 ±2.7	23.1 ±2.7	0.34	0.845
24 h	36.0[a] ±3.7	36.0[a] ±4.6	34.4[b] ±4.0	36.4[a] ±3.6	0.56	0.046
48 h	51.1 ±3.7	50.1 ±4.6	50.9 ±2.5	49.6 ±3.3	0.48	0.072

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.5.1.2 Parameters of degradability and effective degradability

The parameters of degradability and the effective degradability of dry matter of hay are represented in Table 37. The parameters showed no significant differences between the enzyme treatments. Remarkable were the highest numeric values for the soluble fraction (a), the ruminal degradable fraction (b), and the degradable fraction (d) caused by the control. The constant rate of degradation (c) showed relatively low rankings between 2.19 %/h to 3.26 %/h.

The parameters of degradability of the dry matter of hay were not affected by the supplementation with amylase and protease.

Table 37: Parameters of degradability and effective degradability of dry matter of hay (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	11.3 ±0.6	11.0 ±0.7	10.4 ±1.0	11.0 ±0.7	0.27	0.128
b (%)	66.7 ±17.2	59.3 ±20.4	55.8 ±21.7	56.4 ±15.0	6.52	0.578
c (%/h)	2.19 ±1.0	2.83 ±1.5	3.26 ±1.5	2.62 ±0.8	0.41	0.292
d (%)	78.0 ±17.7	70.3 ±20.9	66.2 ±22.6	67.4 ±15.6	6.74	0.558
t_0 (h)	0.00 ±0.0	0.00 ±0.0	0.00 ±0.0	0.00 ±0.0	0.00	-
Effective degradability						
ED2 (%)	42.9 ±2.7	41.6 ±5.0	40.6 ±4.3	41.2 ±3.6	1.12	0.452
ED5 (%)	29.5 ±1.5	29.3 ±2.0	29.1 ±1.5	29.1 ±1.8	0.35	0.796
ED8 (%)	24.1 ±1.3	24.2 ±1.3	24.1 ±1.2	24 ±1.2	0.25	0.928

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$

The enzyme supplementation showed also no effects on the effective degradability of dry matter. At a passage rate of 8 %/h, representing a high level of feed intake, the means of the different treatments were almost equal. Assuming low (ED2) and medium (ED5) rates of passage, the control showed also the highest numeric effective degradability.

The effective degradability of the dry matter of hay was not affected by the supplementation with amylase and protease.

4.2.6 Effects of amylase and protease supplementation on ruminal degradability of the TMR

The TMR was studied concerning the effects of the different enzyme treatments on ruminal degradability of dry matter.

4.2.6.1 Degradability of dry matter

4.2.6.1.1 *In sacco* dry matter disappearance

Table 38 shows the DMD of the administered TMR. Regarding the supplementation with amylase, some significant differences towards the control could be observed, especially at short incubation times. Amylase supplementation showed a significant increase of DMD at 1, 2, 4, and 5 hours of incubation. Supplementation with amylase led, however, to a significant decrease of DMD after 12 hours of incubation. Supplementation with protease showed a significant increase of DMD at 1, 2, 3, 4, 5, 9, and 48 hours of incubation. During these points in time, an average increase by 9.0 %, compared to the control, could be observed. The combination of both enzymes was similar to protease supplementation. The combination of amylase and protease led to an increased DMD at 1, 2, 3, 4, 5, 9, and 24 hours of incubation. During this time frame, an average increase of 8.1 % towards the control was observed.

The dry matter degradability of the administered TMR was affected by the different enzyme supplementations. Protease supplementation and the combination of amylase and protease led to a significant increase of dry matter degradation of the TMR. Supplementation with amylase showed a significant increase only in short incubation times.

Table 38: Ruminal dry matter disappearance (%) of the TMR (± SD) dependent on treatment and incubation time

Time of incubation	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
1 h	26.1^{b} ±3.1	27.8^{a} ±2.2	28.2^{a} ±3.2	28.4^{a} ±1.9	0.48	0.003
2 h	25.9^{b} ±2.3	28.2^{a} ±2.3	28.0^{a} ±3.3	28.3^{a} ±2.9	0.48	0.001
3 h	27.9^{b} ±2.6	28.8^{b} ±2.9	31.0^{a} ±2.2	30.4^{a} ±3.2	0.46	< 0.0001
4 h	28.5^{b} ±3.3	30.8^{a} ±3.5	31.6^{a} ±3.1	31.6^{a} ±2.5	0.52	< 0.0001
5 h	30.5^{c} ±2.8	32.6^{b} ±2.9	34.0^{a} ±3.0	32.6^{b} ±3.3	0.43	< 0.0001
6 h	32.5 ±4.1	33.1 ±4.5	34.3 ±3.2	33.9 ±4.0	0.56	0.097
9 h	36.9^{c} ±5.2	38.2^{bc} ±5.2	40.8^{a} ±6.3	39.5^{ab} ±5.7	0.62	< 0.0001
12 h	43.2^{a} ±5.3	40.4^{b} ±6.7	44.1^{a} ±6.0	44.5^{a} ±6.1	0.76	0.001
24 h	60.6^{b} ±4.9	62.7^{ab} ±7.2	60.7^{b} ±5.6	63.7^{a} ±4.9	0.78	0.011
48 h	77.0^{b} ±5.8	76.9^{b} ±5.0	79.3^{a} ±2.5	77.5^{ab} ±3.5	0.67	0.033

Different superscripts indicating significant differences between treatments ($p < 0.05$) within the respective incubation time

4.2.6.1.2 Parameters of degradability and effective degradability

The parameters of degradability and the effective degradability of dry matter of the TMR are represented in Table 40. All enzyme treatments led to a significant increase of the soluble fraction (a), where supplementation with protease showed with 23.4 % the highest soluble fraction of all treatments. The remaining parameters were not affected by supplementation with the enzymes used in the present study. Values between 90.3 % (Amy) and 95.5 % (Prot) were noticed for the degradable fraction (d). The lag-time between 0.0 h (Prot) and 0.5 h (Amy) indicated a rapid onset of degradation.

The soluble fraction (a) was significantly increased by all enzyme treatments. The other parameters of degradability of the TMR were not affected by the supplementation with amylase and protease.

Table 39: Parameters of degradability and effective degradability of dry matter of the TMR (± SD) dependent on treatment

	Treatment				SEM	p-value
	Con	**Amy**	**Prot**	**Amy+Prot**		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Parameter of degradability						
a (%)	21.2[b] ±0.8	22.6[a] ±1.7	23.4[a] ±0.8	23.1[a] ±1.0	0.44	0.006
b (%)	72.5 ±7.3	67.7 ±10.4	72.1 ±5.8	70.3 ±7.5	2.89	0.587
c (%/h)	3.20 ±0.8	3.58 ±0.9	3.21 ±1.0	3.43 ±1.1	0.23	0.544
d (%)	93.7 ±7.0	90.3 ±11.5	95.5 ±5.6	93.3 ±7.4	3.06	0.645
t_0 (h)	0.25 ±0.7	0.50 ±1.4	0.00 ±0.0	0.04 ±0.1	0.30	0.590
Effective degradability						
ED2	65.0 ±3.2	64.6 ±5.1	66.6 ±1.9	66.2 ±2.3	1.08	0.468
ED5	48.7[b] ±2.8	49.4[ab] ±3.4	50.7[a] ±2.7	50.6[a] ±2.9	0.48	0.013
ED8	41.2[c] ±2.5	42.2[b] ±2.9	43.4[a] ±2.5	43.4[a] ±2.8	0.34	0.0001

a: soluble fraction, b: not soluble, but ruminal degradable fraction, c: constant rate of degradation of b, d: degradable fraction, t_0: lag time; parameters of degradability were estimated using following equation: $p = a + b\ (1 - e^{-c(t-t0)})$; effective degradability was calculated assuming different rates of passage (2, 5, 8 %/h) using following equation: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$; different superscripts indicating significant differences between treatments ($p < 0.05$)

Assuming a passage rate of 2 %/h, no significant differences between treatments were observed. The effective degradability was significantly increased by supplementation with protease and the combination of both enzyme preparations at a passage rate of 5 %/h, representing a medium feed intake level. Assuming a high rate of passage (ED8), a significant increase of the supplementation with amylase towards the control and a significant increase of protease supplementation and the combination with amylase and protease towards amylase supplementation were observed. Supplementation with protease and the combination of both enzymes showed almost identical values and the highest effective degradability.

Assuming a passage rate of 5 %/h, the effective degradability was significantly increased by protease supplementation and the combination of amylase and protease, whereas ED8 was significantly increased by all enzyme treatments. The effective degradability of dry matter of the TMR was not significantly affected by one of the treatments at a passage rate of 2 %/h.

4.3 Effects of amylase and protease supplementation on rumen microbial populations

Rumen fluid samples were analysed concerning the amount of specific rumen microorganisms at different sampling times using qPCR. Selected rumen microorganisms were: total bacteria, archaea, protozoa, anaerobic fungi, *Prevotella spp*., *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Streptococcus bovis*. Figure 3 shows the amount of selected rumen microorganisms in the rumen fluid at the onset of feeding (0 h) and one and three hours after feeding (means across treatments). Subject matter of investigations were not changes of the composition of rumen microorganisms in time course after feeding, but differences within sampling times dependent on enzyme treatments.

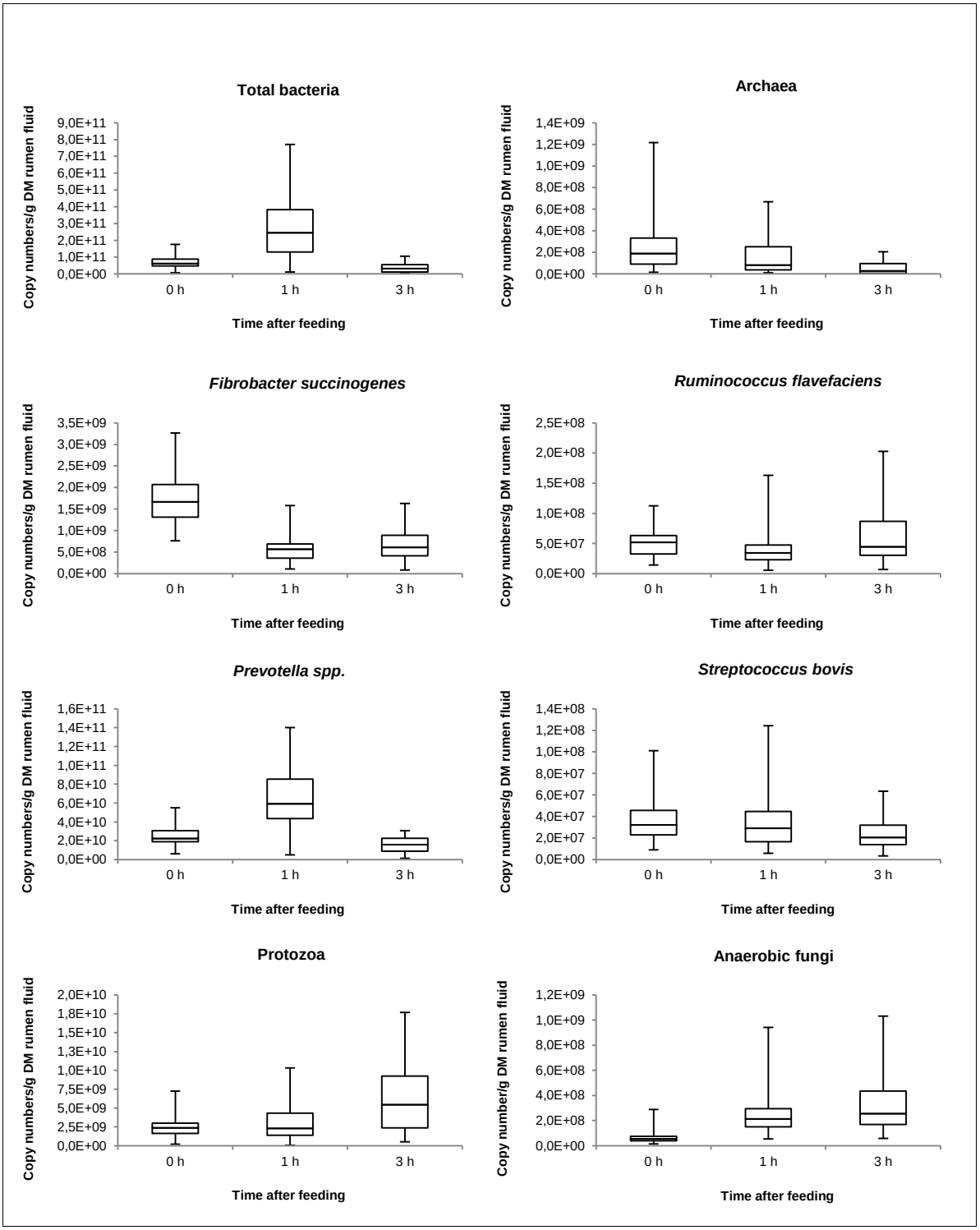

Figure 3: Amount of selected rumen microorganisms in the rumen fluid dependent on sampling time (means across treatments) determined by qPCR analysis

0 h: time of morning feeding (07:00 h); boxplots representing minimum, maximum, and the 25, 50, and 75 % quartiles

4.3.1 Effects on microbial populations straight before feeding

Table 40 shows the concentration of total bacteria, of selected bacterial populations and further rumen microorganisms in the rumen fluid right before the morning feeding. The amount of total bacteria in the rumen fluid was not affected by supplementation with amylase and protease, as well as *Prevotella spp*. in total and the proportions. *Prevotella spp*. showed on average with 56.7 % a high proportion in total bacteria. *F. succinogenes* had a percentage in total bacteria of 3.56 % to 4.25 % and was not affected by one of the enzyme treatments either. *R. flavefaciens* showed no differences in total, but in the proportions. Amylase supplementation slightly decreased the proportion of *R. flavefaciens* in the rumen fluid prior to feeding (0.08 % vs. 0.13 %). The percentage of archaebacteria was also affected by the enzyme treatments. Protease supplementation showed a higher proportion of archaea (0.45 %) than the control (0.22 %) and the combination of both enzymes (0.23 %). Amylase supplementation showed a numeric, but not statistically significant, increase of total archaebacteria and of the proportion relative to total bacteria. The concentration of total archaea was not affected by one of the enzyme treatments. Supplementation with amylase and protease had no effects on the concentration and the proportion of *S. bovis*, protozoa and anaerobic fungi in the rumen fluid straight before feeding.

Amylase supplementation decreased the proportion of *R. flavefaciens* and protease supplementation increased the proportion of archaebacteria relative to total bacteria in the rumen fluid straight before feeding. Further selected rumen microorganisms showed neither in total amount nor in percentage relative to total bacteria statistically significant effects of amylase and protease supplementation.

Table 40: Selected rumen microorganisms (Log_{10} 16S rRNA (respectively 18S rRNA for protozoa and anaerobic fungi) copy number per g rumen fluid dry matter) in the rumen fluid right before the morning feeding (± SD) dependent on treatment

Item	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
Total bacteria	10.6 ±0.2	10.8 ±0.1	10.6 ±0.3	10.6 ±0.1	0.06	0.177
Prevotella spp.	10.3 ±0.1	10.4 ±0.3	10.3 ±0.2	10.3 ±0.2	0.07	0.969
% in total bacteria	60.7 ±15.8	53.1 ±17.1	53.8 ±24.4	59.1 ±13.0	4.88	0.699
Fibrobacter succinogenes	9.16 ±0.1	9.26 ±0.2	9.22 ±0.2	9.16 ±0.2	0.05	0.405
% in total bacteria	4.17 ±1.5	3.56 ±0.6	4.25 ±2.6	3.99 ±1.2	0.56	0.622
Ruminococcus flavefaciens	7.63 ±0.1	7.59 ±0.2	7.76 ±0.3	7.72 ±0.2	0.08	0.396
% in total bacteria	0.13^{a} ±0.1	0.08^{b} ±0.03	0.14^{a} ±0.1	0.15^{a} ±0.1	0.02	0.034
Streptococcus bovis	7.46 ±0.2	7.49 ±0.2	7.62 ±0.3	7.49 ±0.2	0.07	0.272
% in total bacteria	0.08 ±0.01	0.07 ±0.01	0.10 ±0.03	0.09 ±0.03	0.01	0.061
Archaea	7.83 ±0.4	8.31 ±0.2	8.13 ±0.8	7.92 ±0.2	0.14	0.153
relative to total bacteria (%)	0.22^{b} ±0.1	0.38^{ab} ±0.1	0.45^{a} ±0.4	0.23^{b} ±0.1	0.06	0.043
Protozoa	9.16 ±0.1	9.12 ±0.5	9.26 ±0.2	9.14 ±0.3	0.10	0.705
relative to total bacteria (%)	4.44 ±2.2	4.63 ±4.1	4.41 ±1.7	4.72 ±3.9	0.95	0.995
Anaerobic fungi	7.76 ±0.3	7.74 ±0.4	7.76 ±0.2	7.82 ±0.4	0.10	0.938
relative to total bacteria (%)	0.22 ±0.2	0.17 ±0.2	0.16 ±0.1	0.26 ±0.3	0.06	0.633

Different superscripts indicating significant differences between treatments ($p < 0.05$)

4.3.2 Effects on microbial populations one hour after feeding

Table 41 shows the concentration of selected rumen microorganisms in the rumen fluid one hour after feeding. Supplementation with amylase and protease showed no statistical significant effects on the concentration of selected rumen microorganisms one hour after feeding, neither in total amount nor in percentage relative to total bacteria. Compared with the onset of feeding (0 h), an increase in total amount of anaerobic fungi, protozoa, *Prevotella spp*., and total bacteria was observed. In contrast, *F. succinogenes*, *R. flavefaciens*, *S. bovis*, and archaebacteria decreased in their total amounts. Regarding the proportions relative to total bacteria on average, a decrease of all selected microorganisms occurred (*Prevotella spp*.: 56.7 % vs. 42.3 %; *F. succinogenes*: 3.99 % vs. 0.42 %; *R. flavefaciens*: 0.13 % vs. 0.03 %; *S. bovis*: 0.09 % vs. 0.02 %; archaea: 0.32 % vs. 0.04 %; protozoa: 4.55 % vs. 1.28 %), except for anaerobic fungi (0.20 % vs. 0.24 %).

The concentration of selected rumen microorganisms in the rumen fluid were not affected by supplementation with amylase and protease one hour after feeding.

Table 41: Selected rumen microorganisms (Log_{10} 16S rRNA (respectively 18S rRNA for protozoa and anaerobic fungi) copy number per g rumen fluid dry matter) in the rumen fluid 1 h after feeding (± SD) dependent on treatment

Item	Treatment				SEM	p-value
	Con	Amy	Prot	Amy+Prot		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Total bacteria	11.2 ±0.2	11.3 ±0.4	11.1 ±0.4	10.9 ±0.5	0.16	0.242
Prevotella spp.	10.8 ±0.1	10.8 ±0.3	10.7 ±0.3	10.6 ±0.4	0.11	0.216
% in total bacteria	43.4 ±16.7	33.0 ±8.8	42.3 ±17.5	50.5 ±19.7	5.89	0.259
Fibrobacter succinogenes	8.70 ±0.2	8.81 ±0.3	8.69 ±0.2	8.57 ±0.3	0.09	0.297
% in total bacteria	0.32 ±0.1	0.35 ±0.1	0.41 ±0.2	0.58 ±0.4	0.10	0.195
Ruminococcus flavefaciens	7.50 ±0.2	7.61 ±0.4	7.58 ±0.3	7.34 ±0.3	0.12	0.327
% in total bacteria	0.02 ±0.01	0.02 ±0.01	0.03 ±0.01	0.04 ±0.03	0.01	0.343
Streptococcus bovis	7.45 ±0.3	7.53 ±0.2	7.46 ±0.3	7.24 ±0.3	0.09	0.087
% in total bacteria	0.02 ±0.03	0.01 ±0.01	0.02 ±0.01	0.03 ±0.03	0.01	0.740
Archaea	7.70 ±0.5	7.94 ±0.4	7.81 ±0.7	7.49 ±0.5	0.20	0.669
relative to total bacteria (%)	0.03 ±0.02	0.04 ±0.02	0.05 ±0.04	0.03 ±0.04	0.01	0.785
Protozoa	9.25 ±0.2	9.15 ±0.5	9.31 ±0.3	8.92 ±0.6	0.16	0.306
relative to total bacteria (%)	1.23 ±0.7	0.97 ±0.7	1.63 ±0.8	1.30 ±0.8	0.27	0.340
Anaerobic fungi	8.25 ±0.3	8.46 ±0.3	8.39 ±0.2	8.18 ±0.3	0.09	0.109
relative to total bacteria (%)	0.13 ±0.1	0.20 ±0.2	0.26 ±0.3	0.36 ±0.4	0.10	0.330

4.3.3 Effects on microbial populations three hours after feeding

The concentration of selected rumen microorganisms in the rumen fluid three hours after feeding is represented in Table 42. Supplementation with amylase and protease showed no statistical significant effects on the concentration of selected rumen microorganisms three hours after feeding, neither in total amount nor in percentage relative to total bacteria. Towards the point of one hour after feeding, an increase in total amount of *F. succinogenes*, *R. flavefaciens*, protozoa, and anaerobic fungi was observed. In contrast, *Prevotella spp*., *S. bovis*, archaebacteria, and total bacteria decreased in their total amounts. Regarding the proportions relative to total bacteria on average, an increase of all selected microorganisms was observed (*Prevotella spp*.: 42.3 % vs. 61.5 %; *F. succinogenes*: 0.42 % vs. 4.07 %; *R. flavefaciens*: 0.03 % vs. 0.30 %; *S. bovis*: 0.02 % vs. 0.14 %; archaea: 0.04 % vs. 0.11 %; protozoa: 1.28 % vs. 18.6 %; anaerobic fungi: 0.24 % vs. 1.86 %).

The concentration of selected rumen microorganisms in the rumen fluid were not affected by supplementation with amylase and protease three hours after feeding.

Table 42: Selected rumen microorganisms (Log_{10} 16S rRNA (respectively 18S rRNA for protozoa and anaerobic fungi) copy number per g rumen fluid dry matter) in the rumen fluid 3 h after feeding (± SD) dependent on treatment

Item	Treatment				SEM	p-value
	Con	Amy	Prot	Amy+Prot		
	-	300 KNU	-	150 KNU		
	-	-	15000 PROT	7500 PROT		
Total bacteria	10.3 ±0.3	10.1 ±0.5	10.2 ±0.5	10.1 ±0.6	0.16	0.631
Prevotella spp.	10.2 ±0.2	10.0 ±0.4	10.1 ±0.3	10.1 ±0.4	0.12	0.579
% in total bacteria	68.7 ±18.1	53.4 ±17.6	56.3 ±19.0	67.4 ±21.6	6.73	0.586
Fibrobacter succinogenes	8.90 ±0.2	8.70 ±0.3	8.75 ±0.3	8.66 ±0.4	0.10	0.322
% in total bacteria	3.83 ±1.4	4.47 ±2.4	3.68 ±2.1	4.29 ±2.6	0.77	0.847
Ruminococcus flavefaciens	7.74 ±0.3	7.57 ±0.4	7.67 ±0.5	7.71 ±0.3	0.13	0.753
% in total bacteria	0.30 ±0.2	0.32 ±0.1	0.28 ±0.1	0.30 ±0.1	0.05	0.943
Streptococcus bovis	7.36 ±0.1	7.25 ±0.3	7.30 ±0.4	7.24 ±0.4	0.10	0.812
% in total bacteria	0.11 ±0.1	0.16 ±0.1	0.12 ±0.1	0.15 ±0.1	0.03	0.479
Archaea	7.22 ±0.5	7.07 ±0.7	7.36 ±0.8	7.27 ±0.7	0.24	0.861
relative to total bacteria (%)	0.09 ±0.1	0.08 ±0.1	0.14 ±0.1	0.11 ±0.1	0.03	0.624
Protozoa	9.58 ±0.2	9.23 ±0.5	9.53 ±0.5	9.49 ±0.4	0.14	0.244
relative to total bacteria (%)	19.6 ±12.0	15.2 ±8.1	22.1 ±14.0	17.4 ±4.4	3.52	0.477
Anaerobic fungi	8.46 ±0.3	8.32 ±0.4	8.38 ±0.3	8.48 ±0.2	0.07	0.415
relative to total bacteria (%)	1.65 ±1.3	2.06 ±1.8	1.72 ±1.4	1.99 ±1.5	0.54	0.933

4.4 Effects of amylase and protease supplementation on apparent total tract digestibility

To evaluate the effects of supplementing amylase and protease on the total tract digestibility of the entire ration, faeces samples were collected and the digestibility was determined by using titanium dioxide (TiO_2) as an indigestible marker. The total tract digestibility of dry matter (DM), organic matter (OM), crude fibre (CF), crude protein (CP), total lipids (TL), nitrogen-free extracts (NFE), crude ash (CA), neutral detergent fibre (NDF), acid detergent fibre (ADF), and starch was calculated and is shown in Table 43. Significant differences between treatments were not determined, regarding the total tract digestibility. The amylase supplementation led neither to an increase of fibre digestion (CF, NDF, ADF) nor to an increased digestion of starch. The supplementation with protease showed no increase of the crude protein digestibility of the entire ration. The total tract digestibility of starch was rather high for all different treatments (97.5 % for the control and supplementation with amylase and 97.4 % for protease supplementation and the combination of both enzymes, respectively).

The total tract digestibility of the investigated ingredients was not significantly affected by the supplementation of amylase and protease.

Table 43: Apparent total tract nutrient digestibility (%) (± SD) dependent on treatment

Ingredient	Treatment				SEM	p-value
	Con - -	Amy 300 KNU -	Prot - 15000 PROT	Amy+Prot 150 KNU 7500 PROT		
DM	78.2 ±2.6	76.7 ±2.1	77.3 ±3.1	77.3 ±1.6	0.84	0.630
OM	79.9 ±2.6	78.6 ±2.2	79.1 ±3.0	78.9 ±1.7	0.77	0.637
CF	76.5 ±2.7	75.2 ±3.0	75.6 ±3.6	74.8 ±1.8	1.02	0.617
CP	64.8 ±5.4	62.5 ±3.9	63.4 ±5.9	64.3 ±3.4	1.55	0.700
TL	62.0 ±9.9	65.8 ±8.5	64.3 ±9.9	62.4 ±13.7	4.32	0.901
NFE	84.6 ±2.1	83.3 ±1.9	83.7 ±2.1	83.8 ±1.2	0.53	0.316
CA	44.8 ±6.0	40.1 ±6.4	43.1 ±8.6	45.0 ±5.8	2.89	0.554
NDF	69.8 ±4.5	67.3 ±4.6	68.0 ±5.3	68.2 ±1.6	1.56	0.660
ADF	68.9 ±4.1	66.7 ±3.4	67.1 ±4.6	67.1 ±2.5	1.39	0.646
Starch	97.5 ±0.5	97.5 ±0.6	97.4 ±0.8	97.4 ±0.4	0.21	0.970

DM: dry matter, OM: organic matter, CF: crude fibre, CP: crude protein, TL: total lipids, NFE: nitrogen-free extracts (calculated according to Weender analysis), CA: crude ash, NDF: neutral-detergent fibre, ADF: acid-detergent fibre

5 Discussion

The following passage gives an explanation of methods applied, discusses the results of the present study in regard to other studies and outlines the mode of action and fields of application of amylase and protease in ruminant nutrition.

5.1 Methods applied

Exogenous enzymes are able to act in the feed (when applied prior to consumption), in the rumen (direct hydrolysis or support of microbial enzymes) or in the intestine (direct contribution to digestion and enhancing nutrient utilization) (Hristov et al., 2000; McAllister et al., 2001). In the current study effects of enzyme supplementation on rumen fermentation should be measured, thus exogenous enzymes were applied to TMR right before feeding to preclude enzyme activity prior to consumption and testing feed material was stored at -20 °C to minimise enzyme activity prior to incubation. Furthermore, testing silage material was used as fresh material for measuring effects under practice-like conditions, besides Davies et al. (2000) reported effects of enzyme supplementation to fresh grass silage on *in vitro* gas production, but no effects when silage was dried and ground.

The present study was carried out with rumen cannulated cows and a simple composition of diet to study the nature of exogenous enzyme supplementation on ruminal degradation of typical ruminant forage (maize silage, grass silage, hay) and concentrate (maize grain, soybean meal) feedstuffs. The composition of the diet and the animal model enabled investigations concerning the effects of enzyme supplementation on feedstuffs rich in fibre, protein, and starch *in situ*. Following the recommendations of the manufacturer, which were based on previous studies, a dosage of 300 KNU/kg diet DM was chosen for the amylase treatment and 15000 PROT/kg diet DM for the protease treatment. Additionally, a combination of amylase and protease was selected to study synergistic effects between both enzymes. Thereto, the supplemented enzyme dosage was halved.

5.2 Rumen physiological parameters

5.2.1 pH-value

Ruminal pH-values ranged from 6.51 to 6.93 and were not affected by enzyme supplementation. The increased ruminal starch degradation entailed not a decrease of ruminal pH-value. These results are in line with other authors, who reported also no effects on ruminal pH when exogenous α-amylase was supplemented to dairy cows (Hristov et al., 2008; Nozière et al., 2014), steers (Tricarico et al., 2005) or sheep (Mora-Jaimes et al., 2002). In contrast, Rojo et al. (2005) reported an increase of ruminal pH-value when sheep were supplemented with glucoamylase. Results of Hristov et al. (2000) also showed an increase of ruminal pH when heifers were fed a diet supplemented with a mixture of polysaccharide-degrading enzymes, containing amylase. The only pH decreasing effect was reported by Eun & Beauchemin (2005), who supplemented serine protease from *Bacillus licheniformis* to dairy cows.

Rapid ruminal degradation of high amounts of starch leads to a strong decrease of ruminal pH-value which on the one hand may end in acidosis (Owens et al., 1998) and on the other hand restricts ruminal fibre degradation (Mould & Ørskov 1983; Yang et al., 2002) due to changing conditions to fibrolytic bacteria (Huntington 1997; Zebeli et al., 2012). This was not the case in our study and in other enzyme studies mentioned above. Exogenous enzymes only act beneficially when neither ruminal pH is decreased to ranges which cause acidosis, nor fibre degradability is negatively affected which is undesirable, especially for dairy cows.

5.2.2 Ammonia-nitrogen

Ammonia is produced inside the rumen during protein degradation and deamination of amino acids by bacteria and protozoa (Tamminga, 1979) and is the main nitrogen source for microorganisms (Kopecny & Wallace, 1982). In the current study rumen ammonia-nitrogen (NH_3-N) content was not affected by enzyme supplementation, suggesting that increase in protein degradation inside the rumen due to protease supplementation was only slight. These results are in line with other studies, which reported no effects on ruminal NH_3-N content when exogenous amylase was supplemented to dairy cows (DeFrain et al., 2005; Tricarico et al., 2005; Hristov et al., 2008; Nozière et al., 2014) or sheep (Mora-Jaimes et al., 2002), or when exogenous protease was supplemented to dairy cows (Eun & Beauchemin, 2005). The only decreasing effect on ruminal NH_3-N content was reported by Rojo et al. (2005) when amylase was supplemented to sheep.

Otherwise, an increase of ruminal NH_3-N content was only reported when a mixture of enzymes was supplemented. Gado et al. (2009) observed an increase of ruminal NH_3-N concentration when dairy cows were fed a diet supplemented with a mixture of enzymes containing cellulase, xylanase, amylase, and protease. Hristov et al. (2000) also reported an increase of ruminal NH_3-N concentration in heifers supplementing a mixture of polysaccharide-degrading enzymes containing amylase. These mixtures contained fibrolytic enzyme activity, suggesting an increase in fibre degradation benefits protein degradation. For example, about 10 % of total-nitrogen in alfalfa is linked to NDF-residues anyway (Aufrère et al., 1994).

5.2.3 Volatile fatty acids

Volatile fatty acids are produced by rumen microorganisms during fermentation of feed and served as energy source to the animal (Weimer, 1998). In the current study the concentrations of acetic acid, propionic acid, butyric acid, and valeric acid were determined in the rumen fluid. Total volatile fatty acids, acetic acid, propionic acid, valeric acid, as well as the acetic to propionic acid ratio were not affected by enzyme supplementation. Significant differences were observed for the concentration of butyric acid, but only at two times. At 07:00 h supplementation with amylase and the combination of amylase and protease decreased ruminal concentration of butyric acid, at 16:00 h even all enzyme treatments decreased ruminal butyric acid concentration. Noticeable was that 07:00 h and 16:00 h are the times right before feeding, respectively long time after feeding. However, one hour after feeding no differences among treatments were observed.

In most studies with lactating dairy cows which shows benefits on digestibility and/or on animal performance due to amylase supplementation VFA's were not determined. Effects of supplementation with exogenous enzymes on VFA pattern were mainly reported either by studies using rumen cannulated animals or by those using *in vitro* rumen simulating techniques. An increase of total VFA concentration due to amylase supplementation was also not observed by other studies. Using exogenous amylase of fungal origin, no effects on total VFA concentration were observed in dairy cows (DeFrain et al., 2005; Tricarico et al., 2005) or in sheep (Mora-Jaimes et al., 2002). Amylase supplementation in combination with fibrolytic enzymes also showed no effects on total ruminal VFA concentration in dairy cows (Hristov et al., 2000; Hristov et al., 2008). Nonetheless, changes in VFA composition were reported by Tricarico et al. (2005). The authors determined an increase of acetic and butyric acid, while propionic acid was decreased by supplementation with exogenous amylase to dairy cows. The concomitant increase of

acetic to propionic acid ratio was not a result of increased fibre degradation and the shift in VFA pattern was also no result of increased starch degradation inside the rumen. The authors suggested the shift in VFA pattern is related to microorganisms which benefit from maltodextrins, confirming the cross-feeding theory of Tricarico et al. (2008). An increase in butyric acid concentration may be beneficial especially in heifers, because butyric acid serves as main energy source to rumen epithelium (Weigand et al., 1975; Tricarico et al., 2008). DeFrain et al. (2005) reported a numeric increase of butyric acid concentration in *prepartum* dairy cows supplemented with amylase, suggesting beneficial effects on ruminal absorption in early lactation. Manipulation of ruminal VFA composition has the potential to improve animal performance (Weimer, 1998) and butyric acid has the highest correlation to milk yield of all VFA's (Seymour et al., 2005).

In contrast to Tricarico et al. (2005), a decrease of acetic and butyric acid and increased propionic acid concentration, accompanied by a decreased acetic to propionic acid ratio, due to amylase supplementation to dairy cows were observed by Nozière et al. (2014). The difference between these two studies is that Nozière et al. (2014) reported increased ruminal starch degradation and this is usually associated with a decrease in acetic to propionic acid ratio (San Emeterio et al., 2005). In the current study no effect on acid to propionic acid ratio was observed, although an increased ruminal starch degradation of maize grain was measured.

Protease supplementation showed also no effects on total VFA concentration when applied to dairy cows (Eun & Beauchemin, 2005) or tested *in vitro* (Colombatto et al., 2003a; Eun & Beauchemin, 2007; Eun et al., 2007) but changes in the composition were observed. A decrease of acetic and butyric acid concentrations in dairy cows was reported by Eun & Beauchemin (2005) when exogenous serine protease from *Bacillus licheniformis* was added to a diet containing barley silage and alfalfa hay. Eun & Beauchemin (2007) observed a decrease of acetic acid and an increase of propionic acid due to protease supplementation to alfalfa hay and maize silage in vitro. The authors suggested that the consequent decrease in acetic to propionic acid ratio may be beneficial to dairy cows, especially in early lactation, due to an improved nutrient utilisation because propionic acid acts as a glucogenic precursor.

Gado et al. (2009) reported an increase in acetic and propionic acid concentrations resulting in an increased total VFA concentration in dairy cows when a mixture of exogenous enzymes (cellulase, xylanase, amylase, and protease) was applied to a maize-based diet. The current study showed no increase in VFA concentrations due to enzyme supplementation to a maize-based diet. A reason may be the low level of feed intake, compared to other studies which used dairy cows. Further differences are that

Gado et al. (2009) additionally supplemented fibrolytic enzymes and Tricarico et al. (2005) used amylase of fungal origin and not of bacterial, as in the current study. Protease may have mainly effects on particular feedstuffs, especially alfalfa.

5.3 Ruminal degradation characteristics of tested feedstuffs

5.3.1 Maize grain

The results showed an increased ruminal dry matter degradability of maize grain when a combination of amylase and protease was supplemented. Dry matter degradability of maize grain was increased by 6.7 % on average from 1 h up to 24 h of incubation when animals were supplemented with the combination of amylase and protease. This led to the assumption that the enzymes acted synergistically because supplementation of either amylase or protease showed no increase of dry matter degradability. These findings are in contrast to Gutiérrez et al. (2005), who reported an increase of ruminal dry matter degradability of maize and sorghum grain when rumen cannulated steers were supplemented with α-amylase from *Bacillus licheniformis* and glucoamylase from *Aspergillus niger*, and Crosby et al. (2012), who reported an increased dry matter digestibility *in vitro* of maize and sorghum grain when treated with α-amylase from *Aspergillus niger*. One reason why amylase supplementation in our study showed no effects on ruminal dry matter degradability may be the high proportion of starch in the TMR. High amounts of starch, respectively grains, increased amylolytic activity in the rumen fluid (McAllister et al., 1993; Martin & Michalet-Doreau, 1995; Hristov et al., 1999) and lead to a higher production of amylase inside the pancreas (Russell et al., 1981), compared to a high-forage diet, thus activity of amylases may not be the limiting factor in this case. This is in line with DiLorenzo et al. (2011) who suggested exogenous amylase may be overlayed by bacterial amylases in high-grain-diets.

Supplementation of protease showed no increase of dry matter degradability but supplementation with both amylase and protease did. One explanation could be that both enzymes act synergistically. Supplemented protease may break down protein structures but our results suggested that an exogenous amylase is needed additionally to increase the starch degradability of maize grain. The parameters of degradability showed an increased soluble fraction (a) and an unaffected ruminal degradable fraction (b) of dry matter of maize grain when TMR was supplemented with a combination of amylase and protease. This suggests an accelerated ruminal degradation of maize grain dry matter by the combination of amylase and protease and finally leads to an increase of the effective

degradability of maize grain dry matter inside the rumen for low, medium and high feed intake levels. Similar results were reported by Hristov et al. (2000), who investigated the effects of an enzyme mixture, containing amylase, on ruminal degradability feeding a barley-based diet (85.5 % rolled barley grain) to ruminal cannulated heifers. Authors observed an increased soluble fraction (a), a decreased ruminal degradable fraction (b) and an increased effective degradability of the TMR dry matter due to the enzyme mixture. The difference to our study was that the enzyme mixture contained beside amylase additionally activities of fibre degrading enzymes (carboxymethylcellulase, xylanase, and β-glucanase).

To ensure that degradability of starch was affected by the combination of amylase and protease and to verify whether the protein degradation of maize grain was affected by one of the treatments, ruminal degradability of starch and crude protein was measured. *In situ* starch disappearance of maize grain was neither affected by supplementation with amylase nor by supplementation with protease. The combination of both enzymes led to a numeric increase of starch disappearance at 1 h and 3 h and to a significant increase between 6 h and 24 h of incubation. During this time frame an average increase of 10.6 % was observed. These findings confirm the hypothesis that exogenous protease break down protein structures and thus relieved access of exogenous amylase to starch granules resulting in increased starch degradation and that these exogenous enzymes act synergistically. The parameters of degradation showed only significant differences between treatments for the rate of degradation (c). The combination of both enzymes significantly increased c compared with amylase, respectively protease and increased numerically c compared to the control (3.97 %/h vs. 4.97 %/h). This suggests acceleration in starch degradation of maize grain due to the combination of amylase and protease. Acceleration of ruminal degradation showed effects on effective ruminal degradability, especially when feed intake is high and retention time inside the rumen is low. Thus, the combination of both enzymes led to a significant increased effective degradability of starch from maize grain for medium (ED5) and high (ED8) rates of passage. For ED8 an increase in effective starch degradability of maize grain by 10.1 % was observed.

An increase in ruminal starch degradability of maize grain, when TMR was supplemented with amylase and protease was measured in the current study. Supplementation with amylase showed no effects on ruminal starch disappearance of maize grain. This is in line with Tricarico et al. (2005), who also reported no effects on *in sacco* starch disappearance of maize grain and maize silage incubated in the rumen of cannulated dairy cows and steers when TMR was supplemented with α-amylase from *Aspergillus oryzae*. In contrast to that, other authors reported an increased ruminal starch digestibility of the TMR in dairy

cows (Nozière et al., 2014) and sheep (Mora-Jaimes et al., 2002; Rojo et al., 2005) when α-amylase from *Bacillus licheniformis* was supplemented. Rojo-Rubio et al. (2001) measured an increased starch digestibility *in vitro* of maize and sorghum grain when treated with α-amylase from *Bacillus licheniformis*. In this study supplementation with protease showed no effects on ruminal starch disappearance of maize grain. This is in line with Colombatto et al. (2003a), who reported no effects on starch digestibility of a TMR *in vitro* supplemented with serine protease from *Bacillus licheniformis*.

Enzyme supplementation showed no clear effects on ruminal crude protein degradation of maize grain. No consistent effects of treatment on *in sacco* disappearance of crude protein were observed and parameters of degradation were only affected for the soluble fraction (a), whereas no enzyme treatment showed a significant increase towards the control. Thus, the effective degradability of crude protein from maize grain was not affected by one of the enzyme treatments. As a result, the increased ruminal dry matter degradability of maize grain is based on an increased ruminal degradation of starch.

5.3.2 Soybean meal

The dry matter disappearance of soybean meal showed no consistent effect of treatments. Regarding the parameters of degradation a decreased soluble fraction (a) and an increased ruminal degradable fraction (b) by the supplementation with protease were observed, whereby the degradable fraction (d) was not affected as well as the effective degradability. *In sacco* protein disappearance of soybean meal was not affected by amylase supplementation, but by supplementation with protease and the combination of amylase and protease at short incubation times. Supplementation with both enzymes led to a significant increase up to 6 h and supplementation with protease increased ruminal protein degradation up to 9 h of incubation. This indicates that accelerated ruminal degradability of protein from soybean meal is primarily an effect caused by protease supplementation. This is confirmed by an increased soluble fraction (a) due to the supplementation with protease. This accelerated ruminal degradability led to increased effective degradability of protein from soybean meal at medium (ED5) and high (ED8) passage rates. These findings lead to the conclusion that ruminal protein degradability of soybean meal was mainly affected by supplementation with protease, but the increase of protein degradation is slight so that dry matter degradability of soybean meal was not significantly increased by the enzyme treatments. Colombatto et al. (2003a) observed no effects on crude protein digestibility of a TMR *in vitro*, consisting of 30 % alfalfa hay, 30 % maize silage, and 40 % rolled maize grain, when supplemented with serine protease,

suggesting that exogenous protease cleaves specific bonds between protein and fibre (see also 5.6).

5.3.3 Maize silage

In the current study the dry matter disappearance of maize silage was significantly increased by the supplementation with protease and supplementation with amylase and protease. Protease increased dry matter disappearance from 2 h up to 24 h and the combination of both enzymes from 1 h up to 24 h of incubation (except for 6 h). During the mentioned time frames an increase by 11.8 % was observed for protease supplementation and the combination of amylase and protease, respectively. Furthermore, these two treatments led to an increased soluble fraction (a). This acceleration in dry matter degradability resulted in increased effective degradability of maize silage dry matter for medium (ED5) and high (ED8) rates of passage. According to the degradation of maize grain the combination of both enzymes may have led to an increase of starch degradation of maize silage inside the rumen, but an increase of dry matter disappearance of maize silage by supplementation with protease was unexpected. Apart from that, Colombatto & Beauchemin (2009) reported an increased dry matter digestibility *in vitro* of fresh maize silage and alfalfa hay when supplemented with serine protease. An increase of *in vitro* dry matter digestibility of alfalfa hay due to exogenous protease was also reported by Colombatto et al. (2003b) and Eun et al. (2007). In contrast, Colombatto et al. (2003a) observed no increase of dry matter digestibility *in vitro* of a TMR containing alfalfa hay, maize silage, and rolled maize grain supplemented with exogenous protease.

As with grain maize the ruminal degradation of crude protein and starch from maize silage was determined to verify which nutrient was affected to explain increase of dry matter disappearance. Starch disappearance of maize silage was not affected by treatments. Regarding the parameters of degradation only the soluble fraction (a) was increased by the supplementation with protease. Furthermore, the parameters of degradation showed no significant differences, as well as the effective degradability. Apart from the increased soluble fraction (a) due to protease supplementation, no effects were observed indicating an increased ruminal starch degradation of maize silage by supplementation with protease or amylase and protease. This is in line with Colombatto et al. (2003a) who observed no increase of starch digestibility *in vitro* of a TMR containing alfalfa hay, maize silage, and rolled maize grain supplemented with exogenous protease. In contrast, an increase of *in vitro* starch degradability of maize silage related to protease

supplementation was reported by Young et al. (2012). However, in this study the protease was added to maize silage at the start of ensiling.

Similar results were observed for crude protein degradation. Apart from an increased soluble fraction (a) due to the supplementation with protease, no effects were observed indicating an increased ruminal crude protein degradation of maize silage by supplementation with protease or the combination of amylase and protease. This is once more in line with Colombatto et al. (2003a) who observed no increase of crude protein digestibility *in vitro* of a TMR supplemented with exogenous protease.

In the current study an increased dry matter degradability of maize silage was observed when protease or amylase and protease were supplemented. This increase is neither based on increased starch degradation nor on increased crude protein degradation inside the rumen. Colombatto et al. (2003a) added exogenous serine protease to a TMR containing 30 % alfalfa hay, 30 % fresh maize silage, and 40 % rolled maize grain and reported no effects on *in vitro* digestibility of starch and crude protein, but *in vitro* NDF digestibility was increased by protease supplementation. Based on these findings the authors assumed that exogenous proteases may cleave specific bonds between protein and fibre, resulting in an increased digestibility of NDF, but not of crude protein. Due to protease supplementation increased *in vitro* NDF digestibility of maize silage and alfalfa hay was also reported by Eun & Beauchemin (2007) and of alfalfa hay by Colombatto et al. (2003b) and Eun et al. (2007). An enzyme product containing amylase and protease activity also showed increased *in vitro* NDF digestibility of alfalfa hay (Yang et al., 2011).

In the current study, ruminal NDF degradation of maize silage was not determined, but taking these findings mentioned above into account it cannot be excluded that the increased dry matter degradability of maize silage due to protease supplementation and the combination of amylase and protease is also based on an increased NDF disappearance. Another possibility is that the increased dry matter degradability of maize silage is based on an increased starch or protein degradation, but it was not detectable to methods applied.

5.3.4 Grass silage and hay

Ruminal dry matter disappearance of grass silage was not increased or accelerated by enzyme supplementation. Results showed rather a decelerated effect of amylase supplementation on dry matter degradation of grass silage at short incubation times. Parameters of degradation showed also a decelerated effect due to a decreased soluble fraction (a), in this case by supplementation with protease. Effective degradability was not

affected by treatments. Ruminal NDF degradation of grass silage was also not affected by enzyme supplementation. Similar results were observed regarding ruminal degradation of hay. Dry matter disappearance of hay was not affected by enzyme supplementation.

In the current study no evidence was found regarding an increased ruminal NDF degradability of grass silage and hay. Effects of exogenous enzymes are heavily depending on feedstuffs (Hristov et al., 1999; Colombatto et al., 2003b) or to chemical composition within a feedstuff (Klingerman et al., 2009). Amylase and protease supplementation showed no effects on ruminal degradability of grass silage and hay, but fibrolytic enzymes have shown the possibility to increase apparent digestibility of dry matter, NDF, and ADF of grass forage fed to steers (Feng et al., 1996; Lewis et al., 1996). In contrast to Colombatto et al. (2003a,b), Eun & Beauchemin (2007), and Eun et al. (2007) who reported an increased *in vitro* NDF digestibility of alfalfa hay by supplementing exogenous protease, in this study no effect on ruminal degradability of hay was observed. This may be due to the fact that we measured ruminal degradability *in vivo* and due to the chemical composition of alfalfa hay, which normally has a higher amount of crude protein and thus may have more specific recognition sites for serine proteases (see also 5.6).

5.3.5 TMR

Ruminal degradability of the TMR is similar to that of maize silage, due to the high percentage of maize silage, with little differences. Dry matter disappearance was increased by protease supplementation, the combination of amylase and protease and even by supplementation with amylase at short incubation times. The soluble fraction (a) was increased by all enzyme treatments. This accelerated dry matter degradation inside the rumen resulted in an increased effective degradability at a medium level of feed intake (ED5) for protease supplementation and the combination of amylase and protease and at a high level of feed intake (ED8) for all enzyme treatments. Ruminal degradability of the TMR is an interaction of the degradation characteristics of the single ingredients described above. Additionally, ruminal dry matter degradation of the TMR was affected by supplementation with amylase. The effective degradability showed that an increase of degradation is becoming significant only for high feed intake, which is typical for dairy cows. This may be an explanation for reported increased animal performance in dairy cows supplemented with exogenous amylase.

5.4 Rumen microbial populations

Measured rumen microorganisms were archaea, protozoa, anaerobic fungi, total bacteria, particular genus of bacteria (*Prevotella spp*.) and single bacteria species (*Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *Streptococcus bovis*). *F. succinogenes* and *R. flavefaciens* are one of the most important fibre-degrading bacteria inside the rumen (Weimer, 1996; Stewart et al., 1997) and *S. bovis* is one of the rumen bacteria showing the most amylolytic activity (Cotta, 1988). *Prevotella spp*. are the major group of rumen bacteria (Stewart et al., 1997; Stevenson & Weimer, 2007) and are mainly responsible for protein degradation and deamination of amino acids (Calsamiglia et al., 2007). The percentage of *Prevotella spp*. on total rumen bacteria could be up to 60 % (Stewart et al., 1997).

Rumen microbial populations were determined prior to morning feeding and 1 and 3 hours after feeding. Amylase supplementation decreased proportion of *R. flavefaciens* and protease supplementation increased proportion of archaebacteria relative to total bacteria in the rumen fluid straight before feeding. Further selected rumen microorganisms were not affected by enzyme supplementation, neither in total amount nor in percentage relative to total bacteria. At times of 1 h and 3 h after feeding no effects of amylase or protease supplementation on ruminal microbial populations could be observed.

The only other study which investigated effects of exogenous enzyme supplementation on rumen microbial populations was by Nozière et al. (2014). The authors reported a decrease of *F. succinogenes* (quantity but not ratio to total bacteria) due to amylase supplementation 2.5 hours after feeding. The ratio of *Prevotella spp*. to total bacteria was increased by amylase in a high-starch diet and decreased in a low-starch diet, also 2.5 hours after feeding. No further effects of amylase supplementation on rumen microbiota could be observed prior to feeding or 2.5 hours after feeding. A high-starch diet led to an increase of total bacteria compared to a low starch diet, suggesting that composition of the ration has much more influence on ruminal microbial populations as feed additives. This is in line with Michelland et al. (2011) and Monteils et al. (2012), who reported changes in rumen bacterial composition after dietary changes.

In the current study a decreased ratio of *R. flavefaciens* to total bacteria due to amylase supplementation was observed prior to feeding and Nozière et al. (2014) reported a decrease in *F. succinogenes* 2.5 hours after feeding when amylase was supplemented. This is not evidence of benefitting fibre-degrading bacteria due to amylase supplementation as suggested by Tricarico et al. (2008) but changes of rumen microbial populations in the current study and in this of Nozière et al. (2014) were only slight.

Nozière et al. (2014) reported a decrease of acetic and butyric acid and an increase of propionic acid due to amylase supplementation. Wang et al. (2001) suggested that changes in rumen physiological parameters are related to shifts in rumen microbial populations due to enzyme supplementation, but this may be not the only reason because changes in ruminal microbial populations observed by Nozière et al. (2014) were only minor. Authors also reported that increased ruminal starch degradation is not related to an increase of population of *S. bovis*, which agrees with the current study. A difference between these two studies was, that in the current study slight changes in rumen microbial populations were observed prior to feeding and no changes were determined after feeding. Nozière et al. (2014) reported slight changes 2.5 hours after but not before feeding.

The present study and that of Nozière et al. (2014) showed that amylase supplementation has not the potential to shift rumen microbial populations towards selected fibre-degrading bacteria.

5.5 Total tract digestibility and animal performance

Apparent total tract digestibility was determined for DM, OM, CF, CP, TL, NFE, CA, NDF, ADF, and starch. Neither a single ingredient, nor DM or OM was affected by enzyme supplementation. Apparent total tract digestibility of DM ranged from 76.7 % - 78.2 %, NDF ranged from 67.3 % - 69.8 % and starch was 97.4 %, respectively 97.5 %. Compared to digestibility trials with lactating dairy cows (Klingerman et al., 2009; Gencoglu et al., 2010; Weiss et al., 2011; McCarthy et al., 2013; Nozière et al., 2014), in this study a higher apparent total tract digestibility was observed for OM and NDF. Starch digestibility was also rather high in these studies, except for Weiss et al. (2011), and was in ranges clearly above 90 %. The high digestibility of DM and NDF in our study is related to the low level of feed intake, which is concomitant with a retention time of feed inside the digestive system for a long time, compared to high levels of feed intake, which is typical for lactating cows. Affected apparent total tract digestibility due to enzyme supplementation was mainly reported for dairy cows, suggesting total tract digestibility may be affected only just for higher feed intake levels as in the current study. This was also implied by Beauchemin et al. (2004) who reported that enzyme technology is not likely to benefit ruminants fed at maintenance, but greatest response will be for ruminants fed for maximum productivity.

As mentioned introductory, amylase supplementation to ruminant diet showed beneficial effects on digestion and animal performance in several studies, whereby the exact mode of action is still unknown (Nozière et al., 2014). In recent years trials were carried out using exogenous amylase either from fungal origin (*Aspergillus oryzae* or *Aspergillus niger*) or bacterial origin (*Bacillus licheniformis*) to evaluate effects on digestibility and animal performance in dairy cows, steers, sheep or *in vitro* using rumen simulation techniques. Amylase from *Aspergillus* species was evaluated supplementing either α-amylase to dairy cows (DeFrain et al., 2005; Tricarico et al., 2005; Klingerman et al., 2009), steers (Tricarico et al., 2005; Tricarico et al., 2007) or *in vitro* (Tricarico et al., 2005; Crosby et al., 2012) or glucoamylase to steers (Gutiérrez et al., 2005) or sheep (Mora-Jaimes et al., 2002; Rojo et al., 2005). α-Amylase from *B. licheniformis* was evaluated in dairy cows (Klingerman et al., 2009; Gencoglu et al., 2010; Ferraretto et al., 2011; Weiss et al., 2011; McCarthy et al., 2013; Nozière et al., 2014; Vargas-Rodriguez et al., 2014), steers (Gutiérrez et al., 2005; DiLorenzo et al., 2011), sheep (Mora-Jaimes et al., 2002; Rojo et al., 2005) and *in vitro* (Rojo-Rubio et al., 2001). All studies which evaluated the effects of α-amylase from *B. licheniformis* on digestibility and performance in dairy cows used the same amylase product as in the current study and the supplemented amylase activity was also 300 KNU per kg feed dry matter, except for Vargas-Rodriguez et al. (2014) who supplemented 400 KNU/kg DM and Klingerman et al. (2009). These authors used a liquid formulation in a low (0.88 ml/kg TMR DM) and high (4.4 ml/kg TMR DM) concentration with an amylase activity of 6400 Ceralpha units (CU) per ml, where one CU was the amount of enzyme in the presence of excess amylase required to release one micromole of p-nitrophenol from blocked p-nitrophenyl maltoheptaoside in one minute at pH 6.0 and 40 °C.

An increase of total tract dry matter digestibility due to supplementation with exogenous amylase was observed in dairy cows (Klingerman et al., 2009; Gencoglu et al., 2010; McCarthy et al., 2013) and sheep (Rojo et al., 2005). Rojo et al. (2005), Klingerman et al. (2009), and Gencoglu et al. (2010) additionally reported an increase in total tract organic matter digestibility. In contrast, Weiss et al. (2011) and Nozière et al. (2014) observed no effect on total tract digestibility of dry matter and organic matter when amylase was supplemented to dairy cows. DiLorenzo et al. (2011) also reported no effect on total tract dry matter and organic matter digestibility when amylase was supplemented to feedlot steers, in a concentration of 600 KNU/kg dietary dry matter, fed a 90 % concentrate diet containing mainly maize grain. The majority of studies which evaluated effects of amylase supplementation on nutrient digestibility reported no effect on total tract digestibility of starch (Klingerman et al., 2009; Gencoglu et al., 2010; DiLorenzo et al., 2011; Weiss et

al., 2011; McCarthy et al., 2013; Nozière et al., 2014). Only Rojo et al. (2005) reported that total tract starch digestibility was affected. Glucoamylase supplementation decreased starch digestibility, whereby α-amylase increased starch digestibility of sorghum in sheep.

However, an increased total tract NDF digestibility was often observed. Klingerman et al. (2009), Gencoglu et al. (2010), Weiss et al. (2011), and McCarthy et al. (2013) reported an increase in total tract digestibility of NDF in dairy cows due to supplementation with exogenous α-amylase. This observation is surprising since α-amylase is only able to cleave internal α-1,4-linkages (Bertoldo et al., 2002; Tricarico et al., 2008). Tricarico et al. (2008) suggested the hypothesis that amylase supplementation produces maltodextrins which are used as substrate by non-amylolytic microorganisms, thus fibrolytic bacteria which only grow slowly or not at all on starch are benefited due to amylase supplementation. This may lead to increased NDF digestibility, but not when doses of exogenous enzymes are too high, because this will lead to a strong degradation of substrate up to mono- and disaccharides which may have a contra-productive effect on that cross-feeding mechanism.

Amylase supplementation increased dry matter intake in dairy cows (Klingerman et al., 2009; Gencoglu et al., 2010), while total tract NDF digestibility was also increased. This finding is remarkable, because NDF digestibility usually decreases when dry matter intake is increasing (Huhtanen et al., 2009). Klingerman et al. (2009) also observed an increase in milk yield due to exogenous amylase, as well as Tricarico et al. (2005). Harrison & Tricarico (2007) reported also an increase in milk yield in a case study including 45 commercial dairy herds across the United States and Canada supplemented with an *Aspergillus oryzae* extract containing α-amylase. DeFrain et al. (2005) reported admittedly no increase in milk yield, but observed a decrease in dry matter intake in *prepartum* dairy cows without negative impact on milk yield during the *postpartum* phase up to 70 days in milk when amylase was supplemented, suggesting an improved energy balance and greater ability to maintain blood glucose concentrations due to amylase supplementation. However, Gencoglu et al. (2010) observed an increase in feed conversion ratio in dairy cows supplemented with amylase, as well as Ferraretto et al. (2011), but reported no effect on milk yield. This is in line with Weiss et al. (2011), McCarthy et al. (2013), Nozière et al. (2014), and Vargas-Rodriguez et al. (2014) who also reported no effect on milk yield when amylase was supplemented to dairy cows. Amylase supplementation to steers fed a maize-based diet increased dry matter intake (Tricarico et al., 2007) resulting in an increased average daily gain of finishing beef cattle. This is in contrast to DiLorenzo et al. (2011), who reported no effect of amylase supplementation on average daily gain in feedlot steers fed also a maize-based diet.

Animal performance of sheep was not affected by supplementation with amylase. Mora-Jaimes et al. (2002) and Rojo et al. (2005) observed no effects on dry matter intake, feed conversion ratio and average daily gain due to exogenous amylase.

Compared to supplementation with amylase, data of protease supplementation on digestion and performance of ruminants is limited. Eun & Beauchemin (2005) supplemented a serine protease from *B. licheniformis* to rumen cannulated dairy cows fed a diet based on barley silage and alfalfa hay. On the one hand, authors reported an increase in total tract digestibility of dry matter, organic matter, NDF, ADF, and starch due to protease supplementation. On the other hand exogenous protease led to decreased dry matter intake and milk yield, where milk production efficiency (kg produced milk per kg dry matter intake) was increased.

Exogenous protease was also supplemented to dairy cows in studies of Chen et al. (1995) and Gado et al. (2009). Chen et al. (1995) added an enzyme product of fungal origin containing amylase and protease activity to sorghum grain and observed an increase in total tract digestibility of dry matter, organic matter and NDF in dairy cows. Milk yield, however, was not affected. Gado et al. (2009) supplemented a mixture of enzymes containing cellulase, xylanase, α-amylase, and protease to dairy cows fed a diet based on maize silage. Authors reported an increase in total tract digestibility of dry matter, organic matter and NDF resulting in increased dry matter intake and increased milk production due to supplementation with the mentioned enzyme mixture.

5.6 Possible mode of action of exogenous enzymes on rumen fermentation kinetics

Supplemented enzymes in the current study were α-amylase and serine protease. Serine proteases are endo-acting enzymes which catalyse bond hydrolysis in the middle of a polypeptide chain. Over one third of all known proteolytic enzymes are serine proteases, playing, among others, an important role in digestion (Di Cera, 2009). α-Amylase is also an endo-acting enzyme and cleaves randomly internal α-1,4-linkages of the starch polymer (Bertoldo & Antranikian, 2002; Isaksen et al., 2010), which makes it very important in starch degradation. Starch is an insoluble polymer of α-glucose units composed of a linear polymer bound by α-1,4-linkages (amylose) and a highly branched polymer containing, in addition to α-1,4 glycosidic linkages, α-1,6-linked branch points (amylopectin) occurring every 17 - 26 glucose units (Bertoldo & Antranikian, 2002; Tricarico et al., 2008; Isaksen et al., 2010).

A requirement for exogenous enzymes to act in the rumen, respectively in the intestine is escaping from ruminal proteolysis. Klingerman et al. (2009) reported enzyme activity of α-amylase from *B. licheniformis* and *A. oryzae* remained relatively constant in buffer and rumen fluid over 24 hours. Hristov et al. (1998a,b) investigated stability of polysaccharide-degrading enzymes *in vitro* and *in vivo*. Authors observed increased enzyme activity up to 6 h *in vitro* and xylanase and amylase were resistant against pepsin but inactivated by low pH-values. *In vivo* investigation showed also increased enzyme activities inside the rumen, where maximum activity was after 1.5 h. One of the tested enzyme formulations containing xylanase even increased enzyme activity in duodenal digesta after 6 h. Authors came to the conclusion that exogenous enzymes can resist proteolysis inside the rumen long enough to escape reticulorumen, demonstrate stability in the abomasum and increase duodenal xylanase activity. Morgavi et al. (2000, 2001) investigated stability of exogenous fibrolytic enzymes in rumen fluid of sheep and cows *in vitro*. Some enzymes were stable in rumen fluid up to 6 h, others were almost completely digested after 1 h. Authors came to the conclusion that stability of exogenous enzymes inside the rumen is probably not the limiting factor by the use of exogenous enzymes in ruminants. Morgavi et al. (2000) additionally reported that some plant proteins, for example from soybeans, may protect exogenous enzymes from proteolytic breakdown. This indicates that ruminal and abomasal stability of exogenous enzymes strongly depends on enzyme source and origin and of other factors such as diet (Hristov et al., 2000).

Mode of action of exogenous enzymes can be individually and synergistically (to effect hydrolysis of substrate), they can act as multi-enzyme complexes (individual enzymes are assembled, such as cellulosomes) or increase bacterial attachment to feed particles (Wang & McAllister, 2002; Meale et al., 2014). Amylase supplementation led to increased NDF digestibility in studies of Klingerman et al. (2009), Gencoglu et al. (2010), Weiss et al. (2011), and McCarthy et al. (2013). This may be an example for individually and synergistically mode of action. Tricarico et al. (2008) suggested the hypothesis of a cross-feeding mechanism. Amylase produces maltodexrtins from starch which are used as substrate by non-amylolytic microorganisms, thus fibrolytic bacteria which only grow slowly or not at all on starch are benefited due to amylase supplementation. For example, *F. succinogenes* and *B. fibrisolvens* use rapid available energy to start cell wall degradation (Miron et al., 2002). Tricarico et al. (2008) suggested that correct dosage is important for this cross-feeding mechanism. Excessive doses may lead to an intense degradation of starch, thus may be unfavourable for benefitting fibre-degrading bacteria. Other authors also reported negative effects when dosage of exogenous enzymes was too high (Beauchemin et al., 1995; Kung et al., 2000).

An example for increasing bacterial attachment due to exogenous enzymes may be increased fibre degradability of alfalfa when protease was supplemented, as reported by Colombatto et al. (2003a,b), Eun & Beauchemin (2007), and Eun et al. (2007). Colombatto et al. (2003a) suggested that exogenous protease may cleave specific bonds between protein and fibre, thus NDF degradation was increased but not degradation of crude protein. Colombatto & Beauchemin (2009) observed large disrupted areas in protease-treated alfalfa hay using electron microscopy, which were used by bacteria to attach and colonise substrate rapidly. Increasing the surface area of cellulosic substrate led to increased adhesion of *F. succinogenes* (Miron et al., 2001). Compounds of protein structures and fibre within the cell wall act as structural barriers to microbial digestion (Wallace & Kopecny, 1983) and alfalfa plants contain large amounts of cell wall protein (Bacic et al., 1988). Tyrosine residues in those cell wall proteins could be cross-linking lignin with polysaccharides (Jung, 1997). These tyrosine residues may be the recognition site for serine proteases (Kopecny & Wallace, 1982) which in turn may be an explanation of increased fibre degradation in alfalfa due to exogenous protease. Maize silage may also have specific recognition sites for serine proteases, which might be a reason for an increased dry matter disappearance of maize silage in the current study when protease was supplemented.

Another explanation for these findings may be synergistic effects of exogenous protease. Colombatto et al. (2003b) suggested synergetic effects of exogenous protease with ruminal enzymes resulting in an increase of fibre degradation of maize silage. Eun & Beauchemin (2007) observed synergistic effects of exogenous protease with exogenous fibrolytic enzymes resulting also in increased fibre degradation, but no additive or synergistic effects were observed when applied to alfalfa. This shows the substrate specificity of exogenous enzymes, also reported by Colombatto et al. (2003b) who observed effective enzymes were different for maize and alfalfa silage.

A further example for synergism between exogenous enzymes could be observed in the current study. Combination of amylase and protease led to increased dry matter and starch degradation of maize grain inside the rumen. That indicates that protein barriers of maize grain were broken down by exogenous protease, facilitating access of amylase to starch granules resulting in increased ruminal starch degradation of maize grain.

5.7 Starch digestibility in ruminants and fields of application of amylase and protease in ruminant nutrition

Ruminants do not have salivary amylase (McDougall, 1948), so starch digestion commences inside the rumen by microbial enzymes and perpetuates post-ruminally by endogenous enzymes (Huntington, 1997), resulting in averaged total tract starch digestibility of 90.6 % in dairy cows for a wide range of diets (Firkins et al., 2001). Starch digestion inside the intestine and absorption of glucose is energetically more efficient than ruminal degradation to volatile fatty acids with subsequent gluconeogenesis (Waldo, 1973; Owens et al., 1986; Nocek & Tamminga, 1991), because degradation inside the rumen is associated with energy losses in the form of heat, CH_4 and H_2 (Rowe et al., 1999). However, starch digestion inside the intestine is limited, unlike ruminal digestion (Huntington, 1997; Harmon et al., 2004; Huntington et al., 2006). The capacity to digest starch in the intestine ranges from 45 % to 85 % of starch entering the duodenum, with that capacity apparently limited by the supply of pancreatic amylase (Huntington, 1997). Ruminal digestion of starch has beneficial effects on microbial growth, thus on formation of microbial protein (Ørskov, 1986; Huntington, 1997). Hence, rate and extent of ruminal starch digestion seem to have influence on total tract starch digestibility and animal performance (Poore et al., 1993; Chen et al., 1995; Lykos et al., 1997), where rate is more important for microbial yield than extent (Herrera-Saldana & Huber, 1989). A fast release of available carbohydrates is beneficial for microbial growth (Khalili & Huhtanen, 1991), but that involves the risk that fibre degradation is restricted (Robinson et al., 1987; Martin & Michalet-Doreau, 1996). Ruminal starch digestion is one of the most important factors which affects animal performance of ruminants fed high-grain-diets (Huntington, 1997), but rapid starch degradation within the rumen may lead to acidosis (Owens et al., 1998). Thus, a high rate and extent of ruminal starch digestion, without taking a risk to acidosis and restricted fibre degradation, is desirable.

Starch digestibility in ruminants is depending on animal species and type of grain. Digestibility of starch in cattle is lower compared to digestibility in sheep (Waldo, 1973). There are great differences depending on the type of grain. For example, ruminal and total tract starch digestibility of wheat, barley and oats is higher compared with maize and sorghum. Regarding maize, up to 40 % of starch is able to escape ruminal fermentation (Waldo, 1973; Ørskov, 1986; Owens et al., 1986). These differences can be ascribed to the chemical composition of different grains.

The grain can be segmented in three components: pericarp, embryo and endosperm. The majority of starch is located in the endosperm (Summers, 2001). This part is encased by

the endosperm cell matrix and starch granules are embedded within a protein matrix. This acts as a considerable barrier to access starch to digestion (Kotarski et al., 1992). These protein matrix consists of prolamins, in maize called zein, which are not or hardly hydrolysable inside the rumen (Tamminga, 1979; Larson & Hoffman, 2008). Hence, starch digestibility is negatively correlated to prolamin content (Philippeau et al., 2000; Hu et al., 2010). Protein matrix is also mainly responsible for differences in ruminal degradation between maize and for example, barley. Protein matrix in the endosperm of maize is extremely resistant to digestion by ruminal microorganisms, in contrast protein matrix of barley is rapidly digested inside the rumen (McAllister et al., 1993).

To increase starch digestibility several grain processing techniques were developed (Theurer, 1986; Owens et al., 1997). Thus, starch digestibility of grains was increased due to a disruption of protein matrix that surrounds starch granules, but electron microscopy showed that grinding indeed broke up endosperm cells but starch granules remained embedded with a protein matrix (McAllister et al., 1993). Exogenous enzymes may have the potential to attack such protein barriers, thus facilitating access and attachment to microorganisms inside the rumen.

Results of the present study indicate that protease supplementation has the potential to increase ruminal dry matter degradation of maize silage, whereby further research is needed to understand the exact mechanism. Usage of a combination of amylase and protease may be beneficial in ruminant nutrition when amounts of slow-release starch (which is typical for maize and sorghum grain due to encapsulating protein structures) in feeding rations are high and ruminal pH-value is not negatively influenced by increased ruminal starch degradation. Results also showed that protease supplementation has the potential to increase ruminal crude protein degradability of protein-rich feedstuffs, which may be unfavourable when high amounts of ruminal undegraded feed protein is needed for adequate nutrient supply of the animal.

6 Conclusion

The current study showed that supplementation of a combination of amylase and protease increased ruminal starch degradation of maize grain. This indicates additive effects of amylase and protease in disruption of the protein matrix and facilitation of access to starch granules, because no effects on ruminal starch degradation of maize grain were observed when amylase or protease was supplemented exclusively.

Protease supplementation led to increased ruminal crude protein degradation of soybean meal. Increase was only minor, thus ruminal dry matter disappearance of soybean meal was not affected by enzyme supplementation.

Protease supplementation and the combination of amylase and protease led to increased ruminal dry matter degradation of maize silage. Ruminal starch and crude protein degradation of maize silage were not affected by enzyme supplementation, suggesting that fibre degradation might have been increased by exogenous protease due to specific recognition sites or to synergistic effects with fibrolytic enzymes.

On the other hand ruminal dry matter and NDF disappearance of grass silage and dry matter disappearance of hay were not affected by enzyme supplementation, which in turn indicates that fibre degradation of grass silage and hay was not increased.

Effective degradability indicated that effects of enzyme supplementation are more obvious when passage rate is high, for example due to high feed intake, typical for animals with high performance. This may be also the reason why total tract nutrient digestibility was not affected by enzyme supplementation in the current study.

The rumen physiological parameters pH-value and ammonia-nitrogen were not affected by enzyme supplementation, indicating that exogenous amylase and protease do not increase acidosis risk and ruminal protein degradation of the TMR. No effects on ruminal volatile fatty acids were observed for acetic, propionic and valeric acid. Only concentration of butyric acid was affected, but effects were extremely slight.

Rumen microbial populations were also hardly affected by enzyme supplementation. Slight differences were observed before feeding, but one and three hours after feeding, where ruminal changes were greatest, no effects of enzyme supplementation were determined. The current study and those of Nozière et al. (2014) found no evidence that fibrolytic bacteria benefitted from supplementation of amylase.

7 Literature

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Appendix

Table 44: pH-value in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)							
		0	1	2	3	4	5	6	9
Con	1	6.93	6.46	6.43	6.56	6.52	6.57	6.36	6.84
Con	2	6.98	6.56	6.85	6.69	6.88	6.77	6.77	6.82
Con	3	6.91	7.17	7.02	6.79	7.29	6.87	7.11	7.03
Con	4	6.87	6.55	5.87	6.70	5.98	6.23	6.33	6.54
Con	5	6.92	6.67	6.74	6.75	6.74	6.86	6.79	6.88
Con	6	6.72	6.23	6.19	6.32	5.96	6.24	5.94	6.72
Con	7	6.95	7.21	6.94	6.93	7.24	6.82	7.07	6.92
Con	8	6.91	7.00	6.67	6.70	6.76	6.77	6.71	6.72
Amy	1	7.05	6.55	6.35	6.54	6.97	6.64	6.87	6.93
Amy	2	6.83	6.34	6.56	6.40	6.10	6.62	6.67	6.74
Amy	3	7.17	6.72	6.87	6.71	6.94	6.87	6.91	7.06
Amy	4	6.57	6.79	6.69	6.08	6.86	6.10	6.25	6.57
Amy	5	7.19	6.94	6.63	6.93	6.91	6.98	6.97	7.00
Amy	6	6.70	6.50	5.90	6.00	5.22	5.69	6.01	6.44
Amy	7	7.19	6.66	6.96	6.80	6.96	6.96	7.04	7.05
Amy	8	6.66	7.04	6.85	6.62	7.08	6.79	6.88	6.80
Prot	1	6.90	7.01	6.97	6.56	7.04	6.65	6.80	6.94
Prot	2	7.09	6.64	6.71	6.94	6.58	6.75	6.90	6.89
Prot	3	6.98	6.71	6.72	6.80	6.77	6.77	6.71	6.93
Prot	4	6.82	6.37	6.82	6.33	6.15	6.35	6.74	6.93
Prot	5	7.07	7.29	7.20	7.05	7.40	7.10	7.22	7.12
Prot	6	6.78	6.76	6.55	6.63	6.77	6.29	6.34	6.50
Prot	7	6.93	6.59	6.67	6.68	6.62	6.82	6.68	6.97
Prot	8	6.84	6.53	6.69	6.61	6.58	6.69	6.68	6.90
Amy+Prot	1	7.03	6.42	6.62	6.47	6.56	6.63	6.70	6.80
Amy+Prot	2	6.72	6.81	6.93	6.41	6.90	6.68	6.87	6.90
Amy+Prot	3	7.14	6.84	6.70	6.92	6.84	7.01	6.93	7.00
Amy+Prot	4	6.48	6.16	6.31	6.24	5.52	5.83	5.54	6.73
Amy+Prot	5	7.13	6.55	6.71	6.84	6.65	6.90	6.82	7.00
Amy+Prot	6	6.81	6.84	6.50	6.82	6.76	6.31	6.48	6.96
Amy+Prot	7	7.08	6.81	6.67	6.81	6.78	6.86	6.77	7.04
Amy+Prot	8	6.81	6.59	6.57	6.51	6.59	6.67	6.57	6.75

Table 45: Ammonia-nitrogen concentration (mg/l) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)							
		0	1	2	3	4	5	6	9
Con	1	39.6	142	149	97.6	49.6	17.3	11.0	29.8
Con	2	44.9	126	123	90.3	56.0	53.0	36.5	59.0
Con	3	85.3	173	146	98.5	73.1	42.6	39.9	49.7
Con	4	34.4	127	243	101	39.4	95.2	49.7	24.2
Con	5	39.6	128	112	79.4	18.6	19.3	24.2	33.5
Con	6	40.5	190	163	124	77.1	19.4	52.8	25.3
Con	7	50.7	141	142	124	46.3	21.4	19.6	30.3
Con	8	51.4	122	83.2	84.6	73.3	25.8	17.8	62.4
Amy	1	43.2	151	132	78.9	70.1	35.3	20.7	37.8
Amy	2	23.9	109	103	98.9	64.7	22.4	5.78	5.57
Amy	3	65.1	170	107	76.2	82.8	46.6	48.8	48.1
Amy	4	40.3	122	114	120	47.5	53.4	45.0	31.0
Amy	5	52.2	160	137	18.1	44.0	20.4	36.6	40.9
Amy	6	45.9	86.6	121	140	94.0	86.3	67.1	18.8
Amy	7	43.4	136	108	55.1	30.6	29.6	17.7	40.5
Amy	8	71.1	197	167	109	92.8	48.5	68.5	52.8
Prot	1	67.6	169	120	79.4	77.3	19.0	16.0	62.2
Prot	2	36.5	141	132	95.8	86.8	39.7	11.9	21.0
Prot	3	47.1	163	126	86.1	44.5	31.6	25.0	32.1
Prot	4	68.5	152	128	96.9	90.1	64.7	31.0	42.1
Prot	5	66.8	160	125	53.8	48.5	28.8	25.1	43.5
Prot	6	41.8	139	169	111	63.3	57.5	46.6	42.9
Prot	7	30.3	129	115	89.4	16.3	20.7	18.6	27.6
Prot	8	77.1	149	144	103	103	69.3	86.6	73.6
Amy+Prot	1	50.5	162	158	95.7	35.2	18.6	35.8	36.9
Amy+Prot	2	51.0	128	133	116	93.2	41.6	20.7	30.3
Amy+Prot	3	57.1	144	79.8	80.5	25.6	34.7	30.1	51.2
Amy+Prot	4	23.8	121	116	121	103	65.6	58.3	15.4
Amy+Prot	5	56.6	156	110	129	72.0	36.5	23.8	39.9
Amy+Prot	6	32.8	166	182	123	73.5	71.6	9.15	19.6
Amy+Prot	7	40.0	150	139	53.7	23.9	16.8	35.9	28.9
Amy+Prot	8	51.7	136	118	74.0	34.5	31.1	34.5	50.8

Table 46: Acetic acid concentration (mmol/l) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)							
		0	1	2	3	4	5	6	9
Con	1	54.3	52.3	65.1	65.2	68.1	76.1	70.2	54.9
Con	2	48.4	53.5	60.4	55.2	49.5	58.3	55.2	55.1
Con	3	51.3	62.3	61.5	64.4	56.7	58.4	60.0	55.6
Con	4	56.0	48.4	81.3	66.2	80.3	74.8	71.8	61.5
Con	5	52.3	62.5	53.9	50.4	57.6	56.1	55.9	52.1
Con	6	71.4	73.2	78.1	77.9	88.0	83.2	86.7	68.0
Con	7	57.9	59.7	64.1	60.0	58.9	60.9	65.4	61.2
Con	8	58.2	66.1	64.0	68.0	60.3	58.3	61.5	60.3
Amy	1	53.0	54.5	66.4	67.1	79.0	66.8	56.3	54.3
Amy	2	57.3	50.7	48.5	60.9	68.6	60.8	54.7	53.0
Amy	3	48.1	60.4	65.1	65.7	55.8	57.7	59.0	51.8
Amy	4	59.7	59.2	65.9	72.8	72.2	80.9	80.8	64.0
Amy	5	45.6	62.2	64.8	57.1	55.7	51.5	53.5	52.1
Amy	6	64.7	67.1	84.1	68.0	.	93.2	75.5	69.4
Amy	7	50.4	54.9	56.4	62.1	53.4	59.7	54.4	54.2
Amy	8	67.0	66.2	71.1	72.7	65.6	61.9	68.0	63.6
Prot	1	54.2	65.1	60.4	69.8	64.3	64.6	60.4	55.3
Prot	2	51.0	45.2	51.7	54.6	58.4	53.8	49.7	53.0
Prot	3	51.6	48.8	57.3	52.3	62.8	61.8	58.7	51.2
Prot	4	64.6	56.8	63.4	79.8	81.8	82.6	57.4	63.1
Prot	5	50.3	60.2	60.8	52.9	56.9	54.8	58.1	53.9
Prot	6	63.5	67.1	67.2	65.2	60.9	72.8	74.0	69.2
Prot	7	51.5	54.4	57.5	47.2	61.4	51.9	56.7	46.1
Prot	8	61.4	66.1	71.4	71.2	69.1	70.9	65.5	61.6
Amy+Prot	1	51.7	62.4	66.3	65.2	66.9	58.4	61.0	56.7
Amy+Prot	2	58.6	50.1	53.4	64.7	56.3	58.7	62.8	52.7
Amy+Prot	3	50.0	57.9	67.1	58.5	57.4	46.6	54.8	50.1
Amy+Prot	4	63.0	52.8	70.7	57.0	97.7	86.8	87.8	56.0
Amy+Prot	5	53.9	62.0	68.4	66.3	69.7	60.0	62.3	62.5
Amy+Prot	6	59.0	73.4	80.9	55.7	71.4	70.1	77.1	55.9
Amy+Prot	7	58.0	63.0	61.9	56.4	58.0	51.3	63.4	51.0
Amy+Prot	8	55.2	62.5	63.3	57.2	66.4	62.5	60.1	54.3

Table 47: Propionic acid concentration (mmol/l) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)							
		0	1	2	3	4	5	6	9
Con	1	11.4	17.4	20.6	18.7	19.7	20.0	16.6	12.2
Con	2	10.3	16.5	17.0	14.8	12.5	15.5	12.2	13.4
Con	3	8.87	17.3	14.9	14.3	11.7	12.0	11.6	10.0
Con	4	13.0	16.5	27.0	19.2	19.1	19.8	18.5	15.4
Con	5	10.5	21.6	17.8	14.3	15.3	13.5	13.1	11.2
Con	6	14.4	21.3	20.7	19.1	20.3	17.4	18.5	13.6
Con	7	12.0	19.4	17.8	18.4	13.6	16.1	14.1	13.8
Con	8	12.8	19.8	15.3	16.8	14.3	12.6	12.9	13.2
Amy	1	12.7	18.5	22.0	21.6	23.0	17.6	13.2	12.6
Amy	2	12.1	17.3	16.7	18.2	17.0	13.6	12.8	11.4
Amy	3	8.77	16.4	15.9	14.3	12.6	11.7	11.2	9.87
Amy	4	14.7	20.3	19.3	22.1	19.1	23.5	19.3	15.9
Amy	5	8.14	19.2	17.7	13.5	12.6	11.1	10.8	10.3
Amy	6	14.9	18.3	23.4	18.9	30.7	19.7	17.1	14.3
Amy	7	10.7	17.7	16.9	16.2	14.0	13.7	12.8	11.8
Amy	8	13.2	18.2	17.3	17.7	14.9	13.7	15.3	13.3
Prot	1	13.0	21.6	19.1	18.4	18.0	16.4	14.9	13.7
Prot	2	9.95	15.1	16.0	15.2	14.7	12.5	10.8	10.9
Prot	3	8.71	15.2	16.4	12.5	14.8	14.4	12.9	10.1
Prot	4	15.0	16.7	18.5	21.0	21.0	20.2	14.2	14.6
Prot	5	8.26	17.0	15.0	12.5	11.9	11.2	11.3	9.63
Prot	6	14.1	19.4	20.9	19.5	15.1	18.5	16.9	15.6
Prot	7	12.6	23.8	21.1	17.5	19.3	15.4	15.7	12.5
Prot	8	12.4	18.9	17.9	16.2	16.4	15.1	13.8	13.0
Amy+Prot	1	12.7	22.2	21.2	23.5	18.6	18.5	15.5	15.1
Amy+Prot	2	11.6	15.4	15.5	15.6	12.9	12.3	12.4	10.0
Amy+Prot	3	9.22	17.7	17.1	14.8	12.6	10.1	10.7	9.31
Amy+Prot	4	14.4	16.2	20.4	18.1	22.0	17.9	20.1	12.6
Amy+Prot	5	9.61	18.2	16.8	14.7	16.3	12.4	12.8	11.4
Amy+Prot	6	10.3	21.3	25.9	16.8	18.0	20.5	19.0	10.7
Amy+Prot	7	12.3	22.5	19.6	16.6	14.8	13.5	13.4	11.4
Amy+Prot	8	12.1	19.8	18.3	16.3	16.4	14.4	14.2	12.9

Table 48: Butyric acid concentration (mmol/l) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)							
		0	1	2	3	4	5	6	9
Con	1	11.3	13.1	17.0	17.0	19.9	23.9	17.5	13.2
Con	2	8.87	12.6	14.4	12.3	11.5	12.9	12.6	12.4
Con	3	9.28	14.0	14.2	14.3	12.8	12.7	13.5	10.9
Con	4	12.9	12.8	24.3	18.9	20.2	20.1	17.7	16.6
Con	5	9.18	13.5	12.9	12.0	14.1	14.4	14.1	12.1
Con	6	13.5	17.3	18.8	18.9	19.7	19.1	21.7	15.3
Con	7	8.99	14.7	14.7	15.3	14.0	13.4	13.3	12.5
Con	8	11.7	15.7	14.4	17.1	14.8	13.7	14.2	13.3
Amy	1	10.5	13.6	16.8	16.9	20.0	16.3	12.6	11.5
Amy	2	8.20	11.5	12.4	15.1	16.9	13.9	12.5	11.0
Amy	3	9.11	12.5	13.9	14.0	12.1	12.9	13.0	11.1
Amy	4	11.4	14.1	16.6	18.1	17.1	18.1	20.3	13.5
Amy	5	7.17	14.4	15.3	12.7	12.4	11.0	11.7	10.2
Amy	6	10.9	15.1	20.5	19.4	31.5	23.7	18.3	13.8
Amy	7	9.53	11.8	13.2	14.1	12.2	13.1	12.2	11.3
Amy	8	9.81	16.0	15.8	17.6	15.1	13.9	15.3	13.5
Prot	1	10.9	16.7	16.4	17.1	17.0	14.5	14.1	11.3
Prot	2	9.17	11.0	13.3	13.0	13.6	13.0	11.1	11.5
Prot	3	8.72	10.8	12.9	12.2	14.5	13.9	13.5	11.6
Prot	4	12.8	13.5	15.2	18.3	19.4	18.6	14.0	13.0
Prot	5	7.11	13.2	13.4	11.2	11.7	11.3	11.6	10.1
Prot	6	12.9	15.8	18.3	18.2	14.9	18.5	18.1	15.8
Prot	7	9.26	12.6	14.4	11.5	15.8	15.0	14.6	11.0
Prot	8	12.3	16.2	17.5	15.5	17.9	16.5	15.1	13.6
Amy+Prot	1	9.48	14.5	15.5	16.8	15.7	12.9	14.7	11.5
Amy+Prot	2	10.1	11.9	13.3	14.5	11.9	11.9	12.6	10.1
Amy+Prot	3	8.80	12.3	13.6	13.8	11.2	10.9	10.5	10.0
Amy+Prot	4	11.7	12.9	18.2	15.4	21.0	18.0	23.0	13.5
Amy+Prot	5	8.77	11.9	14.1	12.8	13.8	11.7	12.5	11.1
Amy+Prot	6	9.94	17.3	20.4	13.0	18.1	16.9	18.0	12.6
Amy+Prot	7	8.72	14.7	14.7	13.5	13.0	11.3	12.9	9.65
Amy+Prot	8	10.3	15.7	16.2	14.1	15.0	14.3	14.7	14.0

Table 49: Valeric acid concentration (mmol/l) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)							
		0	1	2	3	4	5	6	9
Con	1	2.72	2.23	3.55	3.40	3.37	3.71	3.72	2.75
Con	2	2.37	2.89	3.82	3.75	2.97	3.95	3.43	3.15
Con	3	2.20	3.84	3.80	3.88	3.01	3.16	2.65	2.19
Con	4	2.68	2.93	5.25	4.53	5.10	4.60	4.06	3.37
Con	5	1.91	2.42	2.80	2.49	2.63	2.40	2.21	1.97
Con	6	2.83	3.88	4.75	4.37	5.11	4.50	5.21	3.34
Con	7	2.01	3.14	3.84	3.67	3.09	2.90	3.07	2.57
Con	8	2.61	3.17	2.64	3.88	2.87	3.11	2.42	3.02
Amy	1	2.53	2.89	3.61	3.57	4.96	3.89	2.96	2.76
Amy	2	2.32	2.21	2.61	3.20	3.35	2.88	3.01	2.70
Amy	3	2.07	3.46	3.83	3.97	3.02	3.05	2.84	2.20
Amy	4	2.76	3.13	3.60	3.75	3.66	4.35	4.08	2.85
Amy	5	1.73	3.50	3.82	2.85	3.07	2.47	2.43	2.27
Amy	6	2.86	3.39	4.91	4.79	6.05	5.20	4.21	4.18
Amy	7	2.29	3.11	3.64	3.77	3.29	3.55	3.03	2.78
Amy	8	1.77	3.59	3.56	3.66	2.82	2.74	2.76	2.46
Prot	1	3.07	3.59	4.24	3.99	4.57	3.94	2.98	2.66
Prot	2	2.11	2.38	3.18	3.72	3.74	3.49	2.40	2.85
Prot	3	1.83	2.36	3.38	2.72	3.11	2.75	2.96	2.13
Prot	4	3.02	3.01	3.92	5.05	5.30	4.71	3.37	3.16
Prot	5	1.58	3.39	3.42	3.25	2.89	2.72	2.45	2.15
Prot	6	3.01	4.12	4.44	4.70	4.14	4.56	4.02	3.77
Prot	7	1.83	2.19	3.09	2.63	2.81	2.45	2.56	1.81
Prot	8	2.41	3.59	3.66	3.58	3.58	3.31	2.92	2.67
Amy+Prot	1	2.75	3.68	4.52	4.33	4.13	3.32	4.43	2.84
Amy+Prot	2	2.40	2.93	3.11	3.67	3.00	2.86	2.61	1.94
Amy+Prot	3	1.98	3.48	4.16	3.39	3.57	2.63	2.76	2.37
Amy+Prot	4	2.89	2.71	4.51	3.50	4.63	3.79	5.34	3.30
Amy+Prot	5	1.86	2.58	3.05	3.68	3.28	2.81	2.46	2.36
Amy+Prot	6	2.45	4.18	4.93	4.64	4.46	4.87	4.32	3.14
Amy+Prot	7	2.05	2.99	3.33	3.06	3.02	2.41	2.39	2.05
Amy+Prot	8	2.11	2.78	3.40	3.16	3.05	2.46	2.66	2.46

Table 50: Ruminal dry matter disappearance (%) of maize grain of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Con	1	28.4	30.7	29.6	31.7	37.1	32.4	39.0	41.7	70.1	90.2
Con	1	28.7	31.5	31.1	34.0	33.0	34.2	35.1	41.3	68.2	93.7
Con	1	27.4	29.5	27.9	32.5	35.9	32.8	39.3	46.6	66.4	96.3
Con	1	27.5	29.6	30.1	33.4	33.1	34.2	40.3	46.1	69.8	96.7
Con	2	29.8	31.1	34.2	33.3	36.3	38.2	41.6	43.3	60.0	86.0
Con	2	29.0	33.1	32.7	32.6	35.8	36.8	38.1	40.5	67.4	91.7
Con	2	26.4	28.8	29.5	31.1	37.9	37.3	48.4	48.0	62.3	88.3
Con	2	27.0	29.0	29.7	32.0	37.1	34.7	40.1	46.8	59.0	87.9
Con	3	26.9	31.7	33.7	32.0	39.0	40.5	45.3	50.7	69.1	96.6
Con	3	30.4	33.0	31.0	34.5	37.7	40.0	45.4	54.7	69.5	96.8
Con	3	28.5	31.0	31.8	32.4	37.7	40.6	45.4	51.8	74.3	96.4
Con	3	26.5	31.7	31.7	31.0	34.1	35.4	47.5	56.2	72.1	96.9
Con	4	31.0	29.4	34.0	32.8	34.3	36.3	40.3	43.4	69.8	95.6
Con	4	27.9	27.4	32.2	32.5	33.4	34.6	38.4	39.3	71.4	95.8
Con	4	28.5	29.7	42.6	26.5	33.3	33.1	38.4	36.7	68.6	96.6
Con	4	29.4	29.1	31.0	32.1	33.0	32.9	38.1	42.2	63.9	96.6
Con	5	29.1	29.3	32.4	32.2	32.6	37.3	45.9	48.5	64.3	93.2
Con	5	28.0	30.9	30.9	32.1	33.9	36.1	44.1	51.1	65.3	94.8
Con	5	28.2	30.5	32.4	30.4	32.6	37.8	40.1	44.3	64.9	95.8
Con	5	28.1	29.6	30.9	32.6	32.4	36.5	40.2	45.3	66.2	95.9
Con	6	27.5	28.8	30.1	36.4	36.0	37.5	40.8	45.4	75.6	94.6
Con	6	28.5	29.6	32.4	37.1	36.9	36.6	42.4	44.7	77.0	93.7
Con	6	27.9	30.1	32.1	33.6	32.7	40.2	36.3	43.8	70.7	95.7
Con	6	27.5	30.8	30.1	34.5	34.9	38.1	40.5	43.0	70.9	95.6
Con	7	26.3	29.6	33.9	33.2	40.0	39.0	43.3	53.1	63.1	95.7
Con	7	27.2	29.1	32.3	33.9	35.8	39.0	46.0	52.0	60.1	95.8
Con	7	26.7	27.7	31.4	33.3	34.5	35.0	35.8	47.4	61.7	96.0
Con	7	28.2	27.9	31.0	34.8	34.9	34.1	38.8	47.2	58.0	94.6
Con	8	29.1	31.9	32.3	35.5	35.3	39.9	41.1	47.4	70.5	95.6
Con	8	30.0	31.0	32.0	37.2	34.4	39.2	40.7	46.2	69.4	95.4
Con	8	27.8	31.7	32.8	38.2	35.2	38.6	42.2	47.1	67.0	93.4
Con	8	27.4	30.9	31.6	35.9	35.0	39.0	42.7	47.5	70.2	95.5
Amy	1	27.9	31.1	32.4	33.9	34.4	33.8	40.4	42.4	52.6	87.2
Amy	1	27.8	30.1	33.4	34.6	34.8	33.8	41.9	42.7	51.4	90.5
Amy	1	28.0	30.0	34.0	31.6	33.2	34.2	39.4	41.2	58.5	93.2
Amy	1	28.1	30.3	32.6	30.9	35.4	34.2	39.1	42.5	54.2	95.5
Amy	2	28.8	29.5	31.5	30.9	34.0	33.7	36.0	44.4	76.7	94.3
Amy	2	29.3	28.8	30.7	33.3	33.6	34.3	36.4	40.5	64.2	96.4
Amy	2	26.9	28.2	30.9	33.0	33.2	35.2	37.9	47.2	82.6	95.3
Amy	2	27.7	28.0	29.3	30.4	32.6	33.5	37.9	40.1	80.7	95.3
Amy	3	28.0	31.1	34.3	35.6	36.9	42.2	43.6	52.3	78.8	95.8
Amy	3	28.2	30.3	35.0	37.2	35.9	40.4	47.6	52.1	77.8	96.6
Amy	3	28.6	31.5	32.3	34.2	38.1	37.8	42.6	52.9	73.7	96.7
Amy	3	28.6	31.0	32.3	33.3	36.9	40.0	45.4	48.8	75.4	96.3

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Amy	4	26.9	28.6	31.5	30.6	32.8	31.7	35.8	37.2	67.7	87.7
Amy	4	27.1	32.0	29.8	31.8	32.4	33.2	36.1	36.3	68.8	85.4
Amy	4	28.5	30.5	29.7	31.1	33.5	36.3	36.3	41.1	64.3	90.3
Amy	4	26.2	28.9	30.5	28.2	31.5	33.9	37.6	37.3	66.8	86.8
Amy	5	28.5	30.0	33.9	33.5	36.8	40.6	38.8	46.0	75.3	96.8
Amy	5	26.4	29.7	33.3	36.6	36.5	41.6	40.0	46.9	61.9	97.1
Amy	5	26.7	30.7	33.6	31.7	36.1	37.2	40.5	48.4	72.7	97.6
Amy	5	27.1	31.1	31.6	32.9	35.1	37.2	40.3	45.9	66.9	97.6
Amy	6	26.4	32.3	30.6	30.6	35.4	33.7	39.5	46.0	67.4	75.6
Amy	6	28.7	28.5	30.3	32.2	35.5	36.4	36.4	49.6	64.7	77.3
Amy	6	28.3	30.6	29.7	29.9	35.5	33.8	37.2	45.7	70.4	82.6
Amy	6	28.6	29.2	31.7	33.3	37.0	34.8	36.5	44.6	72.9	81.3
Amy	7	25.8	27.9	29.7	33.4	35.7	37.0	39.5	43.5	59.1	97.3
Amy	7	27.9	29.0	31.0	32.3	33.6	34.8	41.7	47.3	66.4	97.4
Amy	7	26.4	28.1	31.1	30.9	33.9	39.5	41.8	45.6	69.1	97.3
Amy	7	26.5	29.4	29.2	30.3	34.7	36.9	42.6	46.0	68.0	97.2
Amy	8	28.4	30.5	33.0	35.9	35.5	34.9	41.2	45.2	75.7	90.0
Amy	8	29.8	31.4	31.0	34.5	34.1	35.3	42.9	52.4	68.7	90.8
Amy	8	27.5	28.9	30.3	34.2	35.8	34.2	40.7	46.0	77.0	92.6
Amy	8	28.0	30.3	30.9	34.0	35.9	34.5	40.4	44.9	76.7	91.0
Prot	1	28.0	30.8	31.1	28.4	33.0	34.9	39.1	42.2	63.4	87.3
Prot	1	27.6	29.9	30.2	31.3	33.0	37.7	40.7	45.6	57.9	88.1
Prot	1	26.0	30.1	30.1	32.9	32.1	38.8	39.2	40.1	71.2	93.6
Prot	1	26.4	29.0	30.6	30.2	33.1	32.9	38.0	38.6	73.9	92.5
Prot	2	27.8	29.1	31.6	30.8	34.8	33.0	39.6	43.1	56.4	96.4
Prot	2	28.8	27.1	30.6	28.7	35.6	33.6	35.8	41.9	47.4	95.0
Prot	2	27.2	27.9	29.3	29.3	33.2	32.2	37.7	49.1	57.9	95.8
Prot	2	25.3	28.6	29.6	29.2	31.4	34.9	39.8	47.0	59.3	95.5
Prot	3	31.0	28.4	32.2	33.2	32.4	34.9	46.2	47.9	62.7	94.2
Prot	3	29.8	27.4	32.1	34.3	32.4	37.6	48.6	46.7	67.2	93.3
Prot	3	29.0	28.9	32.7	30.7	31.7	32.9	47.1	52.1	69.0	94.8
Prot	3	30.4	29.1	32.7	29.7	31.9	33.4	45.9	54.2	65.4	96.0
Prot	4	26.2	29.0	30.0	31.2	33.0	30.4	38.4	48.0	63.2	94.9
Prot	4	26.0	29.9	29.2	30.8	31.9	34.9	40.1	42.2	63.9	94.3
Prot	4	24.1	28.4	27.6	25.0	34.0	31.9	37.9	46.9	68.3	96.4
Prot	4	26.7	27.9	28.6	31.3	33.4	31.6	38.7	43.8	66.4	96.6
Prot	5	30.5	28.8	31.9	36.2	36.0	34.3	43.8	51.8	70.6	94.7
Prot	5	27.7	29.5	34.8	34.1	35.6	32.8	46.6	51.4	74.0	95.5
Prot	5	25.6	27.5	30.4	31.7	35.2	34.3	43.9	46.7	61.7	96.6
Prot	5	26.1	28.1	31.8	31.7	36.8	36.6	45.2	51.5	64.1	96.1
Prot	6	27.8	27.7	32.7	39.4	34.9	35.7	41.5	38.3	67.6	94.5
Prot	6	28.3	30.9	32.9	32.3	33.4	35.0	40.1	38.1	66.8	94.8
Prot	6	24.5	28.5	29.6	34.7	35.9	32.6	39.9	44.5	62.7	94.4
Prot	6	26.7	29.7	32.1	34.1	33.7	34.0	43.7	40.8	69.3	94.5
Prot	7	27.6	28.7	29.2	31.1	33.2	37.8	41.0	47.5	59.8	91.8
Prot	7	27.7	28.4	29.1	30.6	30.1	36.0	41.2	47.7	66.6	94.1

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Prot	7	27.9	28.1	30.2	23.0	35.6	32.5	39.0	53.8	66.1	96.0
Prot	7	21.1	26.6	30.9	29.5	33.8	33.2	38.5	48.7	.	95.9
Prot	8	28.5	30.0	30.5	33.9	33.9	36.9	44.6	47.1	70.0	94.6
Prot	8	29.0	28.9	30.7	34.2	34.1	36.9	43.2	43.1	71.8	94.4
Prot	8	28.6	31.5	31.5	33.8	32.0	34.5	41.2	43.9	66.9	95.6
Prot	8	28.6	31.3	32.5	34.5	32.7	34.6	41.4	48.5	66.0	96.7
Amy+Prot	1	32.2	32.1	35.9	34.3	36.0	40.3	40.7	49.7	74.9	94.0
Amy+Prot	1	32.5	33.3	41.7	36.6	35.9	39.2	45.1	51.3	65.0	94.0
Amy+Prot	1	29.9	32.6	34.7	37.2	38.3	38.2	43.2	46.6	62.7	94.0
Amy+Prot	1	30.3	32.2	37.1	35.9	37.4	37.4	42.9	46.2	70.1	94.9
Amy+Prot	2	31.3	32.5	35.2	35.4	34.7	40.9	40.9	48.0	64.5	89.3
Amy+Prot	2	31.6	32.1	34.4	36.8	35.4	40.6	43.0	48.6	63.6	89.7
Amy+Prot	2	30.2	32.4	34.4	33.4	36.1	40.3	41.1	51.5	69.2	92.3
Amy+Prot	2	29.8	31.2	33.5	32.8	34.7	38.9	40.2	49.3	68.7	90.0
Amy+Prot	3	29.3	32.2	35.4	36.1	44.4	41.1	49.4	61.9	71.9	97.5
Amy+Prot	3	28.6	33.9	36.7	36.2	41.5	41.2	49.5	59.8	83.1	97.1
Amy+Prot	3	26.0	30.8	36.3	32.8	42.9	43.6	50.4	54.2	77.1	97.4
Amy+Prot	3	24.1	30.4	39.3	32.4	40.2	43.4	55.0	.	77.6	97.6
Amy+Prot	4	29.2	31.9	33.8	34.0	35.2	33.9	41.1	40.7	68.4	95.0
Amy+Prot	4	27.4	33.4	34.7	34.0	35.4	35.7	40.1	43.1	71.5	94.4
Amy+Prot	4	29.3	31.0	32.8	34.0	34.8	37.3	38.6	38.4	69.0	93.6
Amy+Prot	4	.	31.4	33.7	34.3	35.0	37.2	39.9	41.6	70.6	95.0
Amy+Prot	5	31.2	33.6	34.6	37.3	37.3	39.3	44.4	45.2	69.7	95.4
Amy+Prot	5	31.9	34.3	35.2	38.1	36.4	38.8	46.0	53.6	67.5	95.6
Amy+Prot	5	30.1	30.7	32.3	34.9	36.1	39.6	45.0	50.4	69.1	96.2
Amy+Prot	5	29.4	32.0	33.0	34.9	34.7	38.6	43.9	50.9	75.1	96.5
Amy+Prot	6	31.5	32.9	36.5	.	37.3	37.3	41.1	57.1	75.4	90.7
Amy+Prot	6	35.0	34.1	35.1	38.3	39.3	37.4	44.3	52.0	76.1	93.4
Amy+Prot	6	31.7	31.0	34.8	34.4	37.9	36.1	43.0	56.5	75.7	92.1
Amy+Prot	6	30.5	32.6	35.7	35.1	40.6	39.1	42.7	.	76.9	93.5
Amy+Prot	7	28.7	31.8	34.7	35.1	38.1	37.4	43.0	51.0	72.5	95.9
Amy+Prot	7	32.0	32.3	33.1	35.9	37.8	37.2	45.2	48.2	71.0	96.6
Amy+Prot	7	29.3	30.2	33.5	28.6	34.6	37.5	.	51.0	63.1	96.1
Amy+Prot	7	29.9	31.4	34.0	33.3	35.3	37.5	45.8	51.5	67.7	96.6
Amy+Prot	8	30.2	33.6	36.0	35.8	36.1	39.9	45.4	50.2	.	92.5
Amy+Prot	8	30.6	31.7	36.6	36.1	37.5	40.4	42.9	48.5	71.3	90.8
Amy+Prot	8	30.8	33.0	34.3	35.8	37.7	39.3	42.9	47.1	72.4	83.8
Amy+Prot	8	30.7	33.5	33.7	36.9	36.2	37.3	42.3	50.2	74.8	83.8

Table 51: Ruminal dry matter disappearance (%) of soybean meal of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Con	1	31.6	33.1	34.4	35.5	36.4	38.7	49.1	44.9	89.1	98.8
Con	1	30.6	33.6	33.8	36.6	37.0	39.4	46.8	58.2	88.0	97.7
Con	1	31.1	32.8	34.3	44.1	39.4	48.5	47.0	75.0	92.8	97.9
Con	1	30.3	33.1	35.2	36.5	40.4	40.4	58.9	81.0	94.4	98.5
Con	2	30.6	34.5	37.6	40.0	37.4	40.8	57.2	55.5	77.4	97.8
Con	2	31.7	34.9	37.4	38.0	37.5	38.8	46.6	52.2	75.4	92.5
Con	2	31.1	34.0	35.8	41.2	44.9	38.7	67.9	.	86.6	98.4
Con	2	30.6	33.1	36.6	39.3	39.9	40.0	62.4	57.5	92.4	98.2
Con	3	32.5	36.2	35.7	38.5	51.0	49.7	75.6	86.6	96.9	98.3
Con	3	33.3	40.2	38.5	35.9	49.5	49.2	75.4	84.9	96.4	98.4
Con	3	31.2	34.2	37.7	42.3	48.7	49.0	74.5	86.4	97.4	97.9
Con	3	30.7	35.9	37.7	42.8	46.0	44.8	80.3	85.8	97.3	98.1
Con	4	31.2	31.4	35.8	36.8	42.1	43.9	66.6	61.9	93.8	98.7
Con	4	30.7	33.4	36.0	36.6	41.6	46.9	58.9	69.2	90.5	98.5
Con	4	30.2	34.1	35.6	35.2	39.0	43.5	48.2	60.8	95.6	97.9
Con	4	30.3	33.7	38.4	35.0	39.1	42.8	47.7	67.6	95.4	99.3
Con	5	31.6	33.8	35.7	37.7	42.6	43.7	58.4	64.8	96.6	97.6
Con	5	31.6	32.8	35.1	40.7	42.3	48.6	75.5	76.0	96.6	98.2
Con	5	31.4	34.3	35.8	40.4	42.9	48.3	62.4	78.9	96.1	98.3
Con	5	30.9	34.9	36.8	41.6	44.6	44.3	74.3	73.3	95.4	97.9
Con	6	29.9	31.9	33.6	37.9	36.2	43.0	46.2	61.3	94.4	98.1
Con	6	29.6	35.3	33.7	37.3	35.8	39.5	44.6	61.9	95.2	97.9
Con	6	29.5	31.2	33.8	38.9	40.9	40.2	49.2	58.9	94.9	98.0
Con	6	29.5	31.5	34.0	37.7	40.8	40.8	41.5	63.3	95.2	98.3
Con	7	30.5	34.6	39.7	35.2	45.7	56.6	71.3	77.8	94.9	97.8
Con	7	30.5	34.1	37.1	38.5	50.0	.	70.4	76.7	94.8	97.7
Con	7	31.0	32.7	35.6	40.1	46.5	51.4	54.1	77.2	93.7	98.2
Con	7	30.8	32.3	37.2	41.6	45.1	47.0	56.7	73.7	95.4	97.9
Con	8	31.2	34.6	38.1	42.8	39.1	49.1	54.7	64.6	96.1	98.7
Con	8	30.5	34.4	39.3	42.5	39.9	46.9	56.5	59.7	95.6	98.7
Con	8	31.5	32.6	35.2	45.1	42.6	50.0	62.0	74.4	95.2	98.6
Con	8	31.0	33.7	34.7	41.5	39.8	50.9	60.1	67.3	96.4	98.6
Amy	1	30.3	33.0	34.7	36.6	39.5	41.7	49.9	41.7	74.0	98.2
Amy	1	31.4	31.5	35.8	36.0	37.5	38.9	51.7	45.3	74.7	98.2
Amy	1	30.3	32.6	37.1	36.6	41.9	41.5	54.6	69.4	89.3	97.9
Amy	1	31.5	32.0	36.6	35.5	41.2	42.4	53.0	72.9	78.7	98.1
Amy	2	30.9	32.4	33.0	32.9	37.6	37.7	48.5	53.1	95.1	98.5
Amy	2	30.6	31.7	32.5	33.1	34.8	42.4	37.5	52.0	94.5	98.2
Amy	2	30.9	31.4	32.6	36.0	41.8	36.6	42.4	56.1	96.5	98.0
Amy	2	30.7	31.2	33.2	36.1	39.5	39.5	41.0	56.1	95.4	98.3
Amy	3	31.9	33.7	37.7	47.1	48.7	50.1	70.2	84.5	97.1	98.6
Amy	3	31.2	34.6	38.0	44.7	48.3	51.3	65.9	82.7	96.6	98.4
Amy	3	31.0	33.2	35.6	43.4	48.3	54.5	71.1	79.1	96.1	98.3
Amy	3	32.0	34.0	37.3	43.8	44.2	52.9	77.1	75.5	96.8	98.4

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Amy	4	30.2	31.4	35.6	37.3	35.6	39.0	47.0	47.3	82.5	95.9
Amy	4	29.7	32.1	34.0	38.2	36.7	38.5	52.7	47.6	82.9	95.9
Amy	4	30.2	31.0	34.4	36.7	35.1	39.6	46.1	45.3	85.8	97.6
Amy	4	30.9	31.3	35.9	36.1	35.0	37.0	43.9	48.7	88.9	97.0
Amy	5	31.0	36.1	40.4	45.2	50.3	63.4	72.0	82.4	97.6	98.5
Amy	5	32.7	36.9	39.4	40.3	49.5	64.1	65.1	81.6	97.7	98.5
Amy	5	31.4	33.6	40.2	39.8	47.5	52.9	73.9	85.2	96.6	98.5
Amy	5	31.6	34.2	41.0	39.0	47.7	54.9	74.5	79.1	97.0	98.5
Amy	6	33.0	34.7	33.1	41.2	38.1	50.8	40.6	62.8	94.4	94.1
Amy	6	34.0	36.2	33.8	38.0	35.9	46.5	44.6	71.8	92.2	.
Amy	6	29.8	34.2	33.2	38.4	37.4	54.9	54.3	47.7	95.5	99.0
Amy	6	31.5	33.9	33.3	38.0	46.0	52.6	51.5	46.8	94.5	96.9
Amy	7	30.2	32.4	37.0	40.7	49.8	45.5	.	59.8	96.9	98.6
Amy	7	30.6	31.8	36.3	43.0	46.3	43.8	63.9	78.1	84.2	98.3
Amy	7	29.6	33.1	33.9	36.9	39.6	51.9	65.9	69.5	94.4	98.3
Amy	7	30.5	31.9	35.9	38.5	42.0	50.2	67.8	66.1	93.6	98.4
Amy	8	31.1	33.5	34.4	44.7	41.9	41.2	58.9	77.4	89.4	98.2
Amy	8	30.2	33.7	34.2	42.3	41.9	38.9	58.5	79.6	92.2	98.1
Amy	8	31.0	33.6	35.3	37.7	45.0	39.7	58.4	61.9	95.6	98.2
Amy	8	30.3	34.0	34.0	37.7	44.8	39.6	61.8	64.2	95.5	98.4
Prot	1	31.0	33.0	39.6	36.8	38.6	41.9	43.9	47.4	80.7	97.7
Prot	1	31.7	36.2	37.6	38.4	41.2	41.2	46.1	43.8	77.4	97.9
Prot	1	30.5	33.8	35.8	41.0	49.3	45.0	57.5	54.6	95.0	97.8
Prot	1	30.6	35.3	34.4	38.1	48.8	42.7	52.3	54.9	95.2	97.8
Prot	2	31.5	32.2	35.6	38.4	39.2	40.5	48.9	47.5	85.5	98.0
Prot	2	30.1	32.7	35.7	34.8	39.1	44.7	46.1	47.1	88.9	98.1
Prot	2	29.8	30.3	36.3	34.0	39.6	40.2	50.0	79.0	84.7	97.8
Prot	2	29.0	30.4	37.1	33.6	39.2	40.0	60.3	56.8	86.5	97.7
Prot	3	33.1	34.8	37.8	42.1	45.9	48.5	74.4	73.4	96.1	98.2
Prot	3	33.3	36.1	39.2	45.0	44.7	44.0	75.4	76.7	94.8	98.2
Prot	3	31.3	35.1	46.9	38.2	45.4	50.0	74.8	72.1	94.7	98.2
Prot	3	31.2	34.9	36.7	39.1	46.9	48.4	77.8	73.6	95.5	98.1
Prot	4	28.8	33.1	34.6	36.8	40.3	43.2	52.9	58.7	87.1	97.7
Prot	4	30.2	30.2	32.8	37.3	40.3	49.0	48.2	71.2	85.8	98.1
Prot	4	28.8	32.8	33.8	34.7	42.1	42.8	51.7	77.3	93.3	97.8
Prot	4	30.6	32.3	36.0	36.9	42.0	41.3	54.5	77.1	89.8	97.8
Prot	5	33.6	36.9	37.9	42.0	50.3	47.2	75.1	86.4	97.1	98.5
Prot	5	33.5	34.5	37.6	42.5	50.5	48.6	76.8	83.3	96.2	98.7
Prot	5	31.7	34.3	36.4	41.4	52.6	45.8	69.9	83.5	93.4	98.2
Prot	5	30.4	34.2	35.6	43.1	56.4	50.1	71.0	84.7	95.0	98.4
Prot	6	31.0	36.0	42.3	42.1	49.0	50.7	58.1	53.9	89.2	98.1
Prot	6	30.8	36.2	39.8	43.3	49.9	47.7	68.5	56.2	87.5	98.4
Prot	6	30.1	35.5	40.5	42.6	46.4	46.4	65.0	71.3	92.9	98.0
Prot	6	29.1	33.6	39.4	44.3	40.7	48.0	63.2	68.6	91.7	98.2
Prot	7	30.5	33.9	36.3	35.0	38.3	51.6	70.6	71.6	93.9	97.6
Prot	7	31.3	34.6	37.4	37.3	38.1	56.0	72.4	72.5	95.5	98.3

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Prot	7	31.5	33.2	35.2	42.0	42.2	42.1	69.8	84.8	95.3	97.8
Prot	7	31.5	33.7	35.5	39.8	40.9	43.6	67.9	84.6	94.5	97.9
Prot	8	30.1	32.6	35.7	45.6	39.9	47.5	70.5	54.0	92.8	98.0
Prot	8	31.2	33.0	41.8	41.8	40.5	47.5	70.3	53.0	91.6	98.3
Prot	8	.	32.1	.	42.8	36.9	48.0	67.4	50.9	88.2	98.3
Prot	8	30.1	33.8	34.6	41.7	38.0	41.3	70.2	55.0	91.1	98.1
Amy+Prot	1	39.4	33.0	39.9	37.9	38.5	45.2	57.9	63.4	86.3	97.8
Amy+Prot	1	32.1	32.7	38.4	38.7	37.0	38.8	60.1	69.2	84.7	98.1
Amy+Prot	1	30.6	32.7	38.2	38.0	39.4	45.1	55.4	60.5	88.7	98.3
Amy+Prot	1	31.3	33.1	36.8	39.3	38.1	43.0	58.1	64.1	91.5	98.0
Amy+Prot	2	31.5	33.1	35.8	35.4	41.0	39.3	53.7	61.2	89.2	97.9
Amy+Prot	2	29.6	31.8	33.8	36.3	40.3	39.8	61.5	59.8	73.6	97.5
Amy+Prot	2	28.7	30.3	32.6	35.2	36.0	47.4	47.6	69.4	86.2	98.2
Amy+Prot	2	30.8	31.2	32.8	34.5	36.1	45.5	52.0	72.7	89.8	98.2
Amy+Prot	3	30.0	33.1	39.0	42.5	49.4	50.0	74.9	87.0	97.3	98.3
Amy+Prot	3	30.0	35.1	39.2	35.9	47.8	50.7	65.7	86.6	96.8	98.2
Amy+Prot	3	30.3	35.7	39.0	40.8	52.2	57.8	78.2	85.8	97.0	98.5
Amy+Prot	3	30.1	35.2	39.1	40.2	.	62.3	79.5	86.4	97.5	98.4
Amy+Prot	4	31.5	34.2	33.4	35.0	36.8	38.5	51.1	54.4	93.0	97.5
Amy+Prot	4	30.4	33.4	33.7	36.8	38.3	38.7	53.4	72.1	92.9	97.2
Amy+Prot	4	30.2	32.0	36.3	33.1	39.7	39.7	51.1	56.7	93.4	97.4
Amy+Prot	4	30.8	33.0	34.9	34.7	37.6	39.6	44.1	45.3	93.6	97.7
Amy+Prot	5	31.3	35.1	39.1	43.7	47.4	50.2	76.6	80.2	95.3	98.7
Amy+Prot	5	31.1	35.6	36.7	40.8	48.1	48.1	76.6	78.9	90.5	98.3
Amy+Prot	5	30.0	34.2	38.9	42.8	48.7	54.2	65.8	82.6	96.3	98.3
Amy+Prot	5	30.3	33.4	39.6	42.2	47.3	52.5	75.3	81.3	93.4	98.3
Amy+Prot	6	32.1	32.1	39.1	40.3	40.8	51.7	68.7	74.0	94.3	97.6
Amy+Prot	6	32.0	33.2	39.6	43.0	44.2	54.4	65.1	73.0	95.6	98.2
Amy+Prot	6	30.3	32.4	38.6	36.7	48.9	44.9	73.1	65.0	95.1	98.0
Amy+Prot	6	31.2	31.5	38.2	39.0	48.9	55.6	70.2	65.6	93.7	98.1
Amy+Prot	7	31.1	34.0	37.2	40.6	42.7	46.2	62.8	58.0	95.0	98.3
Amy+Prot	7	30.2	33.3	38.3	42.6	41.6	40.0	50.7	62.2	96.1	98.5
Amy+Prot	7	28.8	32.4	37.2	38.9	44.0	44.0	69.4	79.1	94.7	98.2
Amy+Prot	7	30.1	31.9	37.6	39.0	46.0	47.6	69.0	80.5	95.1	98.2
Amy+Prot	8	31.6	34.1	35.5	39.8	41.2	43.7	56.8	79.6	93.5	98.0
Amy+Prot	8	30.6	33.5	34.8	36.9	43.8	45.5	47.3	.	94.2	97.8
Amy+Prot	8	32.7	32.4	35.4	43.3	40.9	42.3	53.1	75.1	92.8	97.5
Amy+Prot	8	31.3	33.9	36.6	44.2	44.2	40.5	52.2	78.4	90.1	98.1

Table 52: Ruminal dry matter disappearance (%) of maize silage of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Con	1	23.9	30.0	27.7	24.7	23.9	27.4	32.5	34.6	61.4	71.8
Con	1	28.3	22.6	29.1	30.0	34.0	29.0	28.5	36.2	56.6	68.6
Con	1	31.5	25.4	27.8	38.8	28.3	18.6	31.8	41.7	53.4	77.7
Con	1	28.1	28.4	27.9	26.4	27.5	29.2	37.1	43.4	57.1	83.7
Con	2	29.2	31.2	28.7	24.7	33.9	39.0	35.2	38.4	47.2	56.2
Con	2	31.1	.	32.1	.	32.4	34.6	32.0	.	49.6	70.5
Con	2	29.8	28.5	24.7	18.8	27.5	39.1	45.3	40.7	49.2	75.1
Con	2	29.0	28.8	27.8	26.9	35.6	33.8	36.8	39.6	47.3	69.8
Con	3	26.2	32.7	38.3	34.1	33.2	34.8	51.4	45.9	62.9	76.4
Con	3	28.1	25.7	32.3	36.0	38.0	40.9	45.9	46.8	60.9	77.7
Con	3	29.9	30.2	34.7	34.0	44.8	40.2	44.6	50.5	66.2	78.8
Con	3	32.6	34.8	35.6	33.8	35.0	37.1	42.1	47.5	65.7	78.0
Con	4	23.5	27.4	33.3	34.2	28.9	35.2	33.0	40.1	61.8	75.3
Con	4	29.3	24.7	25.4	30.9	29.8	35.9	40.6	41.8	59.0	77.5
Con	4	18.5	23.3	29.8	23.3	30.0	26.2	32.2	37.2	63.2	77.9
Con	4	27.7	20.9	26.2	29.6	27.5	32.2	33.2	35.7	58.9	77.7
Con	5	32.2	28.0	31.1	34.5	39.1	40.3	47.5	49.6	61.3	79.3
Con	5	28.7	27.9	31.5	33.4	34.7	42.9	46.0	43.8	57.9	78.1
Con	5	31.2	29.2	25.2	36.4	39.3	40.1	40.2	50.0	61.8	78.7
Con	5	32.2	31.4	31.3	26.0	32.4	36.3	36.6	46.5	65.2	77.4
Con	6	31.1	35.2	27.9	24.7	32.9	32.4	30.5	42.8	65.1	73.6
Con	6	31.2	26.6	31.9	26.3	35.6	31.9	40.5	46.3	66.7	77.0
Con	6	22.6	31.8	21.9	26.7	29.0	29.5	37.3	47.1	60.0	79.7
Con	6	24.0	25.3	28.9	31.7	21.3	28.4	31.1	34.0	64.7	79.6
Con	7	.	23.9	28.8	30.6	38.2	42.8	44.0	44.3	61.7	77.4
Con	7	23.4	29.1	32.1	33.2	36.8	36.0	46.1	52.6	61.5	75.5
Con	7	28.6	29.0	30.6	32.2	28.9	37.2	31.3	39.2	59.7	74.6
Con	7	29.3	27.5	27.4	30.8	38.8	30.2	39.1	41.9	56.1	79.5
Con	8	28.0	26.6	35.9	32.5	26.9	37.9	34.0	43.4	56.9	76.3
Con	8	27.3	27.4	28.9	26.6	31.1	37.8	41.2	42.6	58.0	77.2
Con	8	21.8	26.0	29.0	33.6	42.6	34.7	29.1	38.5	60.8	71.3
Con	8	26.0	24.4	30.6	35.7	29.6	35.1	31.8	46.6	63.1	70.5
Amy	1	26.3	24.1	35.4	30.9	28.9	27.9	39.8	40.0	47.6	77.7
Amy	1	24.8	20.8	33.7	30.8	29.8	47.9	25.6	41.6	43.6	71.4
Amy	1	31.1	24.7	36.6	35.6	29.4	35.0	32.6	37.8	46.1	75.4
Amy	1	26.8	23.2	31.6	37.9	35.2	27.2	41.8	45.0	52.7	76.3
Amy	2	28.5	32.6	28.2	23.7	34.7	37.0	34.8	41.3	48.8	74.9
Amy	2	29.7	30.3	25.3	27.5	29.6	33.4	37.7	45.7	58.5	78.9
Amy	2	28.7	27.4	24.0	27.2	32.6	30.5	35.0	43.8	69.7	79.2
Amy	2	24.6	25.7	31.1	31.1	36.9	34.7	37.5	44.5	69.6	78.4
Amy	3	34.4	30.9	33.6	34.9	37.9	47.2	48.3	54.0	67.8	81.4
Amy	3	33.7	35.0	35.3	38.2	42.8	42.6	48.6	56.4	62.4	80.2
Amy	3	34.8	32.1	33.6	40.0	42.2	45.3	44.8	52.7	70.1	79.7
Amy	3	29.5	30.2	36.4	38.1	41.0	.	51.6	52.4	69.5	79.9

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Amy	4	32.0	28.3	28.0	33.4	32.5	25.3	36.7	36.1	57.8	72.8
Amy	4	18.2	28.9	32.5	29.3	25.3	28.6	35.0	37.7	64.8	73.4
Amy	4	27.5	25.9	31.2	31.0	27.7	32.4	31.5	39.0	55.5	72.9
Amy	4	25.9	29.3	29.0	30.0	28.6	27.0	31.9	36.5	58.4	73.9
Amy	5	33.1	28.5	29.6	39.1	38.5	40.1	44.0	48.9	59.5	80.6
Amy	5	28.0	35.2	36.5	34.2	43.2	44.7	42.1	45.5	64.2	81.8
Amy	5	28.0	27.5	35.4	29.2	41.8	38.1	48.5	47.4	59.9	78.2
Amy	5	25.8	32.1	36.3	30.9	38.7	35.4	46.0	48.0	59.2	79.9
Amy	6	32.2	30.3	29.5	35.6	36.0	39.8	39.4	39.9	62.3	65.1
Amy	6	30.0	20.3	27.2	33.2	44.6	33.0	38.6	47.2	63.2	61.1
Amy	6	29.6	29.8	28.2	31.6	39.2	30.8	30.7	31.2	59.1	69.3
Amy	6	29.3	25.2	33.0	35.6	34.5	39.6	37.8	30.5	56.5	68.9
Amy	7	37.4	35.7	40.9	30.3	35.5	40.1	43.3	41.5	65.1	79.0
Amy	7	31.2	30.4	33.7	31.0	40.1	29.4	43.5	41.4	58.5	81.3
Amy	7	21.4	29.2	35.2	38.6	31.1	37.9	41.6	45.8	62.8	82.3
Amy	7	25.6	34.3	29.9	30.9	29.9	44.1	45.5	44.3	63.5	80.1
Amy	8	24.5	28.0	33.8	40.3	34.9	30.7	39.4	49.5	55.9	76.2
Amy	8	36.6	25.0	31.8	39.6	29.2	30.8	45.1	46.8	63.1	73.6
Amy	8	25.8	29.8	29.0	32.5	40.2	33.3	41.3	42.6	63.9	71.9
Amy	8	30.3	29.7	32.0	33.4	38.0	29.4	47.4	50.0	64.8	73.2
Prot	1	35.9	27.6	37.7	31.2	41.2	31.9	34.0	39.8	49.6	60.6
Prot	1	24.4	28.2	37.5	30.2	36.6	38.8	34.4	35.6	55.4	67.8
Prot	1	32.6	30.9	28.2	28.7	32.4	43.7	34.1	37.5	65.6	80.4
Prot	1	28.6	25.4	29.6	29.3	36.1	40.7	36.7	37.9	66.8	80.0
Prot	2	32.6	30.4	33.9	29.4	38.2	33.1	41.5	36.9	52.0	79.7
Prot	2	33.5	31.6	35.9	29.1	40.1	36.5	50.0	42.3	57.1	77.1
Prot	2	30.7	20.0	32.2	36.2	35.4	31.5	36.6	46.0	58.7	81.6
Prot	2	26.2	34.8	35.6	31.6	35.7	36.1	35.2	36.5	65.1	80.3
Prot	3	33.2	31.5	37.2	42.9	40.6	43.0	51.3	50.7	66.1	73.7
Prot	3	27.2	33.5	35.3	42.2	17.7	43.9	54.5	52.5	66.2	76.3
Prot	3	28.5	32.5	33.7	36.3	44.2	41.5	52.1	51.2	57.9	75.0
Prot	3	27.2	30.9	33.4	35.9	44.9	37.8	47.9	53.4	65.4	76.6
Prot	4	35.0	24.3	25.6	37.0	34.2	37.2	40.8	45.6	61.5	75.0
Prot	4	33.8	29.8	31.2	29.4	28.9	33.5	41.8	49.9	65.9	77.2
Prot	4	25.6	34.5	33.8	33.1	35.6	36.7	46.1	47.1	62.3	81.5
Prot	4	28.9	35.7	33.3	33.0	34.0	40.4	42.8	52.4	63.1	74.6
Prot	5	24.1	32.3	38.0	39.0	44.6	39.4	51.1	55.9	63.2	75.1
Prot	5	20.0	26.4	36.7	43.8	40.1	35.9	53.0	51.8	67.0	76.1
Prot	5	31.5	29.6	27.6	38.7	40.3	39.7	45.1	47.9	65.1	81.5
Prot	5	31.4	31.8	35.8	38.6	42.9	30.4	46.4	53.4	63.8	77.0
Prot	6	32.3	34.1	34.9	33.8	43.3	42.5	47.5	39.9	65.2	73.6
Prot	6	30.5	34.3	31.9	36.4	42.7	45.5	50.1	37.2	64.7	76.8
Prot	6	27.5	27.7	34.9	32.2	34.7	33.0	43.7	49.3	63.2	73.6
Prot	6	27.7	35.3	32.3	35.3	38.2	40.2	43.2	53.2	58.3	78.4
Prot	7	31.7	27.5	37.0	36.5	35.3	39.5	46.4	44.6	61.1	79.9
Prot	7	27.2	28.0	30.9	30.8	35.9	43.5	47.3	46.4	58.2	76.9

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Prot	7	33.5	30.9	27.7	30.7	37.0	40.2	42.9	52.2	67.1	80.7
Prot	7	25.6	31.5	31.2	36.5	41.5	36.8	40.2	45.6	67.8	80.7
Prot	8	32.0	33.3	33.2	43.2	35.5	42.2	.	41.3	56.5	77.4
Prot	8	24.1	39.6	36.0	38.0	28.5	43.5	47.2	39.0	60.4	77.6
Prot	8	30.3	31.7	39.2	41.4	34.9	36.1	50.6	46.7	61.9	74.6
Prot	8	25.3	35.4	37.9	37.8	34.3	38.3	50.9	44.5	58.2	80.5
Amy+Prot	1	37.8	33.6	36.2	33.4	41.4	39.8	39.5	52.1	53.9	76.1
Amy+Prot	1	32.9	36.3	38.6	32.1	38.2	42.4	44.6	38.8	57.4	77.9
Amy+Prot	1	35.5	28.8	34.6	35.4	37.2	35.6	44.3	47.5	54.5	77.4
Amy+Prot	1	36.0	35.0	34.7	33.5	37.6	38.0	42.3	48.7	58.4	73.0
Amy+Prot	2	30.2	32.4	30.9	30.5	32.3	35.9	43.3	44.2	52.8	70.6
Amy+Prot	2	36.8	26.9	31.4	32.0	29.4	21.3	28.0	40.9	55.2	71.5
Amy+Prot	2	25.7	28.8	28.9	37.9	35.1	36.5	40.5	43.3	59.1	67.6
Amy+Prot	2	37.7	30.0	31.0	33.7	31.0	33.5	38.3	39.8	56.0	68.2
Amy+Prot	3	29.6	37.1	35.2	38.9	46.0	41.1	44.0	50.7	63.7	80.1
Amy+Prot	3	34.4	36.1	37.8	35.3	37.4	42.6	50.3	58.0	65.6	80.4
Amy+Prot	3	29.1	37.6	39.8	42.1	45.3	41.3	44.7	57.8	70.0	80.2
Amy+Prot	3	35.6	36.2	37.9	37.5	40.3	39.8	51.8	53.6	70.2	78.0
Amy+Prot	4	29.0	34.4	32.7	28.9	33.3	32.2	32.9	38.1	62.2	78.5
Amy+Prot	4	29.6	31.8	35.6	26.6	33.6	36.9	39.2	49.1	57.9	74.0
Amy+Prot	4	36.0	29.6	32.0	25.1	25.3	32.7	35.0	35.9	65.3	79.0
Amy+Prot	4	32.5	30.0	31.5	27.4	38.0	28.5	32.6	31.8	63.5	77.6
Amy+Prot	5	38.4	37.2	40.0	44.3	45.3	44.7	51.1	61.1	67.9	78.2
Amy+Prot	5	35.2	32.5	41.8	39.1	44.9	44.9	50.1	59.7	67.7	78.9
Amy+Prot	5	36.0	31.8	35.7	36.1	40.3	40.7	44.9	52.4	70.7	78.2
Amy+Prot	5	33.0	30.8	35.5	38.7	40.7	45.5	47.6	55.1	68.5	77.0
Amy+Prot	6	26.5	30.8	30.5	35.1	36.6	28.1	39.3	52.0	67.0	73.7
Amy+Prot	6	31.6	34.7	34.8	31.5	27.7	34.9	43.2	50.8	70.0	72.7
Amy+Prot	6	29.1	27.5	31.4	30.2	37.9	28.3	43.7	45.4	63.3	73.6
Amy+Prot	6	27.8	26.2	29.4	21.0	39.3	26.0	37.5	48.6	67.8	72.6
Amy+Prot	7	32.7	30.4	27.5	31.4	39.1	30.7	37.2	42.0	63.3	75.8
Amy+Prot	7	29.5	31.1	37.3	34.1	35.9	25.6	41.7	43.1	62.1	78.2
Amy+Prot	7	35.7	29.6	31.6	28.4	40.7	33.5	48.0	51.2	56.2	80.5
Amy+Prot	7	29.8	26.5	29.4	29.3	33.2	32.8	44.0	43.0	55.1	79.6
Amy+Prot	8	31.0	21.4	33.6	35.0	37.8	34.6	40.0	49.6	61.7	79.5
Amy+Prot	8	27.0	32.2	33.4	33.8	36.3	36.2	40.5	48.0	63.2	71.7
Amy+Prot	8	33.0	38.4	31.6	30.3	35.6	40.4	36.0	45.6	64.1	75.2
Amy+Prot	8	33.5	32.7	31.4	36.1	36.7	35.9	37.1	47.2	65.0	71.0

Table 53: Ruminal dry matter disappearance (%) of grass silage of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Con	1	33.0	33.3	33.3	33.7	34.1	35.5	42.6	39.7	65.4	80.0
Con	1	33.0	33.6	34.2	35.1	34.8	33.6	42.1	39.5	73.0	79.2
Con	1	32.6	32.7	35.0	34.2	35.2	35.2	38.1	53.2	69.1	83.5
Con	1	32.8	33.2	36.1	34.2	35.4	35.8	38.3	52.9	70.3	83.5
Con	2	32.3	34.7	34.3	36.1	37.7	35.2	45.1	41.4	62.0	72.3
Con	2	32.7	29.1	35.2	35.9	36.6	37.1	43.4	41.5	60.9	66.2
Con	2	30.7	32.2	34.9	34.8	36.3	37.1	48.3	45.6	62.8	79.6
Con	2	30.7	33.1	35.6	35.6	35.0	37.1	45.4	48.2	57.5	81.8
Con	3	34.4	33.4	34.7	35.9	37.6	40.4	43.4	63.5	70.6	83.3
Con	3	33.0	33.9	33.7	37.1	38.3	38.8	59.5	55.7	69.8	85.1
Con	3	32.3	48.9	35.1	35.8	38.7	38.8	47.9	66.7	69.1	82.3
Con	3	31.6	33.2	34.2	36.3	39.4	52.5	58.8	53.9	71.9	84.4
Con	4	30.9	31.8	33.0	33.9	36.8	37.7	39.6	44.4	61.5	82.9
Con	4	30.7	33.0	34.6	33.8	33.6	37.9	39.7	48.3	67.2	82.4
Con	4	29.7	32.5	.	33.9	37.1	35.3	39.3	43.1	73.4	83.3
Con	4	32.5	31.2	33.0	31.9	34.2	35.8	38.3	36.8	71.7	82.6
Con	5	32.7	32.1	35.0	36.0	39.0	39.7	45.5	48.4	69.2	81.1
Con	5	32.2	33.5	35.1	34.8	36.0	37.8	47.5	48.6	68.6	80.9
Con	5	32.7	32.2	33.0	34.1	35.8	38.7	45.6	51.0	69.6	81.7
Con	5	32.1	34.1	34.4	35.9	35.6	38.8	45.0	50.4	67.8	82.5
Con	6	31.9	32.7	34.4	36.7	35.3	37.2	42.5	46.5	72.8	81.2
Con	6	31.0	32.7	35.3	37.3	35.9	38.2	42.4	47.9	72.6	81.1
Con	6	30.9	34.4	34.3	34.0	36.0	39.5	41.4	52.5	68.8	83.5
Con	6	30.8	30.6	34.8	36.4	36.0	40.4	41.0	53.5	71.3	83.4
Con	7	32.6	33.7	33.3	49.5	39.7	54.9	41.7	53.7	68.2	83.7
Con	7	31.6	34.7	31.0	33.0	41.9	40.5	58.3	56.5	70.5	84.5
Con	7	31.1	48.9	34.9	34.1	36.3	37.3	40.2	64.8	65.1	82.2
Con	7	.	32.5	49.4	34.0	36.9	38.4	42.2	54.6	68.2	83.0
Con	8	31.9	33.6	33.5	32.8	34.0	37.6	40.4	40.2	63.1	81.8
Con	8	30.5	32.6	31.7	33.5	34.2	38.0	39.6	42.6	62.8	80.9
Con	8	30.6	30.4	33.7	35.4	35.1	37.5	37.8	47.4	70.3	72.8
Con	8	30.6	30.1	33.0	35.0	35.0	36.3	36.0	45.0	69.8	77.5
Amy	1	31.9	32.2	33.2	31.9	32.4	35.4	37.9	39.4	49.9	78.1
Amy	1	32.3	32.9	34.2	33.0	34.7	39.2	37.7	52.4	48.2	78.9
Amy	1	31.6	30.8	34.7	33.4	34.8	39.2	38.6	47.3	45.3	81.0
Amy	1	31.8	33.3	33.3	34.4	35.0	35.9	37.7	41.9	47.3	80.4
Amy	2	32.8	32.4	31.8	32.6	33.8	36.0	35.4	39.7	70.6	81.4
Amy	2	29.1	32.2	32.9	32.1	33.8	34.3	34.6	42.4	71.1	80.5
Amy	2	32.0	31.8	33.4	31.1	32.4	33.1	39.1	37.4	75.4	80.7
Amy	2	32.4	32.5	32.5	33.5	33.6	33.7	35.6	36.3	73.8	82.1
Amy	3	30.8	32.9	33.2	34.8	35.9	37.9	46.3	54.8	74.1	82.2
Amy	3	30.9	30.0	33.0	35.2	37.1	42.7	44.4	53.8	73.5	82.3
Amy	3	30.2	31.5	32.8	34.6	39.0	41.1	46.7	52.7	71.7	83.5
Amy	3	31.5	31.4	32.7	34.8	37.2	41.4	48.5	56.2	75.1	82.1

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Amy	4	32.0	32.2	34.0	33.8	33.7	35.7	37.5	37.8	65.2	76.4
Amy	4	30.4	33.4	34.2	33.9	34.3	35.6	38.4	38.3	66.8	77.2
Amy	4	29.4	32.0	32.1	33.6	33.0	34.0	37.2	38.4	66.7	78.4
Amy	4	31.1	32.1	31.7	33.8	32.8	34.1	39.6	41.5	67.8	79.2
Amy	5	30.7	33.1	33.2	33.2	37.6	41.1	43.3	50.2	71.6	83.2
Amy	5	30.6	32.2	33.8	34.0	36.9	39.1	44.0	51.2	70.6	84.1
Amy	5	29.1	31.0	34.3	33.7	36.7	41.1	45.1	43.3	74.9	83.6
Amy	5	29.9	32.7	34.1	32.8	38.4	41.2	43.6	44.8	72.1	83.3
Amy	6	31.8	32.7	32.2	33.4	35.5	38.4	44.1	50.2	69.1	70.4
Amy	6	31.6	30.8	31.8	36.5	32.0	38.4	46.2	48.3	70.3	66.2
Amy	6	31.6	31.7	32.8	35.7	40.1	36.6	42.2	48.9	69.4	76.3
Amy	6	31.7	32.7	32.7	35.9	39.5	39.4	40.8	48.4	70.3	73.0
Amy	7	30.1	32.5	33.4	35.1	35.8	38.4	46.1	45.3	66.3	83.5
Amy	7	30.7	32.6	32.2	35.7	35.9	36.0	46.0	48.9	69.9	82.1
Amy	7	30.5	30.3	32.5	35.2	35.6	37.6	43.5	43.2	72.0	83.5
Amy	7	30.7	30.5	32.2	35.7	34.7	38.6	43.4	44.2	67.8	84.5
Amy	8	29.9	32.1	31.2	35.4	36.2	33.1	40.1	46.3	68.4	75.2
Amy	8	31.4	32.0	31.2	34.0	34.5	31.5	40.5	49.6	67.1	73.3
Amy	8	30.5	31.8	32.7	33.7	35.6	35.0	40.5	50.4	69.2	75.7
Amy	8	31.3	32.4	32.3	32.9	35.6	33.9	42.5	48.5	66.6	74.4
Prot	1	32.2	33.3	34.6	33.3	35.6	35.1	36.7	38.5	54.9	75.9
Prot	1	32.4	33.9	33.0	34.6	35.1	35.1	37.6	39.4	58.9	77.3
Prot	1	31.2	33.7	33.2	35.3	35.8	37.2	39.4	39.6	72.2	82.1
Prot	1	32.6	32.7	33.4	34.1	37.9	37.0	36.4	51.2	73.6	80.9
Prot	2	30.2	31.5	32.5	31.6	32.1	34.1	36.9	38.5	53.5	81.1
Prot	2	31.0	31.7	32.9	32.1	35.7	35.6	37.6	42.6	56.9	81.6
Prot	2	30.5	31.6	33.1	32.4	35.4	34.9	36.9	43.4	63.6	82.1
Prot	2	30.4	31.8	32.3	33.5	34.3	35.4	36.0	44.2	65.6	81.5
Prot	3	31.8	33.3	34.1	35.6	38.3	41.2	47.2	51.3	66.5	80.9
Prot	3	31.3	33.8	34.4	34.4	36.9	38.3	42.1	51.0	66.7	80.8
Prot	3	31.4	29.1	34.2	36.0	36.5	39.1	50.5	46.4	64.8	80.8
Prot	3	31.4	32.3	33.1	35.4	36.7	39.2	48.9	49.8	67.4	79.8
Prot	4	31.3	32.4	33.4	33.7	36.8	38.5	39.7	50.0	65.3	80.8
Prot	4	30.9	33.4	32.0	33.4	36.6	38.1	42.1	54.1	69.2	.
Prot	4	29.6	31.3	33.7	35.1	34.9	35.5	41.6	56.7	69.5	82.4
Prot	4	30.7	31.7	32.0	34.1	35.3	38.0	37.7	55.9	71.8	83.0
Prot	5	32.1	33.7	34.9	36.2	40.0	38.1	45.2	51.2	66.5	82.3
Prot	5	32.3	33.1	35.7	36.8	39.1	37.4	47.2	57.5	70.7	82.4
Prot	5	32.7	34.4	34.2	35.9	38.8	37.6	48.1	55.6	70.9	81.1
Prot	5	32.1	32.1	32.0	35.7	38.5	37.6	47.6	56.2	63.9	82.9
Prot	6	31.2	31.7	34.2	35.7	39.9	40.3	48.6	42.0	64.4	80.2
Prot	6	30.5	31.4	34.6	33.9	38.9	41.8	48.8	43.2	68.4	79.0
Prot	6	31.8	30.8	31.8	33.4	36.8	36.7	41.2	49.0	70.9	81.2
Prot	6	30.0	31.9	32.4	33.6	35.5	30.5	41.6	47.8	67.1	81.3
Prot	7	31.0	33.4	33.8	33.8	34.8	40.0	46.6	43.5	70.0	81.1
Prot	7	31.5	31.6	33.1	33.7	35.9	42.9	47.4	42.6	68.6	81.4

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Prot	7	30.0	31.5	33.1	36.4	37.6	39.0	44.4	51.6	69.3	83.5
Prot	7	30.4	31.8	32.8	36.4	37.0	38.5	44.0	47.8	70.6	82.4
Prot	8	29.2	31.7	33.6	36.3	35.8	36.1	45.4	42.5	69.7	79.4
Prot	8	29.9	32.5	34.4	35.0	34.7	35.7	41.6	43.1	63.1	79.5
Prot	8	30.4	33.3	31.6	36.0	33.8	38.0	45.7	37.8	67.0	81.1
Prot	8	30.2	32.8	32.8	35.3	34.2	36.9	41.8	39.0	61.8	81.4
Amy+Prot	1	33.3	33.2	34.1	34.2	35.3	34.6	38.4	50.9	63.8	77.3
Amy+Prot	1	32.5	32.1	35.0	34.6	33.9	37.1	43.0	47.6	67.8	78.9
Amy+Prot	1	32.3	32.6	32.1	34.4	34.5	37.7	47.5	49.9	65.2	78.2
Amy+Prot	1	32.5	32.0	35.1	35.7	35.4	35.7	47.2	48.7	61.0	80.2
Amy+Prot	2	33.4	32.7	30.5	35.6	32.3	35.3	44.9	44.5	61.6	75.8
Amy+Prot	2	29.9	33.0	33.1	34.1	34.6	34.1	45.0	40.1	58.8	76.4
Amy+Prot	2	32.4	32.2	32.1	35.2	36.7	34.5	42.5	43.3	58.7	80.0
Amy+Prot	2	31.6	32.6	33.1	35.6	36.5	36.4	40.7	44.5	63.7	79.4
Amy+Prot	3	31.4	33.6	34.1	35.0	38.5	38.9	47.2	57.6	72.9	83.0
Amy+Prot	3	31.3	32.2	33.7	34.9	38.5	41.3	50.0	56.9	70.3	83.9
Amy+Prot	3	30.8	34.2	35.3	35.9	40.0	41.2	47.8	55.8	74.0	82.2
Amy+Prot	3	31.5	33.2	34.3	36.4	38.7	41.2	48.6	55.8	72.5	84.2
Amy+Prot	4	31.7	32.8	33.0	35.2	33.5	35.8	39.8	50.4	68.9	81.3
Amy+Prot	4	32.8	33.5	33.5	34.5	34.5	36.8	39.3	43.9	65.5	78.9
Amy+Prot	4	32.3	31.5	34.4	34.4	34.8	34.1	40.1	36.3	69.3	81.2
Amy+Prot	4	31.5	33.7	33.8	33.6	33.8	35.0	37.6	35.7	68.7	81.2
Amy+Prot	5	31.2	33.0	33.8	37.0	39.0	39.8	46.7	52.1	65.1	82.9
Amy+Prot	5	32.0	32.9	34.8	36.9	37.8	41.3	46.1	54.5	65.2	82.0
Amy+Prot	5	31.7	33.0	33.6	37.1	38.9	40.0	45.6	56.3	70.4	82.7
Amy+Prot	5	32.6	33.5	34.1	37.2	38.4	40.0	44.4	56.1	.	82.8
Amy+Prot	6	31.3	32.7	34.8	35.7	39.5	33.6	42.5	50.0	70.4	78.2
Amy+Prot	6	31.1	32.8	35.0	34.7	36.2	35.8	41.5	50.6	73.0	78.3
Amy+Prot	6	31.0	31.8	35.7	34.4	38.5	38.2	46.8	41.9	72.0	79.2
Amy+Prot	6	31.2	32.3	33.5	33.3	39.1	34.9	44.9	45.9	71.1	78.4
Amy+Prot	7	33.1	32.5	33.8	34.1	37.7	34.6	43.5	44.0	74.0	80.5
Amy+Prot	7	33.1	32.0	34.0	35.1	37.3	34.0	43.9	49.8	66.9	82.7
Amy+Prot	7	32.6	33.9	34.1	34.3	38.6	39.3	47.5	52.9	59.7	82.9
Amy+Prot	7	32.9	33.4	35.1	34.0	38.8	39.3	44.0	54.5	62.8	82.6
Amy+Prot	8	31.3	30.5	34.6	33.7	36.0	39.9	41.8	48.7	65.7	72.0
Amy+Prot	8	30.8	32.9	35.3	33.5	34.7	38.8	43.6	47.7	67.9	80.1
Amy+Prot	8	31.4	33.0	33.8	32.8	35.5	35.8	41.6	51.4	62.3	71.0
Amy+Prot	8	31.1	32.7	35.6	33.3	35.5	35.1	38.6	48.4	65.3	71.8

Table 54: Ruminal dry matter disappearance (%) of hay of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Con	1	13.7	16.9	15.9	15.9	17.5	18.0	16.9	19.5	35.9	52.7
Con	1	15.2	15.5	17.2	15.9	18.6	18.5	20.5	20.5	39.5	52.9
Con	1	15.3	15.4	17.1	17.4	18.3	19.5	19.3	24.0	35.9	53.7
Con	1	14.7	16.4	16.6	17.1	18.1	19.1	21.0	22.7	36.7	51.9
Con	2	13.4	14.0	16.7	16.6	16.4	16.8	19.1	22.5	26.1	44.4
Con	2	13.3	15.3	16.4	16.5	16.8	16.4	20.2	21.3	26.3	36.5
Con	2	12.8	13.7	16.0	16.7	15.9	18.1	19.8	19.4	32.8	48.3
Con	2	12.7	14.8	15.4	16.6	16.3	17.9	19.8	22.7	32.3	48.9
Con	3	12.9	15.1	15.2	17.1	19.2	19.6	22.6	24.9	37.3	53.1
Con	3	12.3	15.8	15.4	17.0	18.8	19.9	23.5	26.3	40.2	53.1
Con	3	12.1	14.8	14.6	16.4	18.7	18.4	23.8	26.3	39.7	54.7
Con	3	12.4	12.9	15.3	16.6	19.1	18.6	25.4	28.0	36.7	53.4
Con	4	12.8	14.7	14.5	15.7	17.2	18.3	21.2	23.9	34.4	51.8
Con	4	13.4	15.6	15.8	15.5	17.4	18.3	19.8	21.6	29.6	53.8
Con	4	13.0	14.9	15.1	15.4	17.2	18.7	19.8	22.3	40.2	53.4
Con	4	14.3	14.0	14.8	16.8	16.7	18.6	20.0	22.4	39.1	52.4
Con	5	14.9	15.6	16.6	18.8	19.5	18.3	22.7	23.5	36.3	50.8
Con	5	15.6	15.6	16.2	17.9	17.9	19.3	22.6	26.2	34.8	50.6
Con	5	14.8	15.5	15.6	17.2	18.4	19.1	23.3	24.4	37.6	52.6
Con	5	16.3	15.9	17.1	17.7	17.5	18.5	23.3	24.9	37.3	51.5
Con	6	13.6	14.4	15.9	17.8	17.5	17.6	20.0	22.6	35.2	53.0
Con	6	13.2	14.3	15.8	17.0	17.3	18.0	19.1	21.5	39.9	52.5
Con	6	13.0	14.1	16.6	17.7	17.1	17.8	19.2	21.3	40.8	51.1
Con	6	12.1	14.9	16.3	16.1	16.8	16.3	19.9	21.2	39.5	52.4
Con	7	14.0	15.5	16.7	15.3	19.4	17.9	22.9	23.3	38.3	54.7
Con	7	12.9	15.1	16.2	15.8	18.5	19.6	24.2	23.3	35.6	54.4
Con	7	12.7	15.0	16.6	16.6	17.8	18.3	19.1	25.0	33.0	52.9
Con	7	13.2	14.7	16.3	17.2	17.9	18.7	20.8	23.2	34.9	50.7
Con	8	13.0	14.7	15.5	16.0	16.7	19.4	19.3	21.5	36.1	49.2
Con	8	12.3	15.2	15.5	16.2	15.8	18.9	19.7	18.8	33.8	46.4
Con	8	12.6	14.6	14.5	16.6	16.5	19.3	20.8	23.4	39.4	50.4
Con	8	12.4	13.8	15.1	15.5	16.7	18.6	19.6	22.0	36.5	45.9
Amy	1	13.9	15.0	15.7	16.0	17.0	16.8	20.2	19.8	23.6	40.9
Amy	1	14.5	15.3	16.1	17.1	17.0	17.7	19.7	19.2	24.2	45.3
Amy	1	14.5	15.4	16.5	16.2	19.2	18.5	21.4	22.9	33.7	49.2
Amy	1	13.7	15.1	16.5	17.1	19.1	18.2	20.5	21.7	31.6	45.0
Amy	2	13.9	15.0	15.1	14.8	17.1	19.2	18.2	21.8	35.8	.
Amy	2	14.5	16.0	13.6	16.3	17.0	19.0	20.1	20.8	37.7	50.3
Amy	2	14.4	16.0	15.4	16.9	17.8	18.5	19.2	21.7	37.0	49.9
Amy	2	14.7	15.3	15.0	16.4	17.8	18.1	18.5	22.0	39.9	51.2
Amy	3	14.3	14.6	16.3	17.2	18.8	19.0	22.7	26.0	43.1	54.7
Amy	3	14.1	15.0	16.1	16.5	17.1	20.4	22.2	24.4	37.1	54.4
Amy	3	14.4	14.9	15.5	16.6	17.3	19.7	23.5	26.6	41.9	56.4
Amy	3	14.4	15.5	15.6	17.4	18.0	19.7	23.0	25.8	41.3	54.3

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Amy	4	14.8	14.6	16.8	17.4	16.7	18.1	17.1	19.2	31.7	46.9
Amy	4	15.7	15.6	16.7	16.9	17.2	18.0	19.5	19.1	34.5	44.8
Amy	4	14.8	15.8	15.4	16.0	16.2	17.1	18.9	18.4	31.2	48.3
Amy	4	14.3	14.7	16.7	15.9	17.0	16.6	18.2	18.9	31.8	49.8
Amy	5	13.1	15.5	16.7	15.8	18.2	20.2	22.2	24.9	40.0	55.5
Amy	5	12.4	15.3	16.5	16.7	17.9	20.0	23.5	23.8	41.3	54.5
Amy	5	13.7	14.9	16.7	16.3	19.7	20.2	22.9	25.1	39.5	52.6
Amy	5	14.5	15.3	16.6	16.0	18.5	18.8	21.6	26.1	42.2	53.8
Amy	6	16.9	15.6	15.6	17.2	17.6	20.7	22.0	27.1	31.9	49.9
Amy	6	15.1	14.6	15.9	17.8	17.4	20.7	.	26.2	36.3	48.9
Amy	6	15.1	15.3	15.4	17.7	18.6	20.4	23.6	21.8	35.1	42.0
Amy	6	14.7	15.1	16.1	17.8	18.8	19.4	21.2	21.4	34.9	44.6
Amy	7	13.7	13.6	15.7	16.7	16.3	18.4	22.5	21.4	39.7	54.0
Amy	7	13.2	14.0	15.5	16.5	16.2	17.4	23.1	24.9	39.3	55.3
Amy	7	13.8	13.8	14.8	16.6	15.8	20.7	21.5	24.2	36.6	56.2
Amy	7	13.3	14.1	16.2	16.9	17.8	19.4	23.3	23.3	38.7	56.0
Amy	8	14.4	15.7	15.1	16.3	18.2	16.3	19.8	23.0	35.7	49.2
Amy	8	12.3	15.0	15.1	16.6	18.6	17.4	19.3	22.9	36.0	48.7
Amy	8	14.0	15.3	15.8	15.7	17.5	16.4	20.8	21.8	33.7	42.6
Amy	8	12.3	15.4	14.2	15.8	17.5	17.8	21.2	21.9	36.4	47.1
Prot	1	13.2	14.5	15.9	16.7	16.0	18.2	17.1	20.3	26.0	47.5
Prot	1	14.2	14.7	15.9	16.0	17.0	17.0	17.3	18.7	26.8	43.9
Prot	1	14.4	14.8	14.1	15.4	18.1	18.0	18.9	21.2	38.4	46.7
Prot	1	14.0	15.0	14.7	17.6	17.1	18.5	20.4	21.2	37.0	46.8
Prot	2	11.1	15.6	15.2	15.7	16.3	18.9	20.4	21.1	29.5	49.3
Prot	2	11.5	14.8	14.9	15.9	16.9	19.9	19.6	19.6	30.4	49.8
Prot	2	12.0	14.7	.	15.0	16.7	19.4	20.6	22.2	29.8	50.0
Prot	2	14.4	15.4	15.6	15.2	17.0	18.8	19.4	24.0	32.1	52.0
Prot	3	13.3	16.3	16.6	17.0	17.7	19.3	23.2	24.1	40.4	52.8
Prot	3	14.1	15.7	16.7	17.3	17.4	18.6	21.4	25.4	37.8	51.4
Prot	3	15.0	15.7	16.5	18.4	18.6	19.1	22.4	24.8	38.5	53.1
Prot	3	14.4	15.7	16.4	17.4	18.6	21.0	23.6	25.7	41.3	51.8
Prot	4	13.8	15.5	14.5	16.0	17.2	16.8	18.4	22.8	35.7	48.6
Prot	4	13.2	15.4	14.3	16.6	18.2	17.6	18.1	24.6	32.3	46.5
Prot	4	13.8	13.1	14.4	16.8	17.0	16.8	19.0	25.5	30.8	52.2
Prot	4	13.5	13.5	15.5	16.2	16.5	16.9	18.2	26.6	30.0	51.6
Prot	5	13.1	15.2	15.0	16.8	17.8	17.8	22.1	25.9	38.6	51.9
Prot	5	14.5	15.0	16.1	16.8	18.1	17.7	22.1	25.8	37.8	53.1
Prot	5	13.9	15.8	15.5	16.7	18.8	18.3	20.2	25.2	35.4	51.5
Prot	5	12.6	15.1	14.9	15.6	17.3	17.5	22.0	26.0	38.9	52.9
Prot	6	12.9	15.2	16.7	16.4	19.0	19.9	21.9	22.1	29.4	53.9
Prot	6	14.5	15.0	16.5	17.0	19.1	20.1	24.0	23.6	38.4	51.7
Prot	6	14.6	15.4	17.0	16.4	19.1	19.6	21.0	27.7	34.6	49.3
Prot	6	13.7	14.9	16.2	15.7	18.2	18.4	20.8	27.1	33.7	51.5
Prot	7	14.8	15.7	16.5	16.6	17.5	18.7	22.5	20.3	32.4	54.4
Prot	7	14.7	16.5	17.9	16.6	18.0	20.6	21.2	21.3	34.0	52.7

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Prot	7	14.7	15.2	15.5	17.3	17.6	18.7	22.6	25.1	38.5	52.9
Prot	7	15.2	15.6	15.9	17.1	18.7	18.4	21.8	24.1	37.6	53.6
Prot	8	13.7	13.1	14.6	16.7	16.8	19.0	21.3	18.5	34.0	50.0
Prot	8	.	14.6	15.4	16.6	16.6	18.3	22.8	20.0	33.8	51.0
Prot	8	14.0	15.1	15.6	16.0	15.8	17.5	19.7	18.7	32.0	52.4
Prot	8	12.4	14.3	15.2	16.7	16.2	18.9	18.2	19.9	34.0	52.5
Amy+Prot	1	13.2	14.5	15.3	16.5	16.0	17.5	18.9	21.6	31.7	46.8
Amy+Prot	1	13.4	14.9	15.6	15.9	17.0	17.6	18.6	20.6	34.9	45.7
Amy+Prot	1	12.5	13.8	14.9	15.9	15.7	18.3	19.5	24.0	33.6	49.7
Amy+Prot	1	13.4	14.4	14.8	16.1	16.8	18.3	20.7	23.7	38.5	49.0
Amy+Prot	2	14.2	14.7	14.9	17.3	16.4	16.3	21.5	16.9	29.5	48.0
Amy+Prot	2	14.5	14.9	14.7	17.3	16.1	17.4	21.0	21.4	31.5	44.5
Amy+Prot	2	13.1	14.7	14.9	16.3	14.5	18.4	17.6	23.7	31.7	46.5
Amy+Prot	2	13.8	15.5	13.3	16.2	15.9	18.7	19.8	19.3	32.6	45.2
Amy+Prot	3	12.6	14.8	16.4	18.0	16.7	20.1	21.7	26.8	41.2	53.4
Amy+Prot	3	12.9	16.1	16.9	17.3	18.7	19.5	21.4	26.0	41.4	53.6
Amy+Prot	3	13.2	15.1	16.3	17.0	18.1	19.6	23.6	26.1	40.4	54.4
Amy+Prot	3	12.8	15.4	17.6	17.3	18.8	19.8	22.9	26.5	42.9	53.6
Amy+Prot	4	15.1	16.1	16.6	16.7	18.0	16.8	19.4	21.9	35.3	48.7
Amy+Prot	4	14.4	15.4	15.8	17.1	16.9	16.3	19.8	19.9	33.5	51.5
Amy+Prot	4	14.2	15.5	14.7	16.9	16.9	17.1	20.8	21.0	33.5	.
Amy+Prot	4	14.6	15.8	16.3	18.3	16.8	17.4	18.8	19.6	36.8	49.1
Amy+Prot	5	13.4	14.5	16.0	17.4	17.0	19.8	21.6	25.6	36.2	51.7
Amy+Prot	5	13.4	14.2	15.8	18.0	18.6	18.4	21.7	24.8	36.0	52.6
Amy+Prot	5	13.3	14.1	15.9	17.3	16.1	19.1	21.0	27.3	36.7	53.5
Amy+Prot	5	14.1	14.5	15.2	16.9	18.8	19.4	19.9	25.6	40.6	51.5
Amy+Prot	6	13.0	14.7	14.9	16.3	17.6	18.5	19.3	23.4	37.5	47.1
Amy+Prot	6	14.2	14.6	16.7	15.9	17.2	18.5	20.0	25.5	39.2	47.7
Amy+Prot	6	13.6	15.1	15.5	15.5	18.0	18.1	21.8	23.5	39.5	49.8
Amy+Prot	6	13.0	15.4	14.4	16.0	17.9	19.1	21.0	21.1	40.2	48.3
Amy+Prot	7	14.5	15.1	15.0	16.5	17.5	18.9	19.4	20.9	39.3	54.1
Amy+Prot	7	14.7	15.6	16.2	16.6	16.9	17.4	19.8	19.4	40.5	53.4
Amy+Prot	7	14.2	16.0	16.9	16.7	16.4	18.1	21.4	23.5	40.3	52.2
Amy+Prot	7	12.2	15.7	16.3	16.6	17.8	17.4	21.7	24.1	38.1	53.3
Amy+Prot	8	13.1	.	14.7	15.7	17.4	18.6	18.2	21.8	33.3	43.0
Amy+Prot	8	14.1	14.1	14.1	17.2	17.5	18.3	19.3	23.1	34.9	46.7
Amy+Prot	8	13.3	15.0	16.1	16.6	17.5	17.3	19.0	25.2	31.5	46.8
Amy+Prot	8	14.0	14.9	16.0	16.7	17.3	17.0	19.2	26.6	33.5	44.8

Table 55: Ruminal dry matter disappearance (%) of the TMR of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Con	1	29.7	24.1	28.5	32.8	29.3	27.1	33.6	36.4	62.5	76.9
Con	1	27.9	25.6	25.1	29.7	31.3	25.9	31.2	37.2	64.5	76.3
Con	1	27.1	25.3	22.2	29.0	31.8	33.3	31.6	41.7	56.4	81.0
Con	1	24.0	32.0	27.0	25.5	32.0	20.8	25.7	45.1	61.4	81.0
Con	2	23.9	28.7	26.9	27.5	30.1	30.3	36.8	37.2	46.6	57.5
Con	2	24.9	31.1	27.2	34.1	27.8	32.3	34.2	38.6	47.8	57.9
Con	2	23.0	24.6	28.6	29.7	27.1	29.8	38.0	44.8	60.2	75.2
Con	2	28.1	22.4	29.7	24.6	34.7	29.7	32.4	44.7	53.8	78.4
Con	3	30.5	29.3	27.6	31.5	36.1	35.1	45.0	52.3	62.8	77.9
Con	3	27.7	26.6	30.9	31.1	35.8	39.4	45.5	54.9	66.1	83.0
Con	3	28.8	25.6	31.5	32.8	33.5	34.9	46.7	52.1	68.4	80.6
Con	3	27.8	26.5	31.6	27.7	32.0	33.3	49.4	53.4	70.1	81.8
Con	4	27.9	25.2	26.7	24.5	28.0	29.7	33.3	39.5	58.2	78.7
Con	4	27.1	24.5	28.0	24.1	30.1	29.1	32.2	42.3	60.8	80.8
Con	4	18.8	24.6	24.4	23.2	27.6	34.2	34.8	41.4	61.4	78.1
Con	4	23.9	27.7	25.3	24.7	24.1	30.5	31.8	35.7	62.1	79.4
Con	5	29.9	23.6	26.0	31.0	34.1	33.5	38.3	44.6	59.6	77.0
Con	5	22.9	24.8	26.6	.	29.6	38.0	40.6	40.7	60.1	76.8
Con	5	25.6	28.8	29.5	27.7	31.8	35.9	41.1	45.7	58.5	79.3
Con	5	27.7	27.5	30.3	31.4	28.5	36.8	37.1	50.4	61.3	79.0
Con	6	34.7	25.4	33.3	32.9	28.2	30.3	41.2	39.4	65.3	79.0
Con	6	26.3	24.5	30.9	32.1	30.1	30.8	34.1	40.2	60.1	73.6
Con	6	26.7	23.0	27.7	25.2	28.9	25.0	36.1	41.9	65.3	80.0
Con	6	27.3	25.1	27.4	26.0	26.7	33.5	34.6	44.2	61.0	74.1
Con	7	24.3	22.3	29.0	26.2	32.5	36.1	35.3	42.1	60.3	78.7
Con	7	23.4	24.5	25.4	25.0	33.4	38.4	44.1	48.4	60.9	81.4
Con	7	23.6	27.3	31.7	24.8	31.3	32.8	37.5	41.2	59.5	79.8
Con	7	20.4	24.5	25.5	26.4	27.8	34.8	38.2	49.0	59.2	78.8
Con	8	26.0	24.4	24.6	29.7	27.5	33.8	34.3	37.4	59.6	79.7
Con	8	24.8	26.9	29.0	30.4	29.9	35.1	37.3	35.2	57.6	78.8
Con	8	23.0	25.2	24.5	28.6	32.0	33.4	34.2	43.1	66.6	71.3
Con	8	27.7	27.7	29.9	33.7	31.2	34.9	34.7	42.5	61.8	71.2
Amy	1	30.8	24.3	27.1	34.0	29.2	33.6	31.0	35.2	45.7	73.4
Amy	1	25.5	29.3	25.9	25.6	32.8	32.0	32.4	34.3	47.2	73.7
Amy	1	26.4	26.2	25.5	33.5	31.8	28.9	36.4	41.1	45.8	74.6
Amy	1	26.9	27.1	33.4	29.4	34.4	27.6	30.0	36.7	45.9	78.4
Amy	2	27.6	30.1	25.8	25.8	29.7	33.1	29.9	31.6	66.1	77.1
Amy	2	26.5	30.6	25.5	28.6	29.8	36.0	33.7	32.3	65.7	79.1
Amy	2	29.5	27.5	23.6	24.4	.	32.5	35.3	26.1	68.2	78.3
Amy	2	21.4	29.4	24.8	28.9	27.6	34.4	37.3	30.5	70.0	79.5
Amy	3	29.5	30.8	30.8	33.0	32.4	41.9	43.8	45.2	70.8	80.5
Amy	3	32.3	28.3	30.6	30.4	34.9	34.4	43.5	52.1	70.5	81.7
Amy	3	30.1	29.4	33.1	38.5	35.8	35.7	51.0	52.1	68.2	78.7
Amy	3	25.8	27.1	29.9	34.2	37.6	39.2	42.5	50.1	70.3	80.3

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Amy	4	29.8	30.3	28.4	32.2	25.1	29.7	34.1	34.3	61.9	75.0
Amy	4	28.5	29.0	.	27.7	29.9	26.5	31.7	35.5	61.8	75.8
Amy	4	27.6	25.4	30.1	31.7	28.9	25.9	31.1	30.6	59.7	74.7
Amy	4	27.3	26.6	32.5	29.0	31.0	24.5	38.1	32.1	61.9	77.6
Amy	5	24.7	27.6	31.2	29.4	34.5	42.5	40.5	46.4	66.1	82.3
Amy	5	25.2	30.4	30.3	30.4	32.9	36.3	38.1	46.7	68.7	83.5
Amy	5	26.6	23.5	30.9	26.9	35.7	34.1	41.2	45.6	69.0	82.3
Amy	5	28.6	24.2	31.0	28.4	34.9	36.5	39.7	45.9	66.9	83.0
Amy	6	27.1	27.1	26.6	29.2	34.6	34.5	35.8	46.9	58.6	67.0
Amy	6	27.2	29.8	27.5	28.5	30.6	35.2	41.3	40.2	66.0	64.9
Amy	6	28.3	27.3	29.2	28.9	35.1	33.0	32.7	45.8	64.3	67.1
Amy	6	27.2	24.5	23.2	39.9	34.4	25.9	37.9	39.0	61.9	70.2
Amy	7	29.5	26.9	33.8	37.0	35.9	33.6	42.7	43.4	61.9	77.1
Amy	7	27.6	28.3	31.0	31.3	35.0	34.5	45.1	38.8	64.4	82.9
Amy	7	30.5	30.2	27.7	29.9	31.7	37.4	44.2	41.4	66.3	80.5
Amy	7	31.3	28.7	27.2	30.5	34.4	33.4	44.6	41.0	64.1	83.2
Amy	8	25.4	31.3	30.1	33.4	31.2	36.1	36.0	43.9	64.1	77.2
Amy	8	27.5	29.1	28.2	32.2	35.6	33.0	39.0	45.4	62.5	75.4
Amy	8	27.8	33.0	31.3	32.9	34.5	33.5	38.2	40.3	59.8	72.8
Amy	8	28.1	27.8	27.6	30.6	29.9	24.9	42.5	41.8	61.7	71.7
Prot	1	39.6	28.9	34.4	32.1	31.9	32.1	31.9	33.8	53.9	72.9
Prot	1	32.2	31.3	32.1	28.0	29.7	29.4	30.4	35.4	48.8	71.1
Prot	1	28.4	25.1	29.8	33.5	34.4	33.9	34.2	40.5	61.1	78.0
Prot	1	27.6	29.3	28.9	35.2	35.5	33.0	38.8	34.5	62.3	79.9
Prot	2	26.9	22.8	30.3	28.0	31.6	34.4	35.2	33.8	52.5	75.6
Prot	2	30.8	28.1	33.5	28.4	28.4	30.1	36.3	34.2	55.2	80.0
Prot	2	29.5	32.9	31.3	28.3	37.3	.	32.9	42.0	47.9	80.0
Prot	2	30.0	23.8	28.7	28.8	.	31.7	31.4	46.9	51.4	80.0
Prot	3	28.1	28.8	30.5	38.0	36.7	40.1	47.1	50.9	62.7	79.6
Prot	3	31.4	32.1	32.3	34.5	39.0	40.1	49.5	50.2	64.1	78.9
Prot	3	27.5	33.7	33.7	31.0	38.1	39.7	44.2	50.1	69.3	79.7
Prot	3	26.2	27.8	34.4	34.2	36.7	36.1	45.8	53.3	67.2	79.8
Prot	4	30.6	23.4	30.8	27.2	32.9	33.5	32.8	43.2	64.0	81.7
Prot	4	26.5	27.3	32.5	28.1	33.9	32.0	38.3	44.3	59.9	80.5
Prot	4	29.7	29.6	30.0	31.9	31.4	34.2	32.6	51.2	59.9	81.4
Prot	4	23.7	21.8	33.4	27.4	32.9	33.4	32.2	46.2	60.5	81.5
Prot	5	31.6	28.7	27.6	35.9	34.6	32.2	47.9	49.6	65.0	80.7
Prot	5	28.4	29.3	32.6	34.5	38.6	37.2	47.5	47.4	67.2	82.4
Prot	5	24.7	27.2	34.2	37.1	32.3	37.0	44.2	48.2	63.4	82.7
Prot	5	28.6	28.3	31.0	31.5	35.8	33.7	47.8	53.9	67.9	78.5
Prot	6	31.1	32.4	28.8	35.7	37.8	33.0	46.8	40.8	61.4	79.1
Prot	6	24.9	30.9	30.0	27.6	33.4	37.9	46.8	39.4	61.3	79.4
Prot	6	23.4	27.3	30.1	31.0	39.7	28.8	39.4	46.7	61.2	75.8
Prot	6	27.8	28.8	30.7	31.6	31.2	33.5	34.6	44.4	56.8	77.7
Prot	7	28.2	31.1	30.1	28.3	35.7	37.5	44.7	42.9	58.8	81.2
Prot	7	25.1	23.9	31.8	31.3	33.9	30.0	44.3	47.7	59.2	80.7

Treatment	Animal	Time of incubation (h)									
		1	2	3	4	5	6	9	12	24	48
Prot	7	27.5	21.2	32.0	31.3	31.2	37.0	44.5	49.3	67.5	81.1
Prot	7	24.4	29.9	26.8	32.3	35.4	29.1	42.1	48.5	65.0	82.1
Prot	8	30.8	24.7	33.0	34.0	30.7	35.4	46.5	40.8	62.2	79.0
Prot	8	28.5	28.4	26.0	34.1	31.6	36.4	47.1	44.6	55.0	78.2
Prot	8	25.0	26.6	28.0	30.1	29.4	35.6	41.9	37.2	64.9	80.8
Prot	8	25.2	31.1	33.0	31.1	32.6	35.9	46.2	38.5	64.8	78.3
Amy+Prot	1	27.9	32.1	31.1	31.4	29.9	33.4	29.2	42.8	61.1	73.8
Amy+Prot	1	30.7	27.4	31.8	29.9	28.8	32.5	33.6	42.4	61.7	76.3
Amy+Prot	1	30.0	28.7	30.1	30.2	33.2	33.6	35.3	47.2	61.2	78.1
Amy+Prot	1	29.2	30.4	25.9	30.7	33.3	33.1	45.0	45.1	59.2	78.6
Amy+Prot	2	30.2	27.4	27.8	34.1	28.8	34.4	39.8	45.5	58.1	72.3
Amy+Prot	2	28.2	28.8	27.8	32.0	29.4	30.8	41.3	38.7	53.5	73.9
Amy+Prot	2	28.0	25.0	30.9	30.9	31.2	35.4	36.1	44.8	59.0	79.6
Amy+Prot	2	27.4	27.2	32.4	31.0	25.9	30.9	36.6	46.0	51.4	77.0
Amy+Prot	3	28.5	32.1	30.7	31.3	35.9	33.4	.	54.5	72.2	82.3
Amy+Prot	3	26.6	25.9	34.9	30.6	36.5	39.1	45.9	54.8	72.0	82.7
Amy+Prot	3	26.0	29.9	35.1	31.5	36.4	38.0	48.5	52.4	69.7	81.0
Amy+Prot	3	28.3	31.0	32.1	30.0	37.8	42.6	47.1	55.6	71.2	82.5
Amy+Prot	4	31.7	27.5	26.7	30.8	33.0	30.3	29.3	38.2	62.7	75.4
Amy+Prot	4	28.1	27.7	25.5	31.5	32.9	29.9	32.1	42.9	62.2	77.2
Amy+Prot	4	26.0	29.2	24.6	34.0	28.1	30.0	37.5	30.8	63.2	79.5
Amy+Prot	4	29.3	27.6	26.7	28.3	25.9	31.0	26.5	32.7	63.9	79.8
Amy+Prot	5	31.2	24.3	30.7	37.2	38.3	43.6	45.8	49.0	64.4	76.8
Amy+Prot	5	29.5	28.4	35.4	35.2	37.9	38.1	40.4	43.2	65.6	77.3
Amy+Prot	5	28.5	36.0	35.0	35.1	31.9	36.6	46.2	48.4	65.7	79.8
Amy+Prot	5	29.0	30.7	26.4	37.2	37.3	37.5	45.0	51.8	72.0	79.3
Amy+Prot	6	29.2	33.8	34.3	30.4	32.9	34.9	38.5	48.4	63.6	74.4
Amy+Prot	6	30.0	27.8	32.3	37.8	32.0	.	39.1	43.7	64.0	73.6
Amy+Prot	6	25.1	30.0	32.6	31.3	32.1	34.0	44.6	40.6	67.3	76.1
Amy+Prot	6	26.2	29.3	33.5	28.7	35.2	29.8	43.8	35.6	66.8	78.7
Amy+Prot	7	27.3	24.9	31.6	31.8	31.1	27.7	40.4	37.9	66.0	81.2
Amy+Prot	7	28.8	23.5	30.2	30.7	32.9	28.8	37.1	44.3	69.1	82.1
Amy+Prot	7	27.6	28.3	33.6	30.9	30.5	30.6	38.3	43.2	61.8	78.6
Amy+Prot	7	28.8	24.2	28.6	29.7	30.2	35.3	47.7	41.8	61.6	79.9
Amy+Prot	8	30.5	.	25.1	29.2	34.7	38.1	37.7	38.6	63.4	78.7
Amy+Prot	8	22.4	24.8	29.0	28.3	34.0	37.3	42.0	44.1	61.4	72.2
Amy+Prot	8	28.6	26.0	30.8	31.1	32.5	29.0	36.1	50.4	61.2	72.8
Amy+Prot	8	29.3	26.5	30.9	29.6	32.1	32.5	37.9	49.0	60.9	68.0

Table 56: Parameter of degradability and effective degradability of dry matter of maize grain of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	22.1	78.0	3.67	0.25	72.2	54.6	46.1
Con	2	84.2	20.5	63.7	5.37	0.00	66.9	53.5	46.1
Con	3	100	22.4	77.6	4.37	0.00	75.6	58.6	49.8
Con	4	100	22.1	78.0	3.61	0.13	72.1	54.5	46.0
Con	5	100	22.2	77.8	3.69	0.00	72.7	55.2	46.8
Con	6	100	22.1	78.0	3.99	0.07	73.9	56.5	47.8
Con	7	100	22.7	77.3	3.60	0.00	72.4	55.1	46.7
Con	8	100	23.2	76.8	3.86	0.00	73.8	56.7	48.2
Amy	1	100	23.4	76.6	2.93	0.00	68.9	51.7	43.9
Amy	2	100	20.8	79.2	4.07	0.36	73.5	55.7	46.7
Amy	3	100	22.0	78.0	4.60	0.00	76.4	59.4	50.5
Amy	4	100	21.4	78.6	3.16	0.00	69.5	51.8	43.7
Amy	5	100	22.0	78.1	3.99	0.00	73.9	56.6	47.9
Amy	6	92.9	22.7	70.1	3.66	0.00	68.1	52.4	44.7
Amy	7	100	20.9	79.1	3.85	0.00	72.9	55.3	46.6
Amy	8	100	21.8	78.2	3.98	0.00	73.8	56.4	47.8
Prot	1	100	21.6	78.4	3.41	0.00	71.0	53.4	45.0
Prot	2	100	21.3	78.7	3.24	0.00	70.0	52.3	44.0
Prot	3	100	22.0	78.0	3.89	0.00	73.5	56.1	47.5
Prot	4	100	21.2	78.8	3.74	0.59	71.9	53.9	45.1
Prot	5	100	21.6	78.4	4.04	0.00	74.0	56.7	47.9
Prot	6	100	21.8	78.2	3.57	0.00	71.9	54.4	45.9
Prot	7	100	21.2	78.8	3.77	0.38	72.3	54.4	45.7
Prot	8	100	21.9	78.1	3.83	0.00	73.2	55.8	47.2
Amy+Prot	1	100	25.4	74.6	3.70	0.00	73.8	57.1	49.0
Amy+Prot	2	100	25.1	74.9	3.48	0.00	72.7	55.9	47.8
Amy+Prot	3	100	23.2	76.8	5.23	0.02	78.7	62.4	53.5
Amy+Prot	4	100	23.2	76.8	3.59	0.01	72.5	55.3	47.0
Amy+Prot	5	100	24.3	75.7	4.01	0.00	74.8	58.0	49.6
Amy+Prot	6	100	24.7	75.3	4.25	0.00	75.9	59.3	50.8
Amy+Prot	7	100	23.6	76.4	3.97	0.00	74.4	57.4	48.9
Amy+Prot	8	100	25.4	74.6	3.68	0.00	73.7	57.0	48.9

Table 57: Parameter of degradability and effective degradability of dry matter of soybean meal of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	30.3	69.8	7.40	2.89	82.1	66.3	56.8
Con	2	100	26.7	73.3	5.37	0.48	79.6	63.7	55.0
Con	3	99.1	33.2	65.9	17.67	3.62	88.2	76.0	67.1
Con	4	100	30.1	69.9	8.05	2.74	83.1	67.7	58.3
Con	5	100	30.7	69.3	10.65	2.73	85.9	71.8	62.5
Con	6	100	30.6	69.4	7.70	3.64	81.8	65.7	56.1
Con	7	100	28.7	71.3	9.79	1.80	85.8	71.8	62.7
Con	8	100	26.7	73.3	7.58	0.90	83.7	68.9	59.9
Amy	1	100	26.3	73.7	5.16	0.62	78.8	62.6	53.8
Amy	2	100	31.2	68.8	7.73	4.74	80.9	64.2	54.3
Amy	3	100	28.9	71.1	11.6	1.83	87.3	74.2	65.2
Amy	4	100	29.3	70.7	5.31	2.74	77.9	61.1	52.0
Amy	5	100	26.3	73.7	11.0	0.94	87.5	74.7	65.9
Amy	6	100	26.3	73.7	6.11	0.72	81.0	65.4	56.4
Amy	7	100	28.3	71.7	8.74	1.75	84.6	70.1	60.8
Amy	8	100	30.2	69.8	8.86	2.71	84.1	69.2	59.7
Prot	1	100	25.5	74.5	5.20	0.12	79.2	63.3	54.6
Prot	2	100	25.5	74.5	5.73	0.87	79.8	63.6	54.5
Prot	3	100	25.5	74.5	9.47	0.72	86.1	72.5	63.6
Prot	4	100	27.5	72.5	7.48	1.89	82.6	67.1	57.6
Prot	5	100	25.5	74.5	10.8	0.91	87.2	74.2	65.3
Prot	6	100	25.5	74.5	7.08	0.15	83.4	68.8	60.1
Prot	7	99.5	30.2	69.3	12.1	2.89	86.4	72.7	63.3
Prot	8	100	25.5	74.5	6.71	0.48	82.3	67.2	58.2
Amy+Prot	1	100	25.6	74.4	6.34	0.50	81.6	66.2	57.2
Amy+Prot	2	100	29.1	70.9	7.13	2.62	81.6	65.7	56.2
Amy+Prot	3	100	27.9	72.1	13.2	1.83	88.3	75.6	66.7
Amy+Prot	4	100	31.8	68.2	8.06	4.52	81.7	65.4	55.6
Amy+Prot	5	99.7	28.2	71.5	11.6	1.70	87.1	74.0	65.1
Amy+Prot	6	100	25.6	74.4	8.79	0.80	85.3	71.2	62.2
Amy+Prot	7	100	25.6	74.4	8.13	0.95	84.2	69.6	60.4
Amy+Prot	8	100	25.6	74.4	7.66	0.91	83.5	68.6	59.5

Table 58: Parameter of degradability and effective degradability of dry matter of maize silage of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	21.7	78.3	2.28	0.03	63.4	46.2	39.0
Con	2	100	25.1	74.9	1.73	0.00	59.8	44.4	38.4
Con	3	84.3	25.2	59.2	4.48	0.00	66.1	53.1	46.4
Con	4	100	21.7	78.3	2.58	0.40	65.4	47.8	40.2
Con	5	89.0	24.8	64.2	3.69	0.00	66.4	52.0	45.0
Con	6	100	21.7	78.3	2.69	0.00	66.6	49.1	41.4
Con	7	90.1	23.6	66.5	3.31	0.00	65.1	50.1	43.1
Con	8	90.2	23.1	67.1	3.01	0.00	63.4	48.3	41.5
Amy	1	100	25.0	75.0	1.97	0.00	62.2	46.2	39.8
Amy	2	91.5	27.2	64.3	3.53	3.32	65.6	49.7	42.3
Amy	3	84.2	27.1	57.1	5.35	0.00	68.7	56.6	50.0
Amy	4	100	25.3	74.7	2.25	1.87	63.4	46.4	39.5
Amy	5	93.0	27.0	66.0	3.29	0.00	68.0	53.1	46.2
Amy	6	77.2	25.7	51.5	3.48	0.00	58.4	46.8	41.3
Amy	7	79.2	21.7	57.5	5.75	0.00	64.3	52.4	45.7
Amy	8	83.0	25.0	58.0	3.95	0.00	63.5	50.6	44.2
Prot	1	100	27.0	73.0	2.04	0.08	63.8	48.1	41.7
Prot	2	100	27.0	73.0	2.39	0.19	66.6	50.4	43.5
Prot	3	77.6	27.0	50.6	6.03	0.18	64.8	54.4	48.4
Prot	4	89.8	27.0	62.8	3.46	0.53	66.4	52.0	45.1
Prot	5	80.4	27.0	53.4	5.66	0.53	66.0	54.6	48.2
Prot	6	84.9	27.7	57.2	3.80	0.00	65.2	52.4	46.2
Prot	7	92.3	27.0	65.4	3.43	0.37	68.0	53.1	46.0
Prot	8	94.4	29.3	65.1	2.74	0.00	66.9	52.4	45.9
Amy+Prot	1	100	30.4	69.6	2.13	0.00	66.3	51.2	45.1
Amy+Prot	2	99.6	27.3	72.4	1.87	0.00	62.2	47.0	41.0
Amy+Prot	3	84.9	29.1	55.9	4.88	0.00	68.7	56.7	50.3
Amy+Prot	4	83.9	30.8	53.1	5.32	8.01	63.7	49.1	41.9
Amy+Prot	5	81.3	29.0	52.4	5.89	0.00	68.0	57.3	51.1
Amy+Prot	6	75.9	29.3	46.6	7.45	4.60	62.8	51.5	44.9
Amy+Prot	7	100	27.0	73.1	2.47	0.41	67.0	50.6	43.6
Amy+Prot	8	87.9	27.0	60.9	3.30	0.03	64.9	51.1	44.7

Table 59: Parameter of degradability and effective degradability of dry matter of grass silage of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	85.0	33.4	51.6	6.84	7.23	68.0	54.2	46.8
Con	2	92.9	29.1	63.8	2.68	0.00	65.7	51.4	45.1
Con	3	88.7	29.1	59.6	5.25	0.45	71.9	59.0	51.9
Con	4	100	31.4	68.6	3.24	3.84	70.7	53.7	46.0
Con	5	95.5	29.1	66.4	3.45	0.63	70.6	55.4	48.1
Con	6	95.0	30.1	64.8	3.78	1.77	71.1	55.7	48.2
Con	7	92.9	29.6	63.4	3.95	0.00	71.6	57.5	50.5
Con	8	94.9	30.6	64.3	3.15	2.75	67.8	52.2	45.1
Amy	1	100	28.4	71.7	2.03	0.00	64.4	49.0	42.9
Amy	2	81.6	32.6	49.0	13.1	10.9	66.7	53.1	45.3
Amy	3	87.1	30.2	56.9	6.00	2.59	70.7	57.5	50.0
Amy	4	82.5	32.4	50.2	6.35	8.05	64.8	51.1	44.0
Amy	5	98.3	28.3	70.0	3.53	0.88	72.2	56.0	48.2
Amy	6	78.1	28.3	49.8	5.31	0.94	63.8	52.7	46.7
Amy	7	100	28.3	71.8	3.15	0.92	71.3	54.7	47.1
Amy	8	79.7	30.7	49.0	5.79	3.82	64.4	52.4	45.9
Prot	1	100	27.8	72.2	2.51	0.14	67.9	51.8	44.8
Prot	2	100	30.7	69.3	2.76	3.96	67.8	50.9	43.6
Prot	3	92.0	27.8	64.2	3.67	0.13	69.2	54.8	47.8
Prot	4	94.8	27.8	67.0	3.71	0.87	70.5	55.1	47.6
Prot	5	91.5	27.8	63.7	4.11	0.13	70.5	56.3	49.2
Prot	6	96.5	27.8	68.7	3.19	0.42	69.7	54.0	46.7
Prot	7	97.1	27.8	69.3	3.37	0.44	70.9	55.1	47.6
Prot	8	100	27.8	72.2	2.69	0.42	68.9	52.5	45.4
Amy+Prot	1	94.9	28.2	66.7	3.04	0.17	68.3	53.2	46.3
Amy+Prot	2	100	28.2	71.8	2.39	0.13	67.2	51.3	44.5
Amy+Prot	3	90.0	28.2	61.8	5.01	0.73	71.7	58.0	50.6
Amy+Prot	4	85.6	33.0	52.7	6.15	7.73	67.0	52.7	45.3
Amy+Prot	5	92.8	28.2	64.7	3.87	0.11	70.7	56.2	49.1
Amy+Prot	6	90.6	28.2	62.4	3.87	0.71	68.7	54.5	47.4
Amy+Prot	7	100	28.2	71.8	2.94	0.00	70.9	54.8	47.5
Amy+Prot	8	83.2	28.2	55.0	4.03	0.60	64.5	52.0	45.7

Table 60: Parameter of degradability and effective degradability of dry matter of hay of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	61.2	10.9	50.3	3.02	0.00	41.2	29.9	24.7
Con	2	100	12.4	87.6	0.94	0.00	40.4	26.3	21.6
Con	3	71.5	11.0	60.4	2.53	0.00	44.8	31.3	25.5
Con	4	71.7	10.7	61.1	2.26	0.00	43.1	29.7	24.1
Con	5	54.0	11.1	42.9	4.01	0.00	39.7	30.2	25.4
Con	6	93.6	11.3	82.3	1.47	0.00	46.2	30.0	24.1
Con	7	100	12.0	88.0	1.30	0.00	46.7	30.2	24.3
Con	8	72.3	11.2	61.1	1.96	0.00	41.4	28.4	23.2
Amy	1	43.8	10.1	33.7	4.89	0.00	34.0	26.8	22.9
Amy	2	56.7	10.2	46.6	3.43	0.00	39.6	29.1	24.1
Amy	3	77.3	11.2	66.1	2.29	0.00	46.5	32.0	25.9
Amy	4	100	12.1	87.9	1.04	0.00	42.2	27.3	22.2
Amy	5	74.9	11.2	63.7	2.38	0.00	45.8	31.7	25.8
Amy	6	46.6	10.9	35.8	4.92	0.00	36.3	28.6	24.5
Amy	7	95.8	11.1	84.8	1.55	0.00	48.1	31.1	24.8
Amy	8	67.6	11.4	56.2	2.12	0.00	40.3	28.1	23.1
Prot	1	46.7	9.77	36.9	4.34	0.00	35.0	26.9	22.7
Prot	2	49.4	9.29	40.1	4.26	0.00	36.6	27.8	23.2
Prot	3	54.3	10.0	44.3	4.49	0.00	40.7	31.0	25.9
Prot	4	52.9	9.87	43.0	3.65	0.00	37.6	28.0	23.3
Prot	5	75.2	11.1	64.1	2.16	0.00	44.4	30.5	24.8
Prot	6	51.2	9.88	41.4	4.63	0.00	38.8	29.8	25.0
Prot	7	100	12.2	87.8	1.30	0.00	46.8	30.3	24.5
Prot	8	100	11.2	88.8	1.21	0.00	44.7	28.5	22.9
Amy+Prot	1	55.8	10.3	45.4	3.18	0.00	38.2	28.0	23.2
Amy+Prot	2	48.3	10.4	37.9	3.73	0.00	35.1	26.6	22.4
Amy+Prot	3	70.0	11.0	59.0	2.75	0.00	45.2	31.9	26.1
Amy+Prot	4	100	12.1	87.9	1.16	0.00	44.3	28.6	23.2
Amy+Prot	5	76.0	11.4	64.6	2.09	0.00	44.4	30.5	24.8
Amy+Prot	6	63.0	10.9	52.1	2.74	0.00	41.0	29.3	24.2
Amy+Prot	7	62.8	10.2	52.7	3.11	0.00	42.2	30.4	24.9
Amy+Prot	8	63.5	11.6	51.9	2.20	0.00	38.8	27.5	22.8

Table 61: Parameter of degradability and effective degradability of dry matter of the TMR of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	20.2	79.8	2.62	0.00	65.5	47.7	39.9
Con	2	80.4	22.0	58.4	3.09	0.00	57.4	44.3	38.2
Con	3	86.8	21.1	65.8	5.05	0.00	68.2	54.1	46.5
Con	4	100	22.1	77.9	2.83	1.99	66.0	47.6	39.5
Con	5	92.5	21.7	70.8	3.29	0.00	65.7	49.8	42.3
Con	6	99.7	21.3	78.4	2.67	0.00	66.1	48.6	40.9
Con	7	95.4	20.0	75.4	3.24	0.00	66.6	49.6	41.7
Con	8	95.1	21.3	73.8	2.84	0.00	64.6	48.0	40.6
Amy	1	72.1	19.8	52.2	4.46	0.00	55.9	44.4	38.5
Amy	2	100	25.5	74.5	3.01	3.99	66.8	48.4	40.3
Amy	3	88.1	22.8	65.3	4.73	0.00	68.7	54.5	47.1
Amy	4	100	21.9	78.1	2.32	0.00	63.8	46.6	39.4
Amy	5	98.3	21.8	76.5	3.43	0.00	70.1	52.9	44.8
Amy	6	75.1	21.8	53.2	4.59	0.00	58.9	47.3	41.2
Amy	7	100	23.9	76.1	2.89	0.00	68.9	51.7	44.1
Amy	8	89.1	23.3	65.8	3.23	0.00	64.0	49.1	42.2
Prot	1	100	24.7	75.3	2.13	0.00	63.5	47.2	40.5
Prot	2	100	23.3	76.8	2.24	0.00	63.8	47.0	40.0
Prot	3	84.6	23.9	60.8	5.02	0.00	67.3	54.3	47.3
Prot	4	100	22.2	77.8	2.82	0.00	67.7	50.3	42.5
Prot	5	89.4	23.2	66.3	4.32	0.00	68.4	53.9	46.4
Prot	6	97.0	24.0	73.0	2.80	0.00	66.6	50.2	42.9
Prot	7	95.3	22.3	72.9	3.42	0.00	68.3	51.9	44.2
Prot	8	97.7	23.5	74.2	2.89	0.00	67.4	50.7	43.2
Amy+Prot	1	100	23.9	76.1	2.51	0.00	66.2	49.3	42.1
Amy+Prot	2	100	23.8	76.2	2.33	0.00	64.8	48.0	41.0
Amy+Prot	3	88.2	22.2	66.1	5.28	0.02	70.1	56.1	48.4
Amy+Prot	4	100	22.2	77.9	2.52	0.31	65.3	47.8	40.3
Amy+Prot	5	85.8	24.4	61.5	4.52	0.00	67.0	53.5	46.6
Amy+Prot	6	89.8	23.8	65.9	3.42	0.00	65.4	50.6	43.6
Amy+Prot	7	100	22.2	77.8	2.89	0.00	68.2	50.7	42.8
Amy+Prot	8	82.9	22.2	60.7	3.95	0.00	62.5	49.0	42.3

Table 62: Ruminal starch disappearance (%) of maize grain of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)					
		1	3	6	9	12	24
Con	1	32.6	29.2	37.3	38.9	44.7	70.8
Con	2	25.4	27.4	33.5	37.7	39.3	60.3
Con	3	26.3	30.4	35.7	39.0	49.4	72.3
Con	4	33.3	34.9	30.2	33.4	36.4	69.1
Con	5	31.2	31.6	36.7	42.2	45.0	65.0
Con	6	25.7	29.4	33.8	34.8	41.8	73.9
Con	7	26.8	30.3	35.5	38.3	46.1	58.9
Con	8	29.6	33.2	34.7	38.1	45.3	70.2
Amy	1	28.1	29.9	31.4	39.5	40.7	55.0
Amy	2	32.8	32.9	38.0	42.5	45.6	82.1
Amy	3	25.3	30.6	36.1	41.1	47.4	80.2
Amy	4	27.4	31.5	34.0	35.1	36.7	69.3
Amy	5	24.8	28.8	36.2	36.8	44.6	71.5
Amy	6	31.2	31.8	39.4	39.7	48.9	71.7
Amy	7	24.0	26.9	34.7	37.1	43.6	65.3
Amy	8	29.7	30.6	34.4	37.9	44.5	79.2
Prot	1	23.9	27.3	33.8	35.6	38.9	67.5
Prot	2	28.2	29.0	30.1	37.3	44.1	54.7
Prot	3	37.3	38.5	35.4	46.9	53.1	69.6
Prot	4	21.5	26.3	30.5	36.2	42.0	65.2
Prot	5	25.8	29.5	32.4	40.6	47.5	67.3
Prot	6	25.8	28.5	31.7	38.8	39.0	68.2
Prot	7	31.8	34.5	36.8	40.3	51.4	66.8
Prot	8	27.6	27.9	34.0	39.5	43.5	72.0
Amy+Prot	1	29.4	35.2	36.7	40.4	47.8	69.9
Amy+Prot	2	26.9	32.0	37.3	39.6	47.1	69.8
Amy+Prot	3	24.6	34.8	42.2	51.2	60.2	81.9
Amy+Prot	4	32.5	37.4	35.1	39.3	38.4	70.8
Amy+Prot	5	29.5	28.0	36.5	40.7	46.9	71.9
Amy+Prot	6	28.5	32.9	36.2	40.2	57.4	81.5
Amy+Prot	7	30.0	31.3	37.7	45.0	51.8	71.3
Amy+Prot	8	36.2	38.8	37.5	40.2	46.2	74.4

Table 63: Ruminal crude protein disappearance (%) of maize grain of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)					
		1	3	6	9	12	24
Con	1	31.7	29.7	33.4	38.6	38.4	54.1
Con	2	31.0	32.6	37.7	37.8	39.6	51.1
Con	3	29.7	29.9	33.0	38.1	42.1	45.6
Con	4	34.4	35.1	33.1	34.9	37.6	51.3
Con	5	30.7	31.0	34.6	39.7	42.2	48.2
Con	6	30.4	32.4	36.2	36.8	35.1	56.1
Con	7	32.0	31.9	32.5	37.0	35.6	47.2
Con	8	31.6	31.8	34.6	35.8	39.2	46.8
Amy	1	30.2	32.8	31.3	35.7	38.4	42.5
Amy	2	32.5	29.7	32.6	34.4	34.3	57.0
Amy	3	30.3	32.8	34.3	33.7	41.1	47.9
Amy	4	30.1	31.8	32.0	33.2	32.7	44.0
Amy	5	30.0	32.6	32.4	33.6	38.9	40.4
Amy	6	30.7	29.9	30.2	34.0	34.4	52.7
Amy	7	29.5	29.1	33.5	34.1	39.0	47.0
Amy	8	32.7	32.6	33.4	34.8	36.2	43.2
Prot	1	30.9	33.9	37.5	39.3	38.6	43.7
Prot	2	32.1	34.4	34.9	40.3	44.3	44.0
Prot	3	31.4	29.9	30.2	38.5	35.0	47.4
Prot	4	29.6	32.3	32.9	36.5	40.5	47.8
Prot	5	29.3	33.8	34.5	40.5	39.4	48.9
Prot	6	31.6	32.7	36.7	42.2	40.2	46.6
Prot	7	30.1	32.0	33.8	37.5	41.2	51.9
Prot	8	31.2	31.9	35.4	34.3	42.7	47.0
Amy+Prot	1	32.2	34.4	38.8	39.3	40.0	47.4
Amy+Prot	2	32.2	35.4	38.2	34.3	36.8	43.6
Amy+Prot	3	29.9	34.3	37.7	39.1	37.9	48.5
Amy+Prot	4	31.4	34.7	35.4	37.8	37.6	54.9
Amy+Prot	5	31.3	31.9	34.6	39.2	37.0	52.4
Amy+Prot	6	33.8	34.6	31.7	34.6	31.9	44.2
Amy+Prot	7	34.1	34.5	33.5	37.9	37.6	47.6
Amy+Prot	8	31.8	34.1	32.5	38.0	38.9	53.7

Table 64: Ruminal crude protein disappearance (%) of soybean meal of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)					
		1	3	6	9	12	24
Con	1	19.4	23.0	30.6	40.9	57.7	91.5
Con	2	18.7	25.3	28.4	49.5	42.5	78.8
Con	3	20.4	27.1	38.5	74.0	87.3	98.0
Con	4	18.1	25.7	31.7	45.7	55.6	95.2
Con	5	20.5	25.2	35.1	62.6	67.8	97.4
Con	6	17.2	21.1	29.4	33.5	52.0	96.3
Con	7	18.7	27.7	42.4	57.0	72.4	95.6
Con	8	19.8	25.3	37.5	48.7	59.7	97.5
Amy	1	21.2	27.1	30.0	42.8	50.3	75.3
Amy	2	20.1	23.1	27.3	31.3	46.3	98.2
Amy	3	21.3	29.5	46.9	68.5	80.7	98.0
Amy	4	18.0	24.7	26.8	34.6	33.9	82.6
Amy	5	23.1	32.6	53.2	68.0	83.0	98.4
Amy	6	23.1	20.6	41.2	37.1	46.6	97.1
Amy	7	20.1	26.5	39.4	61.5	63.1	92.8
Amy	8	19.4	23.1	30.0	52.4	65.6	94.5
Prot	1	24.5	32.6	37.4	47.5	44.3	86.7
Prot	2	27.0	31.5	33.7	49.8	55.4	90.8
Prot	3	28.9	37.5	44.6	79.9	75.2	97.5
Prot	4	24.4	31.6	39.2	48.5	71.2	90.5
Prot	5	28.8	33.6	43.9	75.2	88.7	97.3
Prot	6	29.5	37.8	44.7	62.6	60.0	94.0
Prot	7	25.8	33.5	44.3	72.3	82.9	97.9
Prot	8	26.1	34.0	42.3	69.7	49.9	95.9
Amy+Prot	1	26.4	32.2	36.3	51.8	61.6	86.9
Amy+Prot	2	22.9	26.4	36.1	47.4	62.1	83.5
Amy+Prot	3	24.6	34.4	51.1	76.9	91.5	98.4
Amy+Prot	4	22.9	27.6	31.9	41.2	49.1	96.7
Amy+Prot	5	23.2	33.8	45.6	74.3	82.0	95.5
Amy+Prot	6	23.0	35.5	45.9	69.0	66.1	97.1
Amy+Prot	7	24.8	31.9	36.5	60.9	69.1	97.0
Amy+Prot	8	25.2	28.8	36.3	45.8	78.6	95.9

Table 65: Ruminal starch disappearance (%) of maize silage of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)					
		1	3	6	9	12	24
Con	1	29.0	38.3	27.9	43.2	53.2	85.0
Con	2	53.2	33.5	64.8	49.6	65.3	73.0
Con	3	61.0	64.2	74.9	64.8	61.7	90.0
Con	4	25.0	36.9	38.3	39.2	47.6	87.8
Con	5	51.4	43.1	56.1	64.8	61.1	81.6
Con	6	52.6	45.4	37.6	54.6	84.0	90.8
Con	7	34.7	44.4	72.4	53.8	80.2	76.7
Con	8	36.0	43.9	50.6	43.8	66.4	86.8
Amy	1	30.3	45.5	34.6	44.9	63.9	66.0
Amy	2	22.5	29.1	46.2	43.3	67.1	84.3
Amy	3	68.9	75.7	84.4	84.1	82.6	92.3
Amy	4	25.3	27.6	35.9	36.7	50.0	96.3
Amy	5	34.8	80.7	59.7	64.0	72.1	86.5
Amy	6	35.9	35.8	55.3	44.0	44.0	86.3
Amy	7	29.6	43.4	72.7	72.3	74.7	83.0
Amy	8	40.8	31.2	26.0	57.4	61.0	78.4
Prot	1	30.2	37.6	68.9	38.0	60.0	77.2
Prot	2	38.0	45.4	39.7	53.5	58.5	88.3
Prot	3	32.2	63.1	55.5	77.0	74.5	83.5
Prot	4	47.2	41.6	47.8	60.9	68.2	82.7
Prot	5	17.6	67.7	72.6	70.2	72.0	84.0
Prot	6	64.2	61.8	59.9	65.8	65.4	87.7
Prot	7	34.8	46.9	56.9	59.9	64.6	86.5
Prot	8	31.0	58.5	58.4	82.8	60.7	86.4
Amy+Prot	1	54.6	46.3	51.4	55.5	64.5	61.7
Amy+Prot	2	39.5	29.7	32.0	45.8	69.9	74.3
Amy+Prot	3	48.1	56.9	52.4	69.9	74.6	90.4
Amy+Prot	4	37.5	44.5	37.4	43.7	51.0	87.4
Amy+Prot	5	52.5	58.0	79.5	61.5	75.5	93.3
Amy+Prot	6	24.7	27.5	32.8	54.6	70.3	84.0
Amy+Prot	7	42.9	37.2	29.6	54.2	63.1	89.9
Amy+Prot	8	32.2	39.1	47.4	44.3	68.6	82.3

Table 66: Ruminal crude protein disappearance (%) of maize silage of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)					
		1	3	6	9	12	24
Con	1	34.1	40.5	37.1	32.2	39.8	44.6
Con	2	48.8	38.4	50.0	41.9	46.1	54.9
Con	3	32.0	33.7	32.6	39.3	37.9	51.6
Con	4	32.8	32.9	30.2	38.4	34.6	49.8
Con	5	46.4	39.5	42.7	46.2	44.5	67.6
Con	6	46.7	40.5	40.3	37.5	53.9	59.8
Con	7	34.9	33.0	25.0	35.9	32.4	38.6
Con	8	32.1	35.2	35.7	32.4	35.4	53.8
Amy	1	40.6	43.6	46.7	41.8	44.6	48.9
Amy	2	45.4	37.4	44.8	38.5	46.0	52.5
Amy	3	59.0	54.7	57.6	55.5	55.9	59.9
Amy	4	30.6	29.0	32.0	29.9	31.4	49.5
Amy	5	41.4	36.6	42.5	44.7	54.1	56.8
Amy	6	45.5	41.1	47.2	36.0	36.2	60.5
Amy	7	53.8	56.0	52.9	52.4	52.2	60.4
Amy	8	36.0	32.5	31.9	34.9	39.4	45.4
Prot	1	37.0	40.5	39.8	15.2	35.7	52.4
Prot	2	40.4	45.5	40.1	44.5	43.1	54.4
Prot	3	43.6	43.6	45.2	48.7	49.5	53.6
Prot	4	58.5	55.8	56.0	53.9	57.9	57.2
Prot	5	33.4	39.1	39.1	43.9	50.1	51.8
Prot	6	45.3	40.8	46.4	44.1	46.4	59.4
Prot	7	47.1	43.4	48.4	40.4	44.2	54.6
Prot	8	51.5	51.4	58.5	54.8	52.5	65.7
Amy+Prot	1	62.1	54.4	56.9	54.0	55.6	53.4
Amy+Prot	2	42.2	27.8	35.4	32.9	42.6	46.3
Amy+Prot	3	33.2	40.4	37.6	23.1	39.7	58.5
Amy+Prot	4	40.6	31.9	38.2	39.8	43.8	55.5
Amy+Prot	5	60.0	42.4	60.1	55.4	59.4	73.4
Amy+Prot	6	34.0	33.6	33.0	40.2	42.5	52.5
Amy+Prot	7	42.9	22.0	28.8	17.4	40.9	52.6
Amy+Prot	8	32.3	28.0	30.8	37.5	45.8	54.6

Table 67: Ruminal NDF disappearance (%) of grass silage of the different animals dependent on treatment and incubation time

Treatment	Animal	Time of incubation (h)					
		1	3	6	9	12	24
Con	1	9.31	9.08	5.36	13.5	21.3	55.5
Con	2	4.47	7.83	9.24	21.5	18.8	40.8
Con	3	6.22	6.99	18.7	31.2	42.0	57.6
Con	4	6.16	7.87	10.1	14.7	17.8	54.2
Con	5	7.08	7.80	9.25	20.4	26.5	54.1
Con	6	3.30	7.93	12.8	16.0	27.7	58.3
Con	7	5.47	11.3	19.3	21.5	38.0	54.3
Con	8	7.26	5.67	11.8	11.7	20.0	50.3
Amy	1	6.26	9.81	10.2	11.4	20.8	23.6
Amy	2	7.77	7.00	5.30	8.84	13.5	60.1
Amy	3	3.15	4.65	14.9	20.2	33.0	61.8
Amy	4	4.58	5.11	8.59	9.25	10.8	50.3
Amy	5	3.77	8.56	14.2	19.2	23.0	59.5
Amy	6	5.75	5.21	9.53	17.4	26.7	53.5
Amy	7	3.11	2.41	11.6	19.3	20.0	54.3
Amy	8	2.95	4.09	7.09	15.1	25.9	52.3
Prot	1	4.41	6.27	6.74	9.67	14.1	47.5
Prot	2	5.39	7.81	9.05	8.91	17.6	40.0
Prot	3	7.39	6.45	12.1	22.6	26.8	49.2
Prot	4	3.65	3.77	9.27	13.3	30.7	54.5
Prot	5	6.04	5.77	11.3	22.8	33.4	52.5
Prot	6	6.16	6.19	10.6	21.0	22.2	52.1
Prot	7	4.85	7.22	15.1	20.6	21.4	54.4
Prot	8	2.66	5.42	11.5	17.2	13.3	48.3
Amy+Prot	1	4.97	5.53	8.68	15.5	22.8	45.2
Amy+Prot	2	4.98	3.84	7.57	17.6	14.6	41.2
Amy+Prot	3	6.19	7.68	15.2	25.8	36.0	59.5
Amy+Prot	4	6.05	6.33	6.97	11.9	15.7	51.6
Amy+Prot	5	5.38	5.78	14.3	20.5	33.5	50.4
Amy+Prot	6	3.13	6.23	6.98	20.1	22.6	57.7
Amy+Prot	7	6.79	8.29	8.86	20.0	29.1	48.2
Amy+Prot	8	5.97	11.8	10.7	15.1	25.3	46.8

Table 68: Parameters of degradability and effective degradability of starch of maize grain of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	21.7	78.3	3.51	0.00	71.6	54.0	45.6
Con	2	71.9	17.8	54.1	5.66	0.00	57.7	46.5	40.2
Con	3	100	19.3	80.7	3.99	0.00	73.1	55.1	46.2
Con	4	100	22.2	77.8	2.81	0.00	67.6	50.2	42.4
Con	5	100	23.0	77.0	3.19	0.00	70.3	53.0	44.9
Con	6	100	18.3	81.8	3.67	0.00	71.2	52.9	44.0
Con	7	72.9	20.7	52.2	5.41	0.00	58.8	47.8	41.7
Con	8	100	21.4	78.6	3.49	0.00	71.4	53.7	45.3
Amy	1	79.4	22.8	56.6	3.46	0.00	58.7	46.0	39.9
Amy	2	100	22.0	78.0	4.32	0.00	75.3	58.1	49.3
Amy	3	100	19.4	80.6	4.51	0.27	74.9	57.1	47.8
Amy	4	100	21.0	79.0	3.02	0.00	68.5	50.7	42.6
Amy	5	100	19.5	80.5	3.65	0.00	71.5	53.5	44.7
Amy	6	100	22.9	77.1	3.71	0.00	73.0	55.8	47.3
Amy	7	100	19.7	80.4	3.22	0.00	69.2	51.1	42.7
Amy	8	100	20.1	79.9	4.01	0.00	73.4	55.7	46.8
Prot	1	100	18.7	81.3	3.19	0.00	68.7	50.4	41.9
Prot	2	75.3	21.7	53.6	4.03	0.00	57.5	45.6	39.6
Prot	3	100	26.4	73.7	3.65	0.00	73.9	57.4	49.4
Prot	4	100	17.6	82.4	3.23	0.00	68.5	50.0	41.3
Prot	5	100	20.0	80.0	3.53	0.00	71.1	53.1	44.5
Prot	6	100	19.4	80.6	3.23	0.00	69.2	51.0	42.6
Prot	7	100	23.9	76.1	3.42	0.00	71.9	54.8	46.7
Prot	8	100	19.5	80.5	3.65	0.00	71.5	53.5	44.7
Amy+Prot	1	100	23.5	76.5	3.49	0.00	72.1	54.9	46.7
Amy+Prot	2	100	21.9	78.1	3.57	0.00	71.9	54.4	46.0
Amy+Prot	3	100	20.2	79.8	5.81	0.01	79.6	63.1	53.7
Amy+Prot	4	77.9	17.6	60.3	6.82	0.00	64.2	52.4	45.3
Amy+Prot	5	100	21.3	78.7	3.71	0.00	72.4	54.8	46.2
Amy+Prot	6	100	20.3	79.7	4.87	0.00	76.8	59.6	50.5
Amy+Prot	7	100	22.7	77.3	3.97	0.00	74.1	56.9	48.4
Amy+Prot	8	81.3	17.6	63.7	7.54	0.00	67.9	55.9	48.5

Table 69: Parameters of degradability and effective degradability of crude protein of maize grain of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	82.9	23.9	59.0	2.89	0.00	58.8	45.5	39.6
Con	2	53.1	23.6	29.5	8.82	0.00	47.6	42.4	39.0
Con	3	46.6	21.7	24.9	12.8	0.00	43.2	39.6	37.0
Con	4	63.3	26.0	37.3	4.50	0.00	51.8	43.7	39.4
Con	5	49.1	22.3	26.8	12.1	0.00	45.3	41.2	38.4
Con	6	100	24.4	75.6	2.08	0.00	62.9	46.6	40.0
Con	7	52.6	24.5	28.2	6.05	0.00	45.6	39.9	36.6
Con	8	47.9	23.6	24.3	9.93	0.00	43.8	39.7	37.0
Amy	1	40.6	21.3	19.3	20.0	0.00	38.8	36.7	35.1
Amy	2	71.3	18.5	52.8	5.09	0.00	56.4	45.1	39.0
Amy	3	49.9	22.6	27.3	9.19	0.00	45.0	40.3	37.2
Amy	4	48.0	23.5	24.5	6.24	0.00	42.1	37.1	34.3
Amy	5	36.0	17.1	18.9	.	0.00	32.3	28.9	26.7
Amy	6	100	22.8	77.2	1.86	0.00	60.0	43.7	37.4
Amy	7	50.6	21.9	28.8	7.91	0.00	44.8	39.5	36.2
Amy	8	36.2	16.8	19.4	.	0.00	32.4	28.9	26.7
Prot	1	39.6	17.6	22.0	.	0.00	37.3	34.6	32.5
Prot	2	41.9	19.4	22.5	.	0.00	38.9	35.6	33.3
Prot	3	54.0	23.0	31.0	5.89	0.00	46.2	39.8	36.2
Prot	4	49.2	21.9	27.3	10.2	0.00	44.7	40.2	37.2
Prot	5	47.6	21.4	26.2	14.3	0.00	44.4	40.8	38.2
Prot	6	43.6	20.2	23.4	27.9	0.00	42.0	40.0	38.3
Prot	7	57.7	22.4	35.3	6.97	0.00	49.9	43.0	38.9
Prot	8	47.3	22.2	25.1	12.3	0.00	43.8	40.1	37.4
Amy+Prot	1	44.4	23.9	20.5	21.5	0.00	42.6	40.5	38.8
Amy+Prot	2	38.0	20.6	17.4	.	0.00	34.4	31.1	29.1
Amy+Prot	3	47.1	24.2	22.9	13.0	0.00	44.1	40.7	38.4
Amy+Prot	4	67.8	25.8	42.0	4.35	0.00	54.6	45.4	40.6
Amy+Prot	5	75.5	25.6	49.9	3.07	0.00	55.8	44.6	39.4
Amy+Prot	6	54.3	27.4	26.9	4.28	0.00	45.7	39.8	36.8
Amy+Prot	7	52.7	26.8	25.9	6.06	0.00	46.3	41.0	38.0
Amy+Prot	8	100	26.1	73.9	1.89	0.00	62.0	46.4	40.2

Table 70: Parameters of degradability and effective degradability of crude protein of soybean meal of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	15.4	84.6	7.56	2.68	78.8	59.9	48.6
Con	2	100	11.7	88.3	4.94	0.00	74.6	55.6	45.4
Con	3	100	11.3	88.7	12.5	0.84	86.5	72.0	61.9
Con	4	100	14.7	85.3	7.76	2.24	79.6	61.1	49.8
Con	5	100	11.3	88.7	9.04	0.64	83.0	66.6	56.0
Con	6	100	16.6	83.4	9.52	5.21	78.7	58.7	46.5
Con	7	100	11.3	88.7	9.33	0.55	83.5	67.5	57.0
Con	8	100	15.6	84.4	8.6	2.19	81.1	63.4	52.3
Amy	1	100	14.1	85.9	4.68	0.00	74.3	55.6	45.8
Amy	2	100	23.1	77.0	.	.	83.2	67.5	57.5
Amy	3	100	13.4	86.6	11.6	0.69	86.2	71.8	61.8
Amy	4	100	15.7	84.3	4.88	2.68	72.4	52.1	41.5
Amy	5	100	13.4	86.6	12.1	0.41	87.1	73.4	63.8
Amy	6	100	19.0	81.0	7.90	3.94	78.7	59.8	48.4
Amy	7	100	16.7	83.3	9.32	1.90	82.7	66.0	55.2
Amy	8	100	16.4	83.6	9.38	2.81	81.5	63.8	52.4
Prot	1	100	17.8	82.2	5.13	0.00	76.9	59.4	49.9
Prot	2	100	17.5	82.5	6.04	0.20	79.2	62.2	52.4
Prot	3	100	17.5	82.5	10.9	0.16	87.0	73.6	64.5
Prot	4	100	17.5	82.5	7.41	0.54	81.8	65.5	55.5
Prot	5	100	17.5	82.5	12.1	0.55	87.5	74.3	65.0
Prot	6	100	19.8	80.2	7.50	0.00	83.1	67.9	58.6
Prot	7	100	17.5	82.5	11.5	0.68	86.8	73.0	63.5
Prot	8	100	17.8	82.2	7.36	0.00	82.4	66.8	57.2
Amy+Prot	1	100	16.2	83.8	6.44	0.00	80.1	63.4	53.6
Amy+Prot	2	100	14.6	85.5	6.22	0.21	78.9	61.4	51.3
Amy+Prot	3	100	14.6	85.5	14.1	0.58	88.5	75.8	66.6
Amy+Prot	4	100	24.2	75.8	12.4	7.49	80.4	61.3	49.5
Amy+Prot	5	100	14.6	85.5	11.8	0.53	86.9	73.1	63.4
Amy+Prot	6	100	14.6	85.5	9.39	0.08	84.9	70.1	60.4
Amy+Prot	7	100	14.6	85.5	8.51	0.28	83.3	67.6	57.6
Amy+Prot	8	100	14.6	85.5	8.23	0.52	82.6	66.3	56.1

Table 71: Parameters of degradability and effective degradability of starch of maize silage of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	23.7	76.3	4.79	0.84	76.6	59.5	50.4
Con	2	75.0	32.8	42.2	11.0	0.00	68.5	61.8	57.3
Con	3	71.4	23.9	47.5	.	0.00	62.7	54.3	48.9
Con	4	100	24.3	75.7	5.15	1.78	76.9	59.5	50.0
Con	5	86.3	33.8	52.6	8.58	0.00	76.4	67.0	61.0
Con	6	100	31.3	68.7	6.93	0.00	84.6	71.2	63.2
Con	7	77.5	23.9	53.6	20.2	0.00	72.6	66.8	62.3
Con	8	100	27.8	72.2	5.74	0.00	81.3	66.4	57.9
Amy	1	79.2	28.5	50.8	6.10	0.00	66.7	56.3	50.4
Amy	2	100	25.1	74.9	6.94	2.25	80.7	64.0	54.1
Amy	3	84.5	28.1	56.4	.	0.00	75.8	66.8	60.7
Amy	4	100	30.6	69.4	21.6	.	92.1	82.5	75.0
Amy	5	72.6	27.7	44.9	.	0.98	64.9	57.0	51.7
Amy	6	100	28.8	71.2	4.32	0.00	77.5	61.8	53.8
Amy	7	77.9	28.7	49.2	.	2.30	68.4	58.8	52.3
Amy	8	83.2	32.6	50.6	15.8	6.00	72.4	61.0	53.3
Prot	1	92.4	33.4	59.1	5.44	0.84	75.8	62.9	55.7
Prot	2	100	33.4	66.6	5.04	0.75	80.4	65.6	57.6
Prot	3	81.8	33.4	48.4	20.8	0.69	76.9	71.1	66.5
Prot	4	100	35.9	64.1	5.25	0.00	82.3	68.7	61.3
Prot	5	75.9	27.4	48.5	.	1.02	67.0	58.1	52.2
Prot	6	100	47.9	52.1	5.05	0.00	85.2	74.1	68.0
Prot	7	100	33.3	66.7	6.10	0.00	83.5	69.9	62.1
Prot	8	80.0	33.4	46.7	22.0	0.90	75.4	69.7	65.2
Amy+Prot	1	55.9	23.1	32.8	.	0.00	51.3	46.4	42.9
Amy+Prot	2	100	24.9	75.1	4.63	0.00	77.4	61.0	52.5
Amy+Prot	3	95.0	33.0	62.0	9.80	0.00	84.5	74.0	67.1
Amy+Prot	4	100	26.9	73.1	4.54	0.00	77.7	61.7	53.4
Amy+Prot	5	79.6	27.9	51.7	36.2	0.00	76.9	73.3	70.2
Amy+Prot	6	86.0	25.1	60.9	19.2	5.33	74.7	62.1	53.2
Amy+Prot	7	100	25.7	74.3	5.57	0.00	80.4	64.9	56.2
Amy+Prot	8	100	25.2	74.8	5.68	0.00	80.5	65.0	56.3

Table 72: Parameters of degradability and effective degradability of crude protein of maize silage of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	33.2	66.8	0.74	0.00	51.2	41.8	38.8
Con	2	61.1	37.9	23.2	4.87	0.00	54.4	49.4	46.7
Con	3	100	29.7	70.3	1.43	0.15	58.9	45.2	40.3
Con	4	100	29.7	70.3	1.23	0.66	56.1	43.2	38.6
Con	5	100	35.1	64.9	2.34	0.00	70.1	55.8	49.8
Con	6	73.4	36.5	36.9	4.12	0.00	61.3	53.2	49.0
Con	7	43.1	30.3	12.7	3.06	0.00	38.0	35.1	33.8
Con	8	100	33.0	67.0	2.80	10.7	64.6	47.1	40.4
Amy	1	45.4	32.4	13.0	.	0.00	42.8	40.4	38.9
Amy	2	49.1	37.4	11.6	13.6	0.00	47.6	45.9	44.7
Amy	3	57.1	32.3	24.8	.	0.00	52.2	47.6	44.8
Amy	4	100	30.9	69.1	2.66	12.2	61.8	43.9	37.4
Amy	5	64.8	34.5	30.3	5.89	0.00	57.1	50.9	47.3
Amy	6	100	36.8	63.2	1.30	0.00	61.7	49.8	45.6
Amy	7	54.8	32.3	22.5	.	0.59	50.1	45.8	43.1
Amy	8	46.3	33.2	13.1	17.0	8.19	43.1	39.9	37.8
Prot	1	100	34.4	65.6	2.51	11.2	63.6	46.9	40.8
Prot	2	61.1	38.9	22.1	3.60	0.00	53.2	48.2	45.8
Prot	3	56.6	40.7	15.9	6.93	0.00	53.0	49.9	48.1
Prot	4	56.6	39.4	17.1	.	0.00	51.8	48.2	46.2
Prot	5	55.3	36.4	18.9	8.67	2.17	51.1	47.2	44.7
Prot	6	100	40.2	59.8	1.36	0.00	64.4	53.0	48.9
Prot	7	63.3	43.2	20.1	4.59	0.00	57.2	52.8	50.5
Prot	8	65.9	45.1	20.7	8.71	0.00	62.0	58.3	55.9
Amy+Prot	1	56.1	36.1	20.0	.	0.00	51.7	47.8	45.5
Amy+Prot	2	52.0	36.2	15.9	10.1	13.9	46.2	41.4	39.1
Amy+Prot	3	100	35.0	65.0	3.84	12.3	68.4	50.3	42.9
Amy+Prot	4	73.3	36.7	36.6	4.22	6.91	58.3	48.6	44.0
Amy+Prot	5	100	45.0	55.0	2.93	0.00	77.7	65.3	59.7
Amy+Prot	6	68.6	34.5	34.1	4.34	6.00	55.2	46.2	41.9
Amy+Prot	7	100	31.4	68.6	3.51	13.5	64.8	45.8	38.5
Amy+Prot	8	55.4	31.8	23.6	20.9	7.67	50.2	44.8	41.0

Table 73: Parameters of degradability and effective degradability of NDF of grass silage of the different animals dependent on treatment

Treatment	Animal	d (%)	a (%)	b (%)	c (%/h)	t_0 (h)	ED2 (%)	ED5 (%)	ED8 (%)
Con	1	100	7.03	93.0	4.44	7.71	62.0	36.8	24.9
Con	2	100	4.48	95.5	2.10	2.05	51.4	30.0	21.3
Con	3	62.6	5.90	56.7	12.2	3.94	50.9	38.9	30.9
Con	4	.	16.5	.	1.80	.	53.9	34.2	26.9
Con	5	100	12.6	87.4	6.05	13.3	62.9	37.2	25.5
Con	6	100	5.24	94.8	4.07	4.91	62.8	38.5	26.8
Con	7	100	4.48	95.5	3.23	0.84	62.5	40.4	30.2
Con	8	100	5.80	94.2	3.19	5.56	57.6	33.6	23.0
Amy	1	30.9	3.82	27.1	5.96	0.00	24.1	18.6	15.4
Amy	2	100	6.34	93.7	6.44	10.8	64.0	37.1	24.0
Amy	3	100	3.54	96.5	4.45	3.80	65.2	41.1	29.0
Amy	4	100	6.07	93.9	4.88	10.9	59.6	32.9	20.9
Amy	5	100	3.29	96.7	3.46	2.70	61.4	37.9	26.8
Amy	6	100	11.2	88.8	14.2	19.4	64.0	36.1	23.2
Amy	7	100	2.78	97.2	3.49	4.08	59.7	35.4	24.1
Amy	8	.	15.8	.	0.59	.	32.4	21.9	19.0
Prot	1	.	4.63	.	.	3.56	59.2	38.7	28.1
Prot	2	60.1	6.36	53.8	6.22	8.22	40.9	26.1	18.5
Prot	3	100	5.30	94.7	2.92	2.81	58.4	35.6	25.5
Prot	4	94.9	3.54	91.4	4.33	4.99	60.1	36.6	25.1
Prot	5	62.3	5.00	57.3	9.24	4.79	47.8	34.3	25.9
Prot	6	100	5.18	94.8	3.33	4.41	59.4	35.6	24.8
Prot	7	100	4.03	96.0	3.08	2.61	59.3	36.1	25.7
Prot	8	100	8.89	91.1	.	.	67.9	44.8	32.9
Amy+Prot	1	100	4.10	95.9	2.85	4.39	55.7	32.1	21.8
Amy+Prot	2	100	3.54	96.5	2.39	4.28	51.7	28.7	19.3
Amy+Prot	3	100	1.80	98.2	3.65	0.84	64.2	41.5	30.6
Amy+Prot	4	100	5.29	94.7	4.03	7.95	59.3	33.7	22.1
Amy+Prot	5	64.2	4.32	59.8	7.35	3.79	47.9	33.8	25.5
Amy+Prot	6	100	3.72	96.3	4.18	5.30	62.3	37.4	25.3
Amy+Prot	7	100	1.80	98.2	2.60	0.52	56.7	34.5	24.9
Amy+Prot	8	100	1.65	98.4	2.31	0.00	54.4	32.7	23.7

Table 74: Total bacteria (Log_{10} 16S rRNA copy number / g rumen fluid DM) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)		
		0	1	3
Con	1	10.7	11.1	10.4
Con	2	10.6	11.3	10.4
Con	3	10.3	11.2	9.89
Con	4	10.5	11.4	10.5
Con	5	10.8	11.4	10.6
Con	6	10.4	10.8	10.0
Con	7	10.7	11.5	10.4
Con	8	10.5	11.0	10.4
Amy	1	10.8	11.2	10.2
Amy	2	10.9	11.1	10.1
Amy	3	10.6	11.2	9.87
Amy	4	10.7	11.2	10.3
Amy	5	10.8	11.7	10.8
Amy	6	10.8	11.7	9.89
Amy	7	10.8	11.7	10.6
Amy	8	.	10.5	9.23
Prot	1	10.9	11.0	9.74
Prot	2	10.8	10.5	10.6
Prot	3	10.6	11.5	10.6
Prot	4	10.2	11.5	9.78
Prot	5	10.9	11.4	10.7
Prot	6	10.5	11.1	10.4
Prot	7	11.1	11.6	10.7
Prot	8	10.3	10.6	9.50
Amy+Prot	1	10.5	11.3	9.77
Amy+Prot	2	10.4	11.2	9.69
Amy+Prot	3	10.4	10.4	10.7
Amy+Prot	4	10.6	9.89	9.02
Amy+Prot	5	10.6	10.9	10.6
Amy+Prot	6	10.6	10.7	10.2
Amy+Prot	7	10.8	11.2	10.8
Amy+Prot	8	10.7	11.4	10.2

Table 75: Archaebacteria (Log_{10} 16S rRNA copy number / g rumen fluid DM) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)		
		0	1	3
Con	1	8.27	7.69	7.28
Con	2	7.80	7.74	7.02
Con	3	7.30	7.27	6.33
Con	4	7.80	7.87	7.84
Con	5	8.24	8.15	7.80
Con	6	7.51	.	6.80
Con	7	8.41	8.34	7.55
Con	8	7.31	6.86	7.16
Amy	1	8.44	7.82	7.18
Amy	2	8.29	7.58	6.67
Amy	3	8.27	7.55	6.27
Amy	4	8.10	7.46	7.02
Amy	5	8.46	8.42	8.12
Amy	6	8.12	8.29	6.53
Amy	7	8.51	8.47	7.70
Amy	8	.	.	.
Prot	1	8.82	7.30	6.17
Prot	2	8.28	6.82	7.69
Prot	3	8.30	8.65	8.14
Prot	4	7.01	8.19	6.26
Prot	5	8.91	8.02	7.94
Prot	6	7.82	7.33	7.43
Prot	7	8.79	8.37	7.88
Prot	8	7.09	.	.
Amy+Prot	1	7.63	7.65	.
Amy+Prot	2	7.90	7.73	6.31
Amy+Prot	3	7.71	.	7.98
Amy+Prot	4	7.76	.	.
Amy+Prot	5	8.01	6.90	7.38
Amy+Prot	6	8.10	6.84	7.25
Amy+Prot	7	8.38	8.26	8.07
Amy+Prot	8	7.88	7.58	6.59

Table 76: Anaerobic fungi (Log_{10} 18S rRNA copy number / g rumen fluid DM) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)		
		0	1	3
Con	1	7.58	7.73	8.18
Con	2	8.20	8.47	8.39
Con	3	8.05	8.57	8.55
Con	4	7.30	8.27	8.35
Con	5	7.87	8.22	8.91
Con	6	7.71	8.02	8.10
Con	7	7.67	8.38	8.63
Con	8	7.73	8.34	8.61
Amy	1	7.62	8.40	8.30
Amy	2	7.17	8.11	8.24
Amy	3	8.24	8.78	8.64
Amy	4	7.63	8.42	8.30
Amy	5	8.37	8.56	9.01
Amy	6	7.55	8.19	7.82
Amy	7	7.83	8.97	8.50
Amy	8	7.51	8.23	7.77
Prot	1	7.49	8.00	7.94
Prot	2	7.87	8.44	8.46
Prot	3	7.69	8.47	8.74
Prot	4	7.86	8.81	8.22
Prot	5	8.10	8.55	8.83
Prot	6	7.66	8.35	8.32
Prot	7	7.83	8.23	8.39
Prot	8	7.61	8.28	8.16
Amy+Prot	1	7.91	8.14	8.05
Amy+Prot	2	7.78	8.32	8.41
Amy+Prot	3	8.18	8.36	8.69
Amy+Prot	4	7.24	7.85	.
Amy+Prot	5	8.46	8.62	8.65
Amy+Prot	6	7.42	7.75	8.41
Amy+Prot	7	7.83	8.29	8.66
Amy+Prot	8	7.73	8.15	8.51

Table 77: Protozoa (Log_{10} 18S rRNA copy number / g rumen fluid DM) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)		
		0	1	3
Con	1	9.21	8.98	9.46
Con	2	9.24	9.41	9.45
Con	3	9.25	9.41	9.56
Con	4	9.04	9.13	9.80
Con	5	9.12	9.09	9.92
Con	6	9.17	9.06	9.15
Con	7	9.25	9.50	9.74
Con	8	9.04	9.38	9.57
Amy	1	9.22	9.32	9.56
Amy	2	8.80	8.54	8.84
Amy	3	9.68	9.58	9.27
Amy	4	8.85	8.71	9.29
Amy	5	9.62	9.63	9.95
Amy	6	9.04	9.04	8.54
Amy	7	9.60	9.84	9.78
Amy	8	8.16	8.55	8.57
Prot	1	9.18	9.27	9.47
Prot	2	9.52	8.95	9.90
Prot	3	9.26	9.52	10.1
Prot	4	9.04	9.72	8.81
Prot	5	9.62	9.32	9.89
Prot	6	9.03	9.06	9.60
Prot	7	9.41	9.60	9.73
Prot	8	9.02	9.02	8.77
Amy+Prot	1	9.22	9.45	9.00
Amy+Prot	2	9.07	8.85	9.05
Amy+Prot	3	9.56	8.77	10.0
Amy+Prot	4	8.45	7.59	.
Amy+Prot	5	9.38	9.24	9.65
Amy+Prot	6	9.06	8.81	9.25
Amy+Prot	7	9.27	9.03	10.0
Amy+Prot	8	9.08	9.60	9.45

Table 78: *Prevotella spp*. (Log_{10} 16S rRNA copy number / g rumen fluid DM) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)		
		0	1	3
Con	1	10.5	10.8	10.4
Con	2	10.3	10.7	10.4
Con	3	10.1	10.9	10.0
Con	4	10.3	10.9	10.3
Con	5	10.5	10.9	10.4
Con	6	10.3	10.7	10.1
Con	7	10.3	11.0	10.2
Con	8	10.3	10.6	10.2
Amy	1	10.6	10.7	10.2
Amy	2	10.7	10.7	10.1
Amy	3	10.1	10.7	9.74
Amy	4	10.5	10.9	10.3
Amy	5	10.3	11.1	10.4
Amy	6	10.6	11.1	9.92
Amy	7	10.4	11.1	10.3
Amy	8	9.78	10.3	9.28
Prot	1	10.4	10.6	9.93
Prot	2	10.3	10.3	10.2
Prot	3	10.0	10.9	10.2
Prot	4	10.1	10.8	9.89
Prot	5	10.5	10.9	10.4
Prot	6	10.3	10.9	10.3
Prot	7	10.7	11.1	10.5
Prot	8	10.3	10.4	9.75
Amy+Prot	1	10.4	10.9	10.0
Amy+Prot	2	10.2	10.8	9.81
Amy+Prot	3	10.1	10.3	10.4
Amy+Prot	4	10.5	9.71	9.15
Amy+Prot	5	10.3	10.6	10.4
Amy+Prot	6	10.4	10.6	10.2
Amy+Prot	7	10.4	10.7	10.5
Amy+Prot	8	10.5	11.0	10.2

Table 79: *Fibrobacter succinogenes* (Log_{10} 16S rRNA copy number / g rumen fluid DM) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)		
		0	1	3
Con	1	9.13	8.52	8.81
Con	2	9.24	8.77	9.11
Con	3	8.96	8.64	8.57
Con	4	9.20	8.77	9.04
Con	5	9.25	8.87	9.21
Con	6	8.97	8.49	8.75
Con	7	9.18	8.90	8.96
Con	8	9.32	8.62	8.70
Amy	1	9.44	8.77	8.66
Amy	2	9.33	8.65	8.65
Amy	3	9.07	8.71	8.63
Amy	4	9.29	8.74	8.87
Amy	5	9.32	8.94	9.09
Amy	6	9.39	9.13	8.72
Amy	7	9.26	9.20	8.86
Amy	8	8.95	8.36	8.17
Prot	1	9.22	8.57	8.40
Prot	2	9.25	8.35	8.95
Prot	3	8.96	8.77	8.83
Prot	4	9.15	8.89	8.56
Prot	5	9.33	8.86	9.10
Prot	6	9.17	8.80	8.85
Prot	7	9.51	8.82	8.98
Prot	8	9.16	8.48	8.36
Amy+Prot	1	9.32	8.85	8.59
Amy+Prot	2	9.05	8.81	8.48
Amy+Prot	3	9.08	8.30	9.10
Amy+Prot	4	9.17	8.04	7.94
Amy+Prot	5	9.22	8.74	8.75
Amy+Prot	6	8.88	8.33	8.63
Amy+Prot	7	9.25	8.68	8.90
Amy+Prot	8	9.32	8.84	8.89

Table 80: *Ruminococcus flavefaciens* (Log_{10} 16S rRNA copy number / g rumen fluid DM) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)		
		0	1	3
Con	1	7.66	7.04	7.50
Con	2	7.77	7.59	7.64
Con	3	7.65	7.54	7.72
Con	4	7.37	7.63	8.31
Con	5	7.72	7.61	7.91
Con	6	7.53	7.53	7.41
Con	7	7.64	7.56	7.57
Con	8	7.74	7.46	7.88
Amy	1	7.72	7.62	7.57
Amy	2	7.44	7.34	7.47
Amy	3	7.38	7.44	7.54
Amy	4	7.69	7.76	7.69
Amy	5	7.75	7.75	8.19
Amy	6	7.84	8.21	7.63
Amy	7	7.46	7.76	7.63
Amy	8	7.45	7.01	6.85
Prot	1	7.75	7.42	7.02
Prot	2	8.05	7.26	8.10
Prot	3	7.91	7.97	8.0
Prot	4	7.42	7.89	7.27
Prot	5	7.94	7.74	8.27
Prot	6	7.78	7.58	7.71
Prot	7	8.05	7.78	7.98
Prot	8	7.16	7.04	7.05
Amy+Prot	1	7.66	7.54	7.40
Amy+Prot	2	7.55	7.52	7.30
Amy+Prot	3	7.79	7.35	8.2
Amy+Prot	4	7.40	6.75	.
Amy+Prot	5	7.71	7.36	7.75
Amy+Prot	6	7.96	7.07	7.65
Amy+Prot	7	7.83	7.49	8.1
Amy+Prot	8	7.84	7.65	7.65

Table 81: *Streptococcus bovis* (Log_{10} 16S rRNA copy number / g rumen fluid DM) in the rumen fluid of the different animals dependent on treatment and sampling time

Treatment	Animal	Time after feeding (h)		
		0	1	3
Con	1	7.47	7.24	7.36
Con	2	7.50	7.34	7.27
Con	3	7.27	7.16	7.20
Con	4	7.37	7.53	7.39
Con	5	7.73	7.68	7.62
Con	6	7.30	7.06	7.19
Con	7	7.65	7.64	7.37
Con	8	7.42	7.97	7.44
Amy	1	7.66	7.21	7.09
Amy	2	7.50	7.50	7.32
Amy	3	7.51	7.38	7.26
Amy	4	7.41	7.47	7.29
Amy	5	7.67	7.61	7.65
Amy	6	7.56	7.86	7.18
Amy	7	7.64	7.69	7.51
Amy	8	6.95	.	6.69
Prot	1	7.94	7.20	6.93
Prot	2	7.66	7.08	7.50
Prot	3	7.74	7.76	7.75
Prot	4	7.26	7.76	7.09
Prot	5	7.79	7.57	7.61
Prot	6	7.34	7.23	7.16
Prot	7	8.00	7.64	7.60
Prot	8	7.25	.	6.79
Amy+Prot	1	7.69	7.39	6.98
Amy+Prot	2	7.08	7.36	6.91
Amy+Prot	3	7.50	6.76	7.55
Amy+Prot	4	7.32	6.88	6.53
Amy+Prot	5	7.60	7.39	7.43
Amy+Prot	6	7.58	7.10	7.30
Amy+Prot	7	7.72	7.62	7.80
Amy+Prot	8	7.46	7.46	7.42

Table 82: Apparent total tract nutrient digestibility (%) of the different animals dependent on treatment

Treatment	Animal	Total tract digestibility (%)									
		CA	TL	CP	CF	NDF	ADF	OM	NFE	DM	Starch
Con	1	41.3	65.1	61.5	72.2	62.9	62.9	76.9	81.7	75.1	97.3
Con	2	51.9	52.8	62.2	76.6	71.7	69.9	79.3	84.6	77.9	97.6
Con	3	39.2	54.4	67.9	80.2	72.8	71.8	81.7	86.1	79.7	98.0
Con	4	51.4	80.8	70.3	78.9	72.4	72.9	82.7	86.1	81.0	97.1
Con	5	38.4	54.5	56.9	74.3	65.8	64.1	76.8	82.2	74.9	96.9
Con	6	48.3	62.6	67.2	76.9	72.8	69.8	81.1	86.0	79.4	98.3
Con	7	38.5	55.5	60.0	74.6	65.2	66.1	77.6	83.0	75.8	97.2
Con	8	49.6	70.7	72.2	78.6	75.0	73.6	83.2	87.3	81.5	97.8
Amy	1	45.8	74.2	59.4	71.5	63.2	64.1	76.0	80.4	74.4	96.8
Amy	2	28.0	49.2	56.6	71.9	61.6	62.4	75.5	81.2	73.1	97.4
Amy	3	45.7	67.8	68.8	78.3	72.6	70.8	81.5	85.5	79.6	98.4
Amy	4	38.1	68.4	62.0	73.1	64.0	63.0	77.3	81.9	75.5	97.3
Amy	5	42.7	65.1	58.3	78.3	71.9	70.6	78.8	83.3	76.9	96.7
Amy	6	36.3	59.0	64.0	73.1	64.0	65.4	78.5	83.9	76.5	97.3
Amy	7	46.9	75.5	66.0	77.8	72.1	68.5	80.9	84.8	79.1	98.3
Amy	8	37.2	67.4	64.8	77.2	68.7	69.0	80.5	85.1	78.5	98.0
Prot	1	40.9	63.2	60.5	71.4	61.5	62.2	75.4	80.1	73.8	96.9
Prot	2	50.6	74.1	63.2	78.7	70.5	71.5	80.4	84.4	78.9	97.0
Prot	3	24.7	62.0	53.6	71.3	61.2	61.5	75.6	81.5	73.1	97.5
Prot	4	47.3	64.9	66.2	76.6	71.3	68.6	80.2	84.7	78.5	98.3
Prot	5	44.1	63.2	59.8	76.7	68.0	68.0	78.9	83.9	77.3	97.4
Prot	6	51.5	73.3	71.7	80.9	75.6	74.3	83.5	87.0	81.8	97.4
Prot	7	39.4	42.8	61.9	71.9	64.3	63.0	76.6	82.5	74.8	96.3
Prot	8	46.3	70.7	70.5	77.4	71.9	67.6	81.8	85.9	80.0	98.6
Amy+Prot	1	46.8	47.0	63.3	72.4	66.1	64.4	76.4	81.6	74.9	97.6
Amy+Prot	2	47.2	48.0	58.0	76.5	68.6	68.5	78.0	83.6	76.5	97.1
Amy+Prot	3	49.1	83.7	69.1	77.6	71.3	71.1	81.2	84.3	79.6	97.4
Amy+Prot	4	36.4	48.8	62.5	74.5	67.2	66.1	78.1	83.5	76.1	97.4
Amy+Prot	5	50.8	64.8	61.8	72.9	67.8	63.6	77.9	83.1	76.5	98.0
Amy+Prot	6	37.1	74.4	64.4	74.2	66.8	66.7	78.9	83.4	76.9	97.5
Amy+Prot	7	50.5	71.4	67.6	74.9	69.4	69.4	80.5	85.0	78.9	96.9
Amy+Prot	8	42.4	60.9	67.5	75.8	68.6	67.3	80.6	85.5	78.7	97.7