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Experimental study on the effect of dose and source of copper supplementation on copper metabolism, rumen microbiota, and rumen fermentation characteristics in cannulated cows

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meiner Familie

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List of Abbreviations

A	Absorbance
a	Soluble fraction
a.m.	Ante meridiem
ADF	Acid-detergent fibre
ANOVA	Analysis of variance
b	Insoluble, but ruminally degradable fraction
bp	Base pair
c	Constant rate of degradation of fraction b
Ca^{2+}	Divalent calcium ion
CA	Crude ash
CF	Crude fibre
cm	Centimetre
Co	Cobalt
$CoSO_4$	Cobalt sulphate
CP	Crude protein
Cu	Copper
Cu^{+}	Monovalent copper ion
Cu^{2+}	Divalent copper ion
$CuCl_2$	Copper chloride
$Cu(OH)_2$	Copper(II) hydroxide
$Cu_2(OH)_3Cl$	Dicopper chloride trihydroxide (Tribasic copper chloride)
CuS	Copper sulphide
$CuSO_4$	Copper sulphate
$CuSO_4 \cdot 5H_2O$	Copper sulphate Pentahydrate
DM	Dry matter
DMD	Dry matter disappearance
DNA	Deoxyribonucleic acid
EC	European Commission
ED	Effective degradability

EDTA	Ethylenediaminetetraacetic acid
et al.	Et alii / et aliae / et alia
Fe	Iron
$FeSO_4$	Iron sulphate
FM	Fresh matter
g	Gramme
GLM	General linear model
h	Hour
H_2	Hydrogen
HNO_3	Nitric acid
H_2O	Water
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulphide
H_2SO_4	Sulphuric acid
H_3PO_4	Phosphoric acid
I	Iodine
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
k	Passage rate
K_2SO_4	Potassium sulphate
kg	Kilogram
l	Litre
log_{10}	Decadic logarithm
m	Metre
mg	Milligramme
Mg^{2+}	Divalent magnesium ion
min	Minute
ml	Millilitre
mm	Millimetre
mmol	Millimole
Mn	Manganese

Mo	Molybdenum
Mol	Mole
mU	Milli units
µg	Microgramme
µl	Microlitre
µm	Micrometre
µmol	Micromole
n	Number
NADPH/$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NaOH	Sodium hydroxide
$Na_2S \cdot 9H_2O$	Sodium sulphide nonahydrate
NDF	Neutral-detergent fibre
NFE	Nitrogen-free extracts
ng	Nanogramme
NLIN	Nonlinear or linear model
NH_3-N	Ammonia-nitrogen
nm	Nanometre
OD	Optical density
OM	Organic matter
p	Disappearance at time t
PCR	Polymerase chain reaction
p.m.	post meridiem
pmol	Picomole
qPCR	Quantitative real-time polymerase chain reaction
rpm	revolutions per minute
rRNA	Ribosomal ribonucleic acid
s	Seconds
S	Sulphur
SD	Standard deviation
Se	Selenium
SEM	Standard error of means

SOD	Superoxide dismutase
t_0	Lag time
TAE	TRIS-Acetate-EDTA
TBCC	Tribasic copper chloride
TDF	Totally degradable fraction
TES	N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid
TiO_2	Titanium dioxide
TL	Total lipids
TMR	Total mixed ration
TRIS	Tris(hydroxymethyl)aminomethane
UV	Ultraviolet
VDLUFA	Verband deutscher landwirtschaftlicher Untersuchungs- und Forschungsanstalten
VFA	Volatile fatty acids
x g	x-fold gravitational acceleration
Zn	Zinc
Zn^{2+}	Divalent zinc ion
°C	Degree Celsius
%	Percent
Σ	Sum
Ø	Diameter
~	About
=	Equal sign

1 Abstract

The objective of this study was to investigate the effect of copper from different sources, supplemented at varying doses, on copper metabolism, rumen microbiota, ruminal degradability, rumen physiological parameters, and on total tract digestibility in cattle. Six rumen cannulated, non-lactating Holstein cows were grouped according to a 6x6 Latin square design and were individually fed 6.5 kg dry matter of total mixed ration (grass silage, maize silage, wheat meal, and soybean meal (solvent-extracted)) in two equal portions per day. Throughout six experimental periods of 21 days each, the cows received six treatment combinations. Copper in form of copper sulphate ($CuSO_4 \cdot 5H_2O$) or tribasic copper chloride (TBCC; $Cu_2(OH)_3Cl$) was supplemented in order to obtain total dietary Cu concentrations of 10, 35, and 50 mg/kg dry matter, respectively. Samples of rumen and duodenal contents were taken in intervals of 1.5 h, starting at 8:00 a.m. and ending at 5:00 p.m. Samples were fractionated in different solid fractions and the liquid fraction prior to copper analysis. Additional samples of rumen fluid were taken to examine rumen microorganisms (total bacteria, *Ruminococcus flavefaciens*, *Firbrobacter succinogenes*, *Streptococcus bovis*, archaea, protozoa, and anaerobic fungi), pH-value, ammonia-nitrogen, and volatile fatty acids. Quantification of rumen microorganisms was performed by quantitative real-time polymerase chain reaction (qPCR). Ruminal degradability was determined using the *in sacco*-method. Feedstuffs were incubated in the rumen of cannulated cows for 1.5, 3, 6, 9, 12, 24 and 48 h, followed by calculations of dry matter degradability, parameters of degradation (soluble fraction, ruminally degradable fraction, rate of degradation, and lag-time), and effective degradability for passage rates of 2, 5, and 8 %/h. Samples of faeces were collected throughout the last seven days of each period. Total tract digestibility was determined using titanium dioxide as an indigestible marker.

While both copper sources were solubilised in the rumen, copper sulphate showed a higher ruminal solubility than tribasic copper chloride. Although the concentration of soluble copper in duodenal digesta was similar for both copper sources, the amount of apparent total tract digested copper was 35.3 % greater at supplementation of 50 mg Cu/kg DM from copper sulphate than from tribasic copper chloride.

With increasing dose of copper sulphate, the occurrence of *Streptococcus bovis* at 1.5 h and the ratio of *Fibrobacter succinogenes* to total bacteria at 1.5 h and 3 h after feeding decreased, whereas tribasic copper chloride showed no effects. The remaining microorganisms were not affected by copper supplementation.

Dry matter degradability was improved by increasing doses of copper sulphate in a range from 1.2 % to 7.8 % for total mixed ration between 3 h and 12 h, for grass silage, maize silage and wheat meal between 6 h and 12 h, and for soybean meal after 6 h and 9 h of incubation. Supplementation of tribasic copper chloride revealed only isolated statistically significant effects on dry matter degradability with no recognisable pattern. Rumen pH was not affected by copper supplementation, neither by copper dose nor by copper source. The maximum ammonia-nitrogen concentration in rumen fluid was delayed from 1.5 h to 3 h after feeding when copper sulphate was supplemented. Volatile fatty acids showed increased values 9 h after feeding at supplementation of 50 mg Cu/kg DM from tribasic copper chloride. Apparent total tract nutrient digestibility was completely unaffected by copper treatments.

In summary, copper sulphate appeared to be more bioavailable than tribasic copper chloride. Copper was not noticeably bound to thiomolybdate due to moderate dietary concentrations of molybdenum and sulphur. Additionally, copper from tribasic copper chloride was presumably not completely solubilised while passing through the abomasum, leading to a loss of absorbable copper.

Copper supplementation induced only a few minor and highly selective negative changes in the rumen microbiota of cattle. Therefore, a sustainable impairment of microbial populations in the rumen can be ruled out.

The stimulation of rumen degradability by increased supplementation of copper sulphate suggests that certain amounts of soluble copper in the rumen may have beneficial effects on the microbial degradation of ingested feed material, independent of microbial growth. Copper supplementation, however, did not improve the total tract digestibility of ingested feedstuffs due to compensation by a slow passage rate.

Copper status parameters in the blood serum were examined in order to receive a general overview of copper status and to affirm results of apparent Cu digestibility. Unfortunately, these parameters are subject to more influencing factors and often described as not reliable. Liver copper concentration, however, directly reflects copper absorption. Therefore, the combined examination of apparent copper digestibility and liver copper concentration is recommended.

The absolute quantification of rumen microorganisms was conducted by qPCR and enabled a direct measurement of changes in microbial concentrations. Therefore, the current study provides for the first time reliable data of absolute quantification of different microorganisms combined with data from dry matter degradation.

2 Introduction

Copper (Cu) is an essential trace element and must be provided to all organisms in a sufficient amount. Cu is on the one hand an essential part of several enzymes which are involved in Cu storage, catalysis of chemical reactions, and the respiratory chain (Linder, 2002). Furthermore, copper plays an important role for cell metabolism, development, and integrity (McDowell, 1992; Suttle, 2010). On the other hand, an oversupply of Cu can cause toxic effects, leading to oxidative stress and damage of DNA and cell structures (Rifkind *et al.*, 1976; Ueda *et al.*, 1980; Halliwell and Gutteridge, 1984). Consequently, the adequate supply of Cu in term of sufficient but not excessive doses is crucial to ensure animal health.

Especially in ruminants, copper deficiency is a major problem around the world (Underwood and Suttle, 1999). Ruminant diets naturally show low copper concentrations between 4 and 10 mg/kg DM (Gooneratne *et al.*, 1989) and the interaction of copper with molybdenum (Mo) and sulphur (S) in the anaerobic rumen environment leads to strongly decreased copper bioavailability through formation of insoluble and poorly absorbable Cu-thiomolybdate complexes (Suttle, 1991; Spears, 2003). These interactions were observed at concentrations of Mo and S which occur naturally in feedstuffs (Mo: 0 - 5 mg/kg DM; S: 1 - 3 g/kg DM) (Gooneratne *et al.*, 1989; Suttle, 1991). Additionally, other trace elements such as iron and zinc, which can be abundant in ruminant diets, are also able to reduce Cu status in cattle (Bremner *et al.*, 1987; WHO, 1998), but interaction of Cu with Mo and S shows the greatest capacity to interfere with Cu metabolism (Suttle, 1991). The absolute dietary Cu requirement in cattle is less than 1.6 mg/kg DM (Gould and Kendall, 2011) but the above listed circumstances result in a Cu absorption less than 10 % (Underwood and Suttle, 1999; Dias *et al.*, 2013). Therefore, the recommended Cu supply was specified at 10 mg/kg DM (GfE, 2001) to avoid Cu deficiency. Clinical signs of a Cu deficiency are reduced weight gain, decreased food intake, reduced efficiency of food conversion, alteration in hair texture and pigmentation, delayed puberty, reduced conception rate, inhibition of oestrus, and swayback (Gould and Kendall, 2011). The current maximum according to feed law in the European Union was set at 35 mg Cu/kg feedstuff (related to 88 % of DM) (EU-Commission, 2003) to avoid possible Cu intoxication.

Apart from its importance as an essential trace element, Cu shows dose-dependent toxic effects on microorganisms. These effects have been utilised in pig fattening for years. Several studies with weaned piglets have demonstrated that supplementation of high amounts of copper promotes growth due to the reduction of the intestinal microflora

(Armstrong *et al.*, 2004; Pérez *et al.*, 2011; Shelton *et al.*, 2011). In ruminants, however, impairment of rumen microorganisms results in deterioration of rumen fermentation along with reduced nutrient supply to the host. In this context, older studies reported inhibited fermentation rates or cellulose digestion due to copper induced impairment of rumen microorganisms (Hubbert *et al.*, 1958; Martinez and Church, 1970; Forsberg, 1978) or showed reduced protozoa counts after Cu supplementation (Essig *et al.*, 1972; Solaiman *et al.*, 2007). Furthermore, even macrophages were reported to use Cu ions to promote killing of undesired bacteria (White *et al.*, 2009; Achard *et al.*, 2012). The toxic potential of Cu is undisputed but the specific mode of action on rumen microorganisms remains to be fully understood. Nevertheless, there are different mechanisms of interference in cell structures and functions described in literature which could also have an effect on rumen microorganisms. Chemical reaction properties of Cu, for example, promote the formation of free radicals which lead to peroxidation of lipid membranes (Chan *et al.*, 1982). Furthermore, Cu is able to alter the structure of proteins and to inhibit their biological function, such as regulation of cell growth, differentiation, and proliferation (Kim *et al.*, 2000).

Additional studies in recent years presented an alternative model of Cu toxicity (Macomber and Imlay, 2009; Chillappagari *et al.*, 2010; Azzouzi *et al.*, 2013). Thereby, Cu^{+} is occupying Fe sites of dehydratases, resulting in impaired key metabolic processes, for example glucose catabolism (Macomber and Imlay, 2009). Another study of Djoko and McEwan (2013) demonstrated that a Cu overload in bacteria increases their sensitivity to hydrogen peroxide. Regarding the impact of Cu on fungi, there is only very limited information available. Borkow and Gabbay (2005) reported electrostatic bonds between Cu ions and negatively charged components of fungi cell walls. The result is a distortion of cell walls along with an increased permeability and a diminished intake of essential nutrients.

In total, it is important to know that the ability of Cu to interact with either complex-building agents or microorganisms in the rumen strongly depends on the respective chemical form and its solubility in rumen fluid (Suttle, 1991; Genther and Hansen, 2015). This was confirmed by Spears *et al.* (2004) who reported higher bioavailability of Cu from ruminally insoluble sources than of ruminally soluble sources, provided that diets are high in Mo and S. However, in a diet low in Mo and S the bioavailability of different Cu sources was comparable (Spears *et al.*, 2004). In most previous studies Mo and S were added to the basal diet to challenge complexation of Cu, demonstrated in an review of Dias *et al.* (2013). In addition, information about the antimicrobial effect of different Cu sources is

scarce, but soluble Cu sources are assumed to have higher toxic potential (Genther and Hansen, 2015).

For these reasons, the current study was conducted to cover both issues, the Cu bioavailability and the antimicrobial effect of Cu in the rumen, in combination. The approach was to reflect moderate and physiologically adequate feeding conditions rather than to simulate an extreme. Therefore, six cannulated Holstein cows, neither Cu - depleted nor compromised with Cu toxicity, were fed a diet containing moderate basal Mo and S concentrations. Cows were supplemented with Cu to receive following total dietary Cu concentrations, which can be found in common feed rations: 10 mg/kg DM (in line with recommendation), 35 mg/kg DM (close to permitted maximum according to feed law), and 50 mg/kg DM (mild excess). Cu from Cu sulphate ($CuSO_4$) and from tribasic Cu chloride (TBCC) was used for supplementation. $CuSO_4$ was assumed to be completely soluble and TBCC to be almost insoluble in rumen fluid (Spears *et al.*, 2004).

The aim of this experiment was to answer following questions:

1. What is the mode of action of different Cu sources in the rumen environment regarding subsequent absorbability in the intestine?
2. Are rumen microorganisms affected by different Cu sources supplemented in various doses?
3. Does Cu supplementation cause changes in microbial fermentation of ingested feed?

3 Material and Methods

3.1 Experimental design

This study was carried out to investigate the effect of different doses and sources of Cu supplementation on rumen fermentation characteristics and rumen microbiota. For this purpose, Cu was supplemented either as Cu sulphate ($CuSO_4$) or as tribasic Cu chloride (TBCC). The mean native Cu content in the dry matter (DM) of the diet was determined and the respective amounts of Cu were added in order to obtain three levels of total Cu concentrations (= dose). Table 1 outlines the six treatments (2 × 3) of combined Cu doses (n = 2) and sources (n = 3) as well as the nutritionally relevance of the different Cu doses.

Table 1: Cu treatment and nutritional relevance of different doses

Treatment		
Cu source	**Cu dose[1]**	**Nutritionally relevance of dose**
$CuSO_4$	10 mg/kg DM	in line with recommendation[2]
	35 mg/kg DM	close to permitted maximum according to feed law[3]
	50 mg/kg DM	mild excess
TBCC	10 mg/kg DM	in line with recommendation[2]
	35 mg/kg DM	close to permitted maximum according to feed law[3]
	50 mg/kg DM	mild excess

[1] Total Cu concentration (native + supplemented), related to 100 % DM content.
[2] GfE (2001), related to 100 % DM content.
[3] EU-Commission (2003): 39.8 mg/kg DM (35 mg/kg related to 88 % DM content).

Six cows received each of the six treatments throughout six consecutive experimental periods, resulting in a 6 × 6 Latin square (Table 2). A period consisted of 13 days adaptation to treatment and eight days sampling and measurements. In total, the experiment lasted 126 days (18 weeks).

Table 2: Experimental design (numbers 1 - 6 represent animals)

Cu source	Cu dose [mg/kg DM]	Experimental periods					
		1	**2**	**3**	**4**	**5**	**6**
$CuSO_4$	10	1	2	3	4	5	6
	35	6	1	2	3	4	5
	50	5	6	1	2	3	4
TBCC	10	4	5	6	1	2	3
	35	3	4	5	6	1	2
	50	2	3	4	5	6	1

3.2 Copper specification

Both copper compounds, Copper Sulphate Pentahydrate ($CuSO_4 \cdot 5H_2O$) and IntelliBond® C (tribasic copper chloride; $Cu_2(OH)_3Cl$), were provided in form of granules by Orffa Additives B.V. (Werkendam, Netherlands). Copper sulphate granules with a particle size less than 630 µm contained 25 % of Cu. Granules of tribasic copper chloride consisted of 54 % Cu and had a mean particle size of 250 µm. The flowability of each compound was declared as “freeflowing”.

3.3 Animals and diet

The feeding trial was conducted at the experimental plant of the Department of Animal Science of the Technical University of Munich. Six non-lactating Holstein cows with a mean body weight of 628 kg (SD ± 13 kg) were housed in a stanchion barn. Every cow was fed individually and the ground was equipped with rubber mats free of litter. The stable was aerated continuously (20 °C) and water as well as salt blocks were offered *ad libitum*. The cows were provided with a rumen cannula (Bar Diamond Inc., Parma, Idaho, USA) at the dorsolateral rumen sac (internal diameter of 10 cm) and with a duodenal cannula placed about 15 cm after the pylorus (internal diameter of 2.0 cm).

The animals received 6.55 kg DM of TMR (total mixed ration) based on grass silage, maize silage, wheat meal, soybean meal (solvent-extracted), and a mineral/vitamin mix offered in two equal portions per head and day (8:00 a.m. and 5:00 p.m.). Grass and maize silage were sampled and analysed for dry matter and nutrient contents right before the beginning of each experimental period (six times in total) and the experimental diet was adjusted to changes of dry matter six times in the course of the trial. Concentrates were bought in one homogeneous batch and subsequently analysed for dry matter and nutrient contents once prior to the trial.

Dry matter, crude protein, total lipids, neutral detergent fibre, and crude ash contents of the individual components of TMR are listed in Table 3. Composition and crude nutrient as well as mineral contents of the experimental TMR are listed in Table 4.

Table 3: Dry matter and nutrient contents of the TMR components

Feedstuff	DM content [%]	Nutrient content [% of DM]			
		CP	TL	NDF	CA
Grass silage[1]	46.9	16.1	3.80	44.6	9.08
Maize silage[1]	41.0	6.86	4.28	36.7	2.74
Wheat meal	86.5	12.4	4.17	12.0	1.26
Soybean meal	87.0	44.6	2.64	25.9	6.88

CP: crude protein, TL: total lipids, NDF: neutral detergent fibre, CA: crude ash.
[1] Mean values of six analyses during the trial.

Table 4: Composition and analysed crude nutrient as well as mineral contents of the experimental TMR

Item	
Ingredient	**% DM of TMR**
Grass silage	49.6
Maize silage	39.7
Wheat meal	4.97
Soybean meal	4.97
Mineral/vitamin premix	0.76
Crude nutrient content of TMR	
CP	13.5
TL	3.92
NDF	38.6
CA	6.00
Mineral content of TMR[1] (including mineral premix)	**mg/kg DM of TMR**
Fe	186
Zn	54.1
Mn	32.2
Mo	2.11
S	1812

Mineral/vitamin premix consisted of: 27.7 % limestone, 27.7 % sodium chloride, 33.5 % monocalcium phosphate, 6.6 % magnesium oxide, 0.8 % Zn (from zinc sulphate heptahydrate), 0.4 % Mn (from manganese sulphate monohydrate), 0.01 % I (from potassium iodide), 0.003 % Co (from cobalt chloride), 0.004 % Se (from sodium selenite), 0.16 % vitamin A, 0.02 % vitamin D3, 0.31 % vitamin E, 0.009 % vitamin B premix, 0.02 % vitamin C, 0.01 % niacin, 0.009 % pantothenic acid.

This ration was fed as of three weeks before starting the feeding trial to ensure an adequate adaptation of rumen microorganisms to the experimental diet. The mineral/vitamin premix was produced on-site (according to the formulation of conventional mineral and vitamin feeds) but without adding copper. This allowed for a defined Cu supplementation related to the different Cu treatments. Mineral/vitamin premix and titanium dioxide (TiO_2) were blended and homogenised thoroughly with ground wheat meal and soybean meal (3.0 mm). Afterwards, this concentrate was gently pelletised (Ø 4.0 mm).

Table 5 explains the composition of total Cu supply and the respective amounts of added granules. Cu concentration of grass and maize silage were analysed right before the beginning of each period. Cu concentration of the homogeneous concentrates was determined once prior to the trial. The amount of supplemented Cu was precisely adjusted to native Cu concentrations in the diet (six times in total) in order to reach the total levels

of Cu supply. The results of this study are based on the total levels of Cu supply rather than the supplemented amount of Cu.

Table 5: Composition of total Cu supply and respective amounts of added granules

Cu supply		Native dietary Cu concentration	Supplemented Cu	Amount of Cu granules
Cu source	Cu dose [mg/kg DM of TMR]	[mg/kg DM of TMR]		[mg/kg DM of TMR]
$CuSO_4$	10.0	7.86[1]	2.14	8.58
	35.0	7.86[1]	27.14	108.58
	50.0	7.86[1]	42.14	168.58
TBCC	10.0	7.86[1]	2.14	3.97
	35.0	7.86[1]	27.14	50.27
	50.0	7.86[1]	42.14	78.04

[1] Calculated from Cu concentration in concentrates and the mean Cu concentration of six analyses of grass and maize silage during the trial; SD (± 0.89).

The TMR was composed directly for every cow before feeding. After weighing the respective shares of grass silage, maize silage, and pelleted concentrate, all components were mixed by hand. At the same time the appropriate amounts of Cu granules were mixed into the TMR.

3.4 Timing scheme

The trial lasted 126 days and was divided into six experimental periods. Each period consisted of 13 days of adaptation to treatment (days 1 - 13) followed by eight days of sampling and measurements (days 14 - 21).

- Days 14 - 21: incubation of feed material in the rumen
- Days 15 - 21: sampling of faeces
- Day 17: sampling of rumen fluid, rumen solid phase, and duodenal digesta
- Day 21: sampling of blood

3.5 *In sacco*-method

The *in sacco*-method according to Ørskov and McDonald (1979) and Madsen and Hvelplund (1994) was used to determine the rumen degradability of the TMR and its single components (grass silage, maize silage, wheat meal, soybean meal). For this purpose, defined amounts of feed material were incubated in the rumen prior to calculation of dry matter disappearance.

First, labelled 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 dried in a forced-air dry oven at 60 °C for 48 h. After cooling down to room temperature in a desiccator, the empty bags were weighed. Subsequently, 4.0 g DM of grass silage, maize silage, wheat meal, soybean meal, and TMR were weighed into the bags, respectively. The single components of the TMR were weighed separately into the bags of TMR, corresponding to the proportions of the ingested TMR by the animals. The filled bags were sealed with common cable ties and stored in a darkened chamber at room temperature. Table 6 shows the dry matter and nutrient contents of incubated feedstuffs.

In preparation for weighing the feedstuff into the bags, grass silage and maize silage were gently dried in a forced-air dry oven at 45 °C for 72 h (López *et al.*, 1995) and afterwards ground through a 5.0 mm screen using a cutting mill (type 880800, Brabender, Duisburg, Germany). By this, the storability of the material was preserved accompanied by simulating the chewing process of the animals. Additionally, homogeneity of grass silage and maize silage was improved. Wheat meal and soybean meal were ground through a 3.0 mm screen using the same cutting mill though without any previous drying step.

Prior to incubation, four bags of each feedstuff (grass silage, maize silage, wheat meal, soybean meal, TMR) were clamped with further cable ties to a cylindrical anchor weight (800 g). Altogether, 20 bags were prepared for each cow and incubation time. The bags were spaced consistently on both ends of the cylindrical anchor weight to reduce possible effects on dry matter degradation caused by different positions inside the rumen. Right before the morning feeding at 8 o'clock, the cylinders were immersed in cold water for 30 s (López *et al.*, 1995). This served to wet the dry feed material, a process naturally happening during mastication and facilitating the association of microorganisms with feed particles (Bowman and Firkins, 1993).

Next to that, the cylinders were put in the ventral sac of the rumen and fixed with a flexible rope at the inside of the screw-cap of the cannula. After the incubation times of 1.5, 3, 6, 9, 12, 24, and 48 h, respectively, all cylinders were removed and instantly immersed in ice

water to inhibit any further activity of microorganisms attached to the remaining feed material within the bags.

Afterwards, the bags were clipped off the cylinders, given into a sink full of cold tap water and washed by hand. In addition to the incubated bags, one bag of each feedstuff (control bags) was added to the washing procedure to determine the dry matter disappearance due to the washing process. The sink was refilled repeatedly until water remained clear. A wash cycle of 19 min (cold water, with no spin cycle) in a customary washing machine finished the washing procedure. Finally, incubated bags and control bags were dried in a forced-air dry oven at 60 °C for 72 h (López *et al.*, 1995), cooled down to room temperature in a desiccator and weighed again.

Table 6: Dry matter and nutrient contents of incubated feedstuffs

Feedstuff	DM content [%]	Nutrient content [% of DM]			
		CP	TL	NDF	CA
Grass silage	91,1	20,5	3,83	42,9	8,65
Maize silage	94,8	7,25	4,41	35,3	2,77
Wheat meal	86.5	12.4	4.17	12.0	1.26
Soybean meal	87.0	44.6	2.64	25.9	6.88

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

3.6 Sampling and sample preparation

3.6.1 Feed samples

Grass and maize silage were sampled before each period of the trial. After determination of dry matter content by drying at 60 °C for 48 h in a forced-air dry oven, samples were ground in a cutting mill (type 880800, Brabender, Duisburg, Germany) through a 1.0 mm screen.

Prior to the start of the trial one sample each of wheat meal, soybean meal, and pelleted concentrate (consisting of wheat meal, soybean meal, mineral/vitamin premix, and TiO_2) were taken. After determination of dry matter content by drying at 103 °C for 4 h the samples were ground in a hammer mill (SR3, Retsch, Haan, Germany) using a 1.0 mm screen.

3.6.2 Samples of rumen fluid

On day 17 of each period, samples of rumen fluid were taken for determination of rumen pH, Cu concentration, rumen microorganisms, NH_3-N concentration, and volatile fatty acid (VFA) concentrations. Sampling times were right before the morning feeding at 8:00 p.m. (denoted as “0 h”) as well as 1.5, 3, 4.5, 6, 7.5, and 9 h after this feeding (in total seven samples per animal).

At each sampling time, approximately 500 ml from the ventral sac of the rumen were collected per animal using a mouth-to-rumen tube (inserted through the cannula) in combination with vacuum.

At first, a fraction of approximately 150 ml was instantly used to measure the pH value of rumen fluid.

Further 50 ml of rumen fluid were instantly frozen at -20 °C for subsequent separation of sub-fractions and determination of the respective Cu concentration. Soluble Cu was assumed to be present in the liquid fraction of rumen fluid. Insoluble Cu, however, was expected to be found in the solid fraction of rumen fluid. For this reason, samples of rumen fluid were thawed, pooled (all samples per animal and period, respectively) and separated in a solid and a liquid fraction via centrifugation at 20,000 x g for 30 min. The supernatant (liquid fraction) was stored at -20 °C until analyses. The pellet consisting of feed particles, protozoa, bacteria, and other solid material (solid fraction) was frozen at -20 °C before lyophilising for 72 h. Finally, the dry pellet was ground in an analytical mill (A10, IKA, Staufen im Breisgau, Germany) with a star shaped cutter for 30 s. During the separation procedure, fresh and dry matter contents of rumen fluid were determined.

For analyses of rumen microorganisms in the rumen fluid, another 50 ml were frozen at -20 °C, immediately. Afterwards, samples were lyophilised for 72 h, homogenised gently by hand in a mortar and stored at -20 °C again.

The residual 250 ml of rumen fluid were centrifuged (Z323, HERMLE Labortechnik, Wehingen, Germany) at 5,000 rpm (revolutions per minute) for 15 min. Afterwards, 10 ml of the supernatant were removed while the rest was stored at -20 °C until determination of NH_3-N concentration. The removed supernatant was admixed with 1.5 ml metaphosphoric acid (25 %) and 0.5 ml formic acid and then centrifuged at 5,000 rpm for 20 min. The new supernatant was stored at -20 °C until analysis of VFA concentrations.

3.6.3 Samples of rumen solid phase

On day 17 of each period, samples of rumen solid phase were collected at the same sampling times as rumen fluid samples. Per animal, 150 g were removed from the upper layer of rumen solid phase. Afterwards, samples were frozen at -20 °C and lyophilised for 72 h. For analyses of Cu concentration, dried samples were pooled (all samples per animal and period, respectively) and ground with a cutting mill (type 880800, Brabender, Duisburg, Germany) through a 1.0 mm screen.

3.6.4 Samples of duodenal digesta

On day 17 of each period, duodenal digesta was sampled at the same times as rumen fluid and rumen solid phase. When sampling, 50 ml per animal were obtained by collecting the outflowing digesta of the duodenal cannula. Duodenal digesta remaining in the tube of the cannula was discarded prior to collection. Right after sampling, the duodenal digesta was frozen at -20 °C. Subsequently, the samples of each animal and period were thawed and pooled to a total sample of 350 ml. In order to measure Cu concentrations in different fractions of duodenal digesta, the total samples were fractionated in solid phase (large particles, small particles, bacteria) and liquid phase referring to Choi *et al.* (2002). In Figure 1 the scheme of fractionation is illustrated. In step 1, the total sample was sieved using a conventional sieve (7 gaps per cm). The retained large particles mainly consisted of rough feed particles. In step 2, the filtrate was centrifuged at 1,000 × g for 10 min at 4 °C (Z 36 HK, HERMLE Labortechnik, Wehingen, Deutschland) to precipitate small particles (minimal feed particles and protozoa). In step 3, the supernatant was centrifuged at 20,000 × g for 30 min at 4 °C (Z 36 HK, HERMLE Labortechnik, Wehingen, Deutschland) to obtain a pellet mainly consisting of bacteria and a supernatant completely free of solid material (liquid phase). Fractions of the solid phase were frozen at -20 °C and lyophilised for 72 h. After that, large particles were ground in an analytical mill (A10, IKA, Staufen im Breisgau, Germany) with a star shaped cutter for 30 s while small particles and bacteria were homogenised by hand in a mortar. The liquid phase was stored at -20 °C until analyses. Dry matter contents of different fractions and the amount of each fraction were determined.

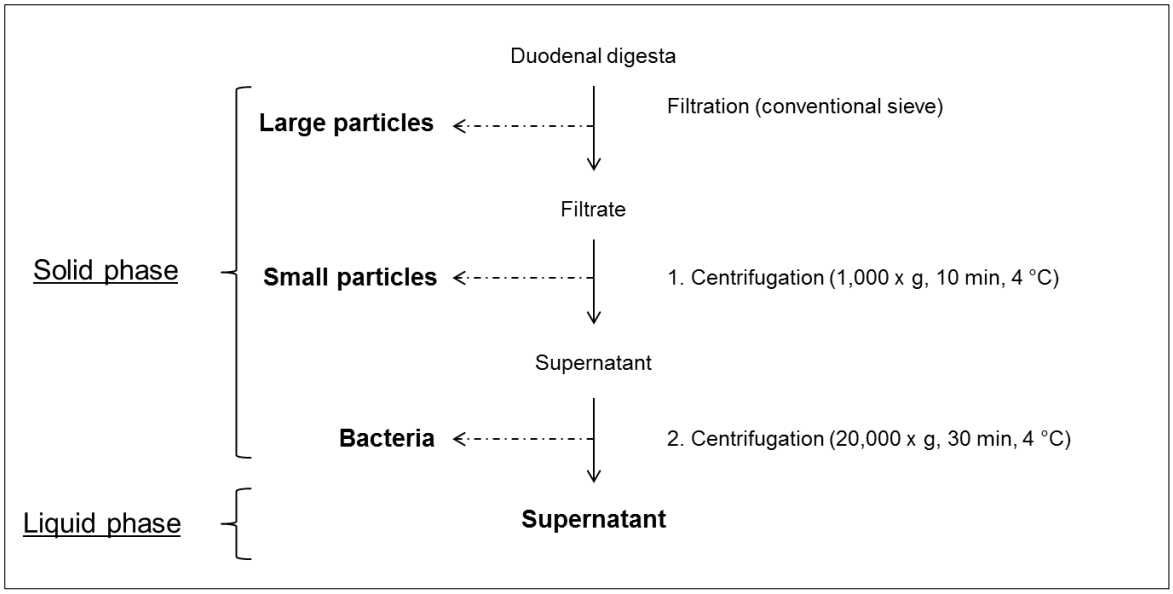

Figure 1: Scheme of fractionation of duodenal digesta (referring to Choi et al. 2002)

3.6.5 Faeces samples

Faeces samples were collected throughout the last seven days of each period (day 15 - 21). Every day between 7:00 and 9:00 a.m., 150 g of faeces were taken of each animal (in total seven samples per animal) and frozen at -20 °C. After that, samples were weighed and lyophilised for 72 h prior to determination of dry matter content. Next to that, dried samples of each animal and period, respectively, were pooled and ground through a 1.0 mm screen using a cutting mill (type 880800, Brabender, Duisburg, Germany).

3.6.6 Blood samples

Blood samples were taken of each animal at 1:30 p.m. at the last day of every period (day 21). For determination of Cu concentration in serum, superoxide dismutase activity in serum, and ceruloplasmin activity in serum, 1 × 9 ml of blood were collected from the *vena jugularis* using serum tubes (S-Monovette Z-Gel, Sarstedt AG & Co, Nürnbrecht, Germany). After 1 h, the coagulated blood was centrifuged at 3,000 × g for 15 min (Rotina 48, Hettich Lab Technology, Tuttlingen, Germany) and the serum was stored at -20 °C.

3.7 Analytical procedures

Prior to analyses, residual water of feedstuffs, rumen solid phase, solid phases of rumen fluid and duodenal digesta, and faeces was determined by drying at 103 °C for 4 h.

3.7.1 Crude nutrient analysis

Crude nutrients (crude protein, total lipids, crude fibre, crude ash) of feedstuffs and faeces were analysed corresponding to “Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten” (VDLUFA) (Naumann and Bassler, 1976, 2012).

Analysis of crude protein (CP) was conducted according to a standard Kjeldahl procedure. The decomposition of 1.0 g DM was performed in the Turbotherm (C. Gerhardt, Königswinter, Germany) with 20 ml of concentrated sulphuric acid and a catalyst (Kjelcat CuTi, C. Gerhardt, Königswinter, Germany). This decomposition process lasted 115 min. Subsequently, samples were alkalised by sodium hydroxide solution (30 %). After steam distillation, ammonia was collected in boric acid (2.0 %) and determined by titration of hydrochloric acid (1.0 %). Steam distillation and titration were carried out with the Vapodest (C. Gerhardt, Königswinter, Germany).

For the analysis of total lipids (TL), 1.5 g DM were hydrolysed in hot hydrochloric acid (15 %) for 75 min and eventually filtered (Hydrotherm, C. Gerhardt, Königswinter, Germany). Afterwards, total lipids were extracted with 140 ml of distilling petroleum ether for 105 min (Soxtherm, C Gerhardt, Königswinter, Germany) followed by drying and weighing.

For the determination of crude fibre (CF), 1.0 g DM was boiled in sulphuric acid (0.128 mol/l) and potassium hydroxide solution (0.223 mol/l) for 92 min (Fibretherm, C. Gerhardt, Königswinter, Germany). After filtration, residual material was washed, dried and weighed. Meanwhile, crude ash content of residual material was determined prior to calculation of CF amount (difference between weight before and after determination of crude ash).

Crude ash (CA) was determined by incinerating 3.0 g DM in a muffle furnace at 550 °C overnight and subsequent weighing.

3.7.2 Fibre analysis

The cell wall components neutral-detergent fibre (NDF) and acid-detergent fibre (ADF) of feedstuffs and faeces were determined according to VDLUFA (Naumann & Bassler, 1976/2012) based on Van Soest *et al.* (1991).

Analysis of NDF started with weighing of 1.0 g DM into FibreBags (C. Gerhardt, Königswinter, Germany) prior to boiling in neutral detergent solution combined with thermostable α-amylase (Termamyl 120L, Univar, Essen, Germany) for 165 min (Fibretherm, C. Gerhardt, Königswinter, Germany). For the determination of ADF, 1.0 g DM was boiled in acid detergent solution without addition of α-amylase for 120 min (Fibretherm, C. Gerhardt, Königswinter, Germany). After boiling, samples were washed with distilled water, dried and weighed. Finally, crude ash content (difference between weight before and after determination of crude ash) of residual material was determined prior to calculation of NDF and ADF amount, respectively.

3.7.3 Determination of titanium dioxide

Titanium dioxide (TiO_2) in feed and faeces was determined according to Brandt and Allam (1987). First, 0.5 g DM were weighed into Kjeldahl-flasks and 10 g K_2SO_4, 2.5 ml $CuSO_4$ (10 %), and 20 ml concentrated H_2SO_4 were added with K_2SO_4 and $CuSO_4$ serving as catalysts. After a boiling step of 160 min (Turbotherm, C. Gerhardt, Königswinter, Germany), this intermixture was filled up to 250 ml with distilled water and subsequently filtered (595 ½, Whatman, Dassel, Germany). Additionally, a solution with 40 ml H_2O_2 (35 %), 120 ml H_3PO_4 (85 %), 200 ml concentrated H_2SO_4, and 360 ml double distilled water was prepared. Thereof, 100 µl were mixed with 1.0 ml of filtered sample in a cuvette to measure TiO_2 content. H_2SO_4 caused hydrolysis of TiO_2 followed by formation of a yellow product with H_2O_2. After 30 min of reaction time, the samples were measured at 405 nm using a spectral photometer (UVmc2, Safas, Monaco, Monaco). Sample blanks were measured as described above but H_2O_2 was replaced by distilled water. For calibration, sulphate solutions with known TiO_2 contents were used.

3.7.4 Determination of Fe, Zn, Mn, Mo, and S concentrations

Concentrations of the elements Fe, Zn, Mn, Mo, and S in the single components of the TMR were analysed at the laboratory of the Bayerische Landesanstalt für Wald und Forstwirtschaft (Freising, Germany). Samples were weighed in quartz vessels (120 - 150 mg DM in duplicate) before 1.0 ml ultrapure HNO_3 (65 %) was added. These vessels

were embedded in pressure vessels (Seiff Aufschlusstechnik, Unterschleißheim, Germany) and heated to 170 °C for 6 h. After cooling, samples were transferred into volumetric flasks and filled up to 15 ml with double distilled H_2O.

The concentrations of Fe, Zn, Mn, and S in digested samples were determined using an ICP-OES (Optima 5300 DV, PerkinElmer, Waltham, Massachusetts, USA) with a GemCone™ nebuliser and a quartz cyclone spray chamber. Yttrium served as internal standard. The plasma observation was either axial or radial, dependent on the element.

The concentration of Mo in digested samples was measured using a ICP-MS (NexION 300XX, PerkinElmer, Waltham, Massachusetts, USA) with an automated sample introduction system (SC-DX FAST, ESI Elemental Service & Instruments, Mainz, Germany), a MicroFlow nebuliser and a quartz cyclone spray chamber. Rhodium served as internal standard.

3.7.5 Determination of Cu concentrations

3.7.5.1 Solid material

Samples of solid material (feed, rumen solid phase, solid phase of rumen fluid, particles and bacteria of duodenal digesta, faeces) were in duplicate (except for solid phase of rumen fluid and bacteria due to lack of sample material) decomposed using a microwave (Ethos 1, MLS, Leutkirch, Germany). The following amounts of samples were weighed into decomposition vessels: feed and faeces (~1.00 g DM), rumen solid phase (~0.83 g DM), solid phase of rumen fluid (~0.37 g DM), duodenal digesta: large particles, small particles, bacteria (~0.71 g DM, ~0.75 g DM, ~0.10 g DM, respectively). Afterwards, 6.25 ml HNO_3 (65 %), 3.0 ml H_2O_2 (30 %) and 5.0 ml double distilled H_2O were added. The acid digestion in the microwave lasted 50 min at temperatures up to 200 °C. Subsequently, the cooled samples were transferred into 25 ml volumetric flasks (10 ml at bacteria samples) and filled up to the calibration mark with double distilled H_2O. Finally, the samples were filtered in ash-free filters to remove residual particles.

After acid digestion, the Cu concentrations of samples were measured using an atomic absorption spectrometer (nova 350, Analytik Jena, Jena, Germany). For calibration, standard solutions (HNO_3, 65 %) with Cu concentrations of 0.0, 0.5, and 1.0 mg/kg were prepared. Every measurement series included two calibrations and each sample was measured twice in reverse order to prevent differences due to varying calibration points. If the Cu concentrations in the samples exceeded 1.0 mg/kg, the samples were further

diluted. The measured values were corrected by the blank value and multiplied with the respective dilution factors.

3.7.5.2 Fluid material

Samples of the liquid phases of rumen fluid and duodenal digesta were analysed directly (without prior acid decomposition) using an atomic absorption spectrometer (nova 350, Analytik Jena, Jena, Germany) as described above. However, the accuracy of measurements can be restricted compared to decomposited samples due to the composition of fluid material. For this purpose, the addition method was applied.

The proportion of Cu present in ionic form in the liquid phase of duodenal digesta was determined by Cu precipitation. First, 100 µl NaOH solution (7.5 molar, ultrapure) were added to 25 ml sample in order to set the pH value to about 6.0 and thus preventing volatilisation of H_2S. After that, solved $Na_2S \cdot 9H_2O$ was mixed to the samples in a five-fold concentration of Cu. This mixture was adjusted to a pH value of 9.0, stirred for 5 min and subsequently centrifuged at 20,000 × g for 15 min. The aim of this procedure is for ionic Cu to precipitate either as CuS or as $Cu(OH)_2$. Finally, the Cu concentration in the supernatant was measured as described above and subtracted of the original Cu concentration.

3.7.6 Determination of Cu status parameter in serum

3.7.6.1 Determination of Cu concentration in serum

Serum samples were in duplicate digested using a microwave (Ethos 1, MLS, Leutkirch, Germany). For acid digestion, 1 ml of the sample, 2.5 ml HNO_3, and 1.5 ml double distilled H_2O were pipetted in a vessel. This vessel was put into a second vessel made of Teflon which was already filled with 5 ml double distilled H_2O and 1 ml H_2O_2. The microwave program lasted 30 min at temperatures up to 210 °C. Afterwards, the cooled samples were transferred into 10 ml volumetric flasks and filled up to the calibration mark with double distilled H_2O. Finally, samples were filtered in ash-free filters to remove residual particles. The Cu concentrations of samples were measured using an atomic absorption spectrometer (nova 350, Analytik Jena, Jena, Germany) as described above.

3.7.6.2 Determination of ceruloplasmin activity in serum

The ceruloplasmin activity in serum was measured using the Ceruloplasmin Activity Colorimetric Kit (Sigma-Aldrich, St. Louis, Missouri, USA) based on substrate oxidation, which results in a colorimetric product (560 nm) proportional to the enzymatic activity. The measurement was performed following the manufacturer's manual. One unit of ceruloplasmin equals the amount of enzyme necessary to oxidise 1 µmol of substrate per minute at 25 °C. The standard curve was generated by non-enzymatic oxidation using a chemical oxidiser. Chloride has inhibiting effects on ceruloplasmin activity, and was therefore removed from serum by pelleting proteins with a saturated ammonium sulphate solution, discarding of supernatant and dissolving the pellet again. For the reaction, 12.5 µl of chloride-free serum were used. The slope of the standard curve was calculated with the absorbance (A_{560}) at 15 min. The linear range of the curve of all samples ranged between 7 min and 15 min. The enzymatic activity was detected using a microplate reader (Ledetect 96, Deelux Labortechnik, Gödenstorf, Germany). Data analysis was carried out with the program MikroWin2010 V 5.1 (Mikrotek Laborsysteme, Overath, Germany). Ceruloplasmin activity was calculated by the following equation:

$$\text{Ceruloplasmin activity}\,[\,\text{mU/l}\,] = \frac{S_K / S_S}{V \times 2}$$

S_K = kinetic slope of the sample (ΔA_{560}/min) in the linear portion of the curve

S_S = slope of the standard curve (ΔA_{560}/nmole)

V = sample volume (mL) added to the well

2 = sample dilution factor for ammonium sulphate precipitated samples

3.7.6.3 Determination of superoxide dismutase activity in serum

The superoxide dismutase (SOD) activity in the serum was determined with the SOD Assay Kit (Sigma-Aldrich, St. Louis, Missouri, USA) following the manufacturer's manual. SOD inhibits the conversion of a tetrazolium salt into a formazan dye and can be quantified by measuring the decrease of absorbance at 450 nm. A microplate reader (Ledetect 96, Deelux Labortechnik, Gödenstorf, Germany) was used for photometric measurement. Data analysis was carried out with the program MikroWin2010 V 5.1 (Mikrotek Laborsysteme, Overath, Germany).

SOD activity was calculated by the following equation:

$$\text{SOD activity [inhibition rate \%]} = \frac{(\text{Blank 1} - \text{Blank 3}) - (\text{Sample} - \text{Blank 2})}{\text{Blank 1} - \text{Blank 3}} \times 100$$

Blank 1 = full enzyme activity

Blank 2 = extinction of sample

Blank 3 = extinction of enzyme solution

Sample = SOD activity of samples

3.7.7 Determination of rumen physiological parameters

3.7.7.1 pH-value of rumen fluid

Straight after collecting rumen fluid samples, pH-value was measured using a calibrated pH meter (CG 842, Schott, Mainz, Germany).

3.7.7.2 Ammonia-nitrogen in rumen fluid

Frozen samples of prepared ruminal fluid 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). This measurement was based on the reaction of ammonia with α-ketoglutarate and NADPH to glutamate and $NADP^+$ under presence of glutamate dehydrogenase. The absorbance, decreasing inversely proportional to ammonia concentration, was measured at 340 nm using a spectral photometer (UVmc2, Safas, Monaco, Monaco). Reaction and calculation were conducted according to the instruction manual of the manufacturer.

3.7.7.3 Volatile fatty acids in rumen fluid

For determination of acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, and isovaleric acid, frozen samples of ruminal fluid were thawed and 250 µl were mixed with 850 µl dilution solution. The dilution solution was prepared by diluting 100 µl 2-methyl valeric acid (serving as internal standard) with meta-phosphoric acid (2.0 %) to a volume of 250 ml. The mixed solution was filtered (syringe filter, 0.2 µm membrane) and subsequently analysed using a gas chromatograph (Clarus 580, PerkinElmer, Waltham, Massachusetts, USA). Sample solution was evaporated in an injector combined with

nitrogen (carrier gas) at 220 °C. This gas mixture was led through a capillary column with a length of 30 m, an inner diameter of 0.25 mm, and a film thickness of 0.25 µm (PerkinElmer, Waltham, Massachusetts, USA). During this process, temperature around the column raised from 100 °C to 235 °C. Finally, volatile fatty acids were detected by a flame ionisation detector at a temperature of 275 °C. For calibration, an analytical standard (Restek, Bellefonte, Pennsylvania, USA) containing the six analysed volatile fatty acids was used.

3.7.8 Determination of rumen microbiota

Concentrations of total bacteria, *Ruminococcus flavefaciens, Fibrobacter succinogenes, Streptococcus bovis,* archaea, protozoa, and anaerobic fungi were determined in the rumen fluid. Samples of rumen fluid were taken right before the morning feeding (0 h) as well as 1.5 h and 3 h after the morning feeding.

3.7.8.1 DNA extraction and quality control

Total genomic DNA was extracted from dried rumen fluid samples using the FastDNA SPIN Kit for faeces (MP Biomedicals, Santa Ana, California, USA). The extraction was performed following the manufacturer's manual with the exception of using 100 mg instead of 500 mg sample. Based on recommendations of the manual, samples were solubilised in 200 µl Sodium Phosphate Buffer prior to the first extraction step in order to optimise DNA recovery from extremely dry samples. For homogenisation and cell lysis of samples, the MP FastPrep®24 instrument (MP Biomedicals, Santa Ana, California, USA) was used. Purified DNA was eluted with 100 µl of TES buffer. Subsequently, amount and purity of eluted DNA were examined by spectrophotometry (NanoDrop 2000, Thermo Fisher Scientific, Waltham, Massachusetts, USA), where the elution buffer served as blank. For evaluation of purity, the optical density (OD) was detected at 230 nm (contamination with reagents), at 260 nm (nucleic acids), and at 280 nm (proteins). Ratios of $OD_{260}/OD_{230} > 1.8$ and $OD_{260}/OD_{280} > 2.0$ were required to assume high DNA quality. Finally, extracted DNA was stored at -20 °C.

3.7.8.2 Primer test

Primers sequences for detection of different microorganisms were acquired from literature: total bacteria (Edwards *et al.*, 2007), *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, anaerobic fungi (Denman and McSweeney, 2006), *Streptococcus bovis* (Stevenson and Weimer, 2007), archaea (Stahl and Amann, 1991; Großkopf *et al.*, 1998),

protozoa (Sylvester *et al.*, 2004). The targeting sequences were 16S rRNA gene sequences for bacteria and 18S rRNA gene sequences for protozoa and anaerobic fungi, respectively. Primers were synthesised by Eurofins (Ebersberg, Germany).

At the beginning of the primer test, extracted DNA of five samples (randomly chosen) was pooled to obtain a representative bulk sample. For determination of optimal reaction conditions, a gradient quantitative real-time polymerase chain reaction (qPCR) with a temperature range of 53.5 °C – 63.6 °C was performed for each primer system using the Bio-Rad CFX Connect™ Real-Time System and software (CFX Manager: version 3.1, Bio-Rad Laboratories, Hercules, California, USA). Due to interfering substances within the extracted DNA, the extract was further diluted (1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1280) to reduce inhibiting effects. The qPCR was carried out in 96-well plates (4titude, Wotton, Surrey, England). The reaction mix contained 7.5 µl SensiFAST SYBR No-ROX Mix (Bioline Reagents, London, England), 0.6 µl forward and reverse primer (100 pmol/µl), respectively, 4.8 µl nuclease-free water, and 1.5 µl DNA extract (15 µl in total).

The qPCR program was set up as follows: initial denaturation (5 min, 95 °C); 30 cycles amplification consisting of: denaturation (20 s, 95 °C), annealing (60 s, 53.5 °C – 63.6 °C), and elongation (30 s, 72 °C); melting curve (20 min, 60 °C – 95 °C).

After finishing the program, melting curves and differences in cycles were evaluated. When there was a distance of exactly one cycle between two consecutive dilution steps but within one temperature step, the higher dilution and its corresponding temperature were selected. Afterwards, desired qPCR products were purified (MinElute®PCR Purification Kit, Quiagen, Hilden, Germany) and put on a 2.0 % agarose gel (peqGold Universal Agarose, Peqlab, Erlangen, Germany) for 60 min at 100 volt. A TAE buffer (pH: 8.0; composition: 242 g TRIS, 57.1 ml acetate, and 100 ml EDTA (0.5 mol/l) filled up to 1 l with double distilled water) diluted 1:50 served as running buffer. The amplicons were visualised using SYBR Safe DNA Gel Stain (Invitrogen, Carlsbad, California, USA) combined with UV light. Finally, specificity and size of amplicons were verified using the peqGold 50 bp DNA ladder (Peqlab, Erlangen, Germany). Table 7 outlines primers used for qPCR analysis and corresponding annealing temperatures and sample dilutions.

Table 7: Primers for qPCR analysis

Target species	Primer sequences (5' - 3')	Amplicon length [bp]	Annealing temperature [°C]	Sample dilution
Total bacteria	f: AGCAGCCGCGGTAAT r: CAGGGTATCTAATCCTGTT	280	61.9	1/160
Ruminococcus flavefaciens	f: CGAACGGAGATAATTTGAGTTTACTTAGG r: CGGTCTCTGTATGTTATGAGGTATTACC	132	60.0	1/40
Fibrobacter succinogenes	f: GTTCGGAATTACTGGGCGTAAA r: CGCCTGCCCCTGAACTATC	121	57.5	1/160
Streptococcus bovis	f: TTCCTAGAGATAGGAAGTTTCTTCGG r: ATGATGGCAACTAACAATAGGGGT	127	54.3	1/10
Archaea	f: ACKGCTCAGTAACACGT r: GTGCTCCCCCGCCAATTCCT	826	63.6	1/40
Protozoa	f: GCTTTCGWTGGTAGTGTATT r: CTTGCCCTCYAATCGTWCT	223	55.6	1/80
Anaerobic fungi	f: GAGGAAGTAAAAGTCGTAACAAGGTTTC r: CAAATTCACAAAGGGTAGGATGATT	120	61.9	1/40

3.7.8.3 Standard preparation

After a successful primer test, bands containing specific amplicons were cut out from the gel followed by subsequent extraction of DNA using the innuPREP Gel Extraction Kit (Analytik Jena, Jena, Germany) and amplification at ideal reaction conditions using the Bio-Rad T100™ Thermal Cycler (Bio-Rad Laboratories, Hercules, California, USA). Resulting PCR products were purified again (MinElute®PCR Purification Kit, Quiagen, Hilden, Germany) prior to measurement of DNA amount of the eluate using the NanoDrop 2000 (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Copy number of DNA fragments per µl was calculated according to Whelan *et al.* (2003) using the following equation:

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

For absolute quantification of samples, a standard series of 10^1 to 10^8 copy numbers per µl was prepared.

3.7.8.4 Quantification of microorganisms in rumen fluid

The quantification of microorganisms in rumen fluid samples [copy number/g dry matter] was performed by qPCR using the Bio-Rad CFX Connect™ Real-Time System and software (CFX Manager: version 3.1, Bio-Rad Laboratories, Hercules, California, USA). The reaction mix and the qPCR program were similar to the ones used in primer tests except for specific annealing temperatures of primers and specific dilutions of extracted DNA samples. The amplification of samples and standard series was carried out in duplicates. The negative control (nuclease-free water instead of a sample) was amplified in four wells distributed across the plate. To evaluate each qPCR run, standard series had to be set up on the same plate together with samples and negative controls. At least five consecutive $\log_{10}$ concentrations of the standard series were required to create a reliable calibration curve. Results of the evaluation were as follows: efficiencies (89.4 % - 102.3 %), slopes (-3.269 - -3.605), intercepts (36.124 - 39.192), and coefficients of determination (> 0.99).

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

3.8 Calculations

3.8.1 Calculation of dry matter disappearance

Dry matter disappearance of all feedstuffs was calculated for every incubation time at each period using following equation:

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

The following calculations of parameters of degradability and effective degradability, dependent on dry matter disappearance, based on the exponential model of Ørskov and McDonald (1979).

3.8.2 Estimation of parameters of degradability

Parameters of degradability were estimated using the equation of McDonald (1981):

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

where p is the disappearance at time t, a is the soluble fraction, b is the insoluble, but ruminally degradable fraction, 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 is the totally degradable fraction (TDF).

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).

3.8.3 Calculation of effective degradability

The effective degradability (ED) was calculated using the following equation of Wulf and Südekum (2005), a modification of the equation of McDonald (1981):

$$ED = a + b\,[(b \times c) / (c + k)]\, e^{-kt_0},$$

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

An increased feed intake reduces the retention time of feed inside the rumen with a subsequent decrease in ruminal degradation. Therefore, different varying feed intake levels were considered by calculating the ED for rates of passage of 2, 5, and 8 %/h. A passage rate of 2 %/h represents a low, of 5 %/h a medium and of 8 %/h a high level of feed intake (ARC, 1984).

3.8.4 Calculation of total tract digestibility

Apparent total tract digestibility was calculated for dry matter, organic matter, crude fibre, crude protein, total lipids, nitrogen-free extracts, crude ash, neutral detergent fibre, and acid detergent fibre. TiO_2 (a non-digestible and non-absorbable marker) was mixed into the TMR in order to obtain a proportion of 0.1 % of DM. The following equation was used for calculation of apparent total tract digestibility:

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

3.9 Statistics

Data were statistically analysed by 2-way analysis of variance with effects of treatment (six treatments resulting from a combination of two Cu sources and three Cu doses) and animal using the GLM-procedure of SAS software (SAS 9.4, SAS Institute, Cary, North Carolina, USA). Based on that, orthogonal contrasts were calculated. On the one hand, differences between Cu sources (comparison of means: $CuSO_4$ vs. TBCC) and on the other hand, linear trends along Cu doses within $CuSO_4$ or TBCC, respectively, were analysed. Comparisons of six treatments were conducted by the Student-Newman-Keuls test. The significance level was $p \leq 0.05$ and tendencies were declared if $p \leq 0.10$. 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 (6 treatments)
$animal_j$	= effect of animal (6 animals)
e_{ij}	= residual error
i	= index of treatment (1-6)
j	= index of animal (1-6)

Results tables show the means of all animals, the standard error of means (SEM), representing the pooled standard error of the respective general linear model, and p-values of ANOVA and orthogonal contrasts. Additionally, different superscripts indicate significant differences between treatments.

The statistical evaluation of the results focused on contrasts between Cu sources and on linear trends along with graduated Cu supplementation within $CuSO_4$ or TBCC.

4 Results

The results of the current study are presented in the following section. At first, the Cu concentrations in rumen and duodenal contents as well as the amount of apparently digested Cu and the status parameters of Cu in the blood serum are shown. Then, the quantity of different rumen microorganisms is depicted followed by the rumen degradation characteristics of TMR, grass silage, maize silage, wheat meal, and soybean meal and the rumen physiological parameters (pH-value, ammonia-nitrogen concentrations, and concentrations of volatile fatty acids). Finally, the apparent total tract digestibility of feed ingredients is shown. The values presented in the tables are the means of the six experimental animals.

4.1 Effect of dose and source of copper supplementation on copper concentration in ruminal and duodenal contents and on copper digestion

The rumen solubility of the applied Cu sources is considered to range between completely soluble and almost insoluble. Thus, one important issue of this study was to investigate the distribution of Cu in the contents of the digestive tract depending on the different Cu sources. Combined with findings from Cu digestibility these results are interesting regarding the physiological relevance of Cu sources.

4.1.1 Copper concentration in rumen contents

The Cu concentrations in rumen fluid and rumen solid are shown in Table 8. The rumen fluid was subdivided into a particle and a liquid fraction. The Cu concentration of the particle and the liquid fraction increased linearly with rising Cu doses, independent of Cu source ($p < 0.0001$). However, there was also a significant effect of Cu source, at least a trend regarding differences between Cu sources (liquid: $p < 0.0001$; particles: $p = 0.10$). The supplementation of Cu in form of $CuSO_4$ led to significantly higher Cu concentrations in the liquid fraction at doses of 35 mg Cu/kg DM and 50 mg Cu/kg DM (0.24 µg/ml vs. 0.20 µg/ml, 0.32 µg/ml vs. 0.26 µg/ml) whereas the Cu concentrations within the particle fraction were numerically higher if TBCC served as Cu source (80.5 µg/g vs. 76.6 µg/g, 116 µg/g vs. 111 µg/g).

The Cu concentrations in the rumen solid were significantly elevated with increasing Cu doses of both, $CuSO_4$ and TBCC ($p < 0.0001$). As in the rumen fluid, a significant effect of

Cu source was observed in the rumen solid (p = 0.02). The Cu concentrations significantly increased to a greater extent at the Cu supplementation of 50 mg/kg DM in form of TBCC, compared to $CuSO_4$ (68.7 µg/g vs. 64.6 µg/g).

The Cu concentration in rumen fluid fractions and in rumen solid was at the same level in both Cu sources if Cu was supplemented with 10 mg/kg DM. The Cu concentration in the liquid fraction of rumen fluid was very low (< 0.5 %), compared to rumen solid and the particle fraction of rumen fluid.

In total, the Cu concentrations within rumen fluid and rumen solid were reflected by the supplemented amounts of Cu, independent of Cu source. However, the comparison of Cu sources demonstrated that $CuSO_4$ caused higher concentrations in the liquid fraction of rumen fluid while TBCC was increased to a greater extend in solid fractions. Furthermore, the majority of Cu measured in rumen digesta was present in solid fractions.

Table 8: Cu concentrations in rumen fluid [particles: µg/g DM; liquid: µg/ml] and rumen solid [µg/g DM] dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Rumen fluid		Rumen solid
		Particles	Liquid	
$CuSO_4$	10	23.7^{c}	0.12^{d}	13.5^{d}
	35	76.6^{b}	0.24^{b}	47.2^{c}
	50	111^{a}	0.32^{a}	64.6^{b}
TBCC	10	23.2^{c}	0.11^{d}	13.7^{d}
	35	80.5^{b}	0.20^{c}	48.2^{c}
	50	116^{a}	0.26^{b}	68.7^{a}
	SEM	4.40	0.02	2.05
P-value	Treatment[1]	<0.0001	<0.0001	<0.0001
	Cu source[2]	0.10	<0.0001	0.02
	linear $CuSO_4$[3]	<0.0001	<0.0001	<0.0001
	linear TBCC[3]	<0.0001	<0.0001	<0.0001

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments (p < 0.05).

4.1.2 Composition and copper concentration of duodenal digesta

In addition to the Cu concentration in rumen contents, the duodenal digesta was analysed with regard to its composition and the Cu concentration of its respective fractions. The composition of duodenal digesta is presented in Table 9. In this context, the amounts of solid components (specified as dry matter) and liquid (specified as fresh matter) are related to 1 g of fresh matter of duodenal digesta.

The amounts of large particles, small particles, and liquid in the duodenal digesta were not affected by Cu supplementation, neither of Cu dose nor of Cu source. The bacteria fraction, however, tended to increase with higher Cu doses from $CuSO_4$ ($p = 0.08$). The liquid was the major fraction with on average 851 mg/g FM of duodenal digesta. The amounts of large particles and small particles were at a similar level with on average 11.5 mg/g FM and 7.87 mg/g FM of duodenal digesta, respectively. Less than 0.05 % of duodenal digesta was originated from bacteria (on average 0.41 mg/g FM).

Table 9: Amounts of dried solid components (large particles, small particles, and bacteria) and liquid in duodenal digesta [mg/g FM] dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Large particles	Small particles	Bacteria	Liquid
$CuSO_4$	10	12.4	7.10	0.36	853
	35	10.9	7.91	0.42	851
	50	11.2	8.44	0.44	845
TBCC	10	11.8	7.30	0.40	847
	35	11.6	8.20	0.40	851
	50	10.9	8.29	0.45	856
	SEM	1.17	0.94	0.03	7.94
P-value	Treatment[1]	0.91	0.83	0.38	0.88
	Cu source[2]	0.95	0.87	0.67	0.76
	linear $CuSO_4$[3]	0.39	0.27	0.08	0.43
	linear TBCC[3]	0.56	0.40	0.29	0.36

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$).

Table 10 represents the Cu concentrations in the solid and the liquid phase of the duodenal digesta. The solid phase is composed of large particles, small particles, and bacteria. The Cu concentration in the liquid phase was examined before and after the precipitation of soluble and potentially precipitable Cu. The Cu concentrations in the respective fractions increased linearly with higher amounts of supplemented $CuSO_4$ ($p \leq 0.001$) and TBCC ($p < 0.001$). Additionally, supplementation of $CuSO_4$ resulted in significantly higher Cu concentrations in bacteria, compared to TBCC ($p = 0.01$), resulting from a greater Cu accumulation at doses of 35 mg Cu/kg DM and 50 mg Cu/kg DM (91.8 µg/g vs. 74.8 µg/g, 116 µg/g vs. 94.9 µg/g). There were no differences between Cu sources in the remaining fractions. The precipitation step in the liquid phase implicated no reduction of Cu concentration. The slight differences regarding the Cu concentrations before and after precipitation were referable to fluctuations of measurements. A significant increase of Cu concentration in the liquid phase due to the graduated supplementation from 35 mg Cu/kg DM to 50 mg Cu/kg DM could not be verified statistically, independent of Cu source.

Table 10: Cu concentrations in solid phase [µg/g DM] and liquid phase [µg/ml] of duodenal digesta dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Solid phase			Liquid phase	
		Large particles	Small particles	Bacteria	Total liquid	Liquid after precipitation
$CuSO_4$	10	20.9^{c}	31.7^{c}	32.4^{d}	0.076^{b}	0.091^{b}
	35	79.1^{b}	108^{b}	91.8^{b}	0.163^{a}	0.163^{a}
	50	107^{a}	149^{a}	116^{a}	0.191^{a}	0.194^{a}
TBCC	10	20.8^{c}	31.1^{c}	32.7^{d}	0.076^{b}	0.087^{b}
	35	78.0^{b}	107^{b}	74.8^{c}	0.183^{a}	0.177^{a}
	50	110^{a}	156^{a}	94.9^{b}	0.208^{a}	0.202^{a}
	SEM	5.99	3.95	5.80	0.026	0.022
P-value	Treatment[1]	<0.0001	<0.0001	<0.0001	0.001	0.001
	Cu source[2]	0.94	0.53	0.01	0.52	0.72
	linear $CuSO_4$[3]	<0.0001	<0.0001	<0.0001	0.001	0.001
	linear TBCC[3]	<0.0001	<0.0001	<0.0001	<0.001	<0.001

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$).

The Cu distribution in 1 g fresh matter of duodenal digesta is depicted in Table 11. The Cu content in total duodenal digesta as well as the respective Cu amounts originating from the different fractions were linearly correlated to the graduated supplementation of $CuSO_4$ and TBCC, respectively ($p \leq 0.002$). The Cu amount from bacteria tended to increase to a greater extend ($p = 0.08$), when 35 mg Cu/kg DM and 50 mg Cu/kg DM were supplemented in form of $CuSO_4$, compared to TBCC (0.038 µg/g vs. 0.028 µg/g, 0.051 µg/g vs. 0.043 µg/g). This corresponds to the Cu concentrations measured in the dry matter of bacteria.

Summarised, the supplementation of different Cu doses and sources did not alter the composition of duodenal digesta, apart from a slight increase of the bacterial fraction along with enhanced doses of $CuSO_4$. The supplemented amounts of $CuSO_4$ and TBCC, respectively, were reflected by Cu concentrations within solid phase and liquid phase. Nevertheless, enhanced doses of $CuSO_4$ led to a higher accumulation of Cu in bacteria, compared to TBCC. The soluble Cu amount present in the liquid phase was not precipitable, independent of Cu source.

Table 11: Cu distribution in the fresh matter of total duodenal digesta (total Cu = Cu content in 1 g FM of duodenal digesta) [µg/g FM] dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Total Cu	Large particles	Small particles	Bacteria	Liquid
$CuSO_4$	10	0.57^{c}	0.26^{b}	0.23^{c}	0.012^{d}	0.065^{b}
	35	1.90^{b}	0.88^{a}	0.85^{b}	0.038^{bc}	0.138^{a}
	50	2.64^{a}	1.18^{a}	1.25^{a}	0.051^{a}	0.162^{a}
TBCC	10	0.55^{c}	0.24^{b}	0.23^{c}	0.013^{d}	0.065^{b}
	35	1.95^{b}	0.89^{a}	0.88^{b}	0.028^{c}	0.156^{a}
	50	2.74^{a}	1.20^{a}	1.32^{a}	0.043^{ab}	0.178^{a}
	SEM	0.08	0.10	0.12	0.004	0.022
P-value	Treatment[1]	<0.0001	<0.0001	<0.0001	<0.0001	0.001
	Cu source[2]	0.50	0.96	0.72	0.08	0.51
	linear $CuSO_4$[3]	<0.0001	<0.0001	<0.0001	<0.0001	0.002
	linear TBCC[3]	<0.0001	<0.0001	<0.0001	<0.0001	<0.001

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$).

4.1.3 Amount of apparently digested copper

Table 12 shows the daily Cu intake, the Cu concentration in faeces, the daily faecal Cu excretion, the apparent Cu digestibility, and the daily amount of apparently digested Cu. The focus is, however, on the amount of apparently digested Cu which was increased ($p < 0.0001$) with rising doses of $CuSO_4$ and TBCC, respectively. At Cu doses of 10 mg/kg DM and 35 mg/kg DM, no differences could be observed between both sources. In contrast, the amount of apparently digested Cu was noticeably higher (even if not statistically significant) at a supplementation of 50 mg Cu/kg DM, if $CuSO_4$ served as source (55.6 mg/day vs. 41.1 mg/day).

In summary, the amount of apparently digested Cu was increased in direct correlation with Cu doses, independent of Cu source. At mild excess (50 mg/kg DM), however, Cu from $CuSO_4$ was absorbed to a higher extent, compared to TBCC.

Table 12: Daily Cu intake [mg/day], Cu concentration in faeces [mg/kg DM], daily faecal Cu excretion [mg/day], apparent Cu digestibility [%], and daily amount of apparently digested Cu [mg/day] dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Cu intake	Cu concentration in faeces	Faecal Cu excretion	Apparent Cu digestibility	Amount of digested Cu
$CuSO_4$	10	65^{c}	44.9^{c}	61.4^{c}	5.47	3.56^{c}
	35	228^{b}	147^{b}	203^{b}	10.9	24.7^{b}
	50	325^{a}	193^{a}	267^{a}	17.1	55.6^{a}
TBCC	10	65^{c}	43.8^{c}	62.4^{c}	5.81	3.78^{c}
	35	228^{b}	147^{b}	207^{b}	11.3	25.7^{b}
	50	325^{a}	210^{a}	282^{a}	12.7	41.1^{ab}
	SEM	0.00	6.96	6.78	4.47	6.14
P-value	Treatment[1]	<.0001	<.0001	<.0001	0.36	<0.0001
	Cu source[2]	.	0.31	0.21	0.71	0.34
	linear $CuSO_4$[3]	<.0001	<.0001	<.0001	0.06	<0.0001
	linear TBCC[3]	<.0001	<.0001	<.0001	0.23	<0.0001

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$).

4.1.4 Status parameters of copper in the blood serum

The status parameters of Cu in the blood serum were analysed to obtain further information about Cu absorption and subsequently about Cu homeostasis and Cu regulation mechanisms within the organism. Cu concentration, ceruloplasmin activity, and superoxide dismutase activity in the blood serum are presented in Table 13.

a) Copper concentration

The Cu concentration in the blood serum remained at the same level in spite of Cu supplementation in different doses and sources. The Cu concentrations ranged from 0.82 µg/ml to 0.88 µg/ml at $CuSO_4$ and from 0.81 µg/ml to 0.86 µg/ml at TBCC. Consequently, the different Cu treatments had no effect on the Cu concentration in the blood serum.

b) Ceruloplasmin activity

Ceruloplasmin activity in the blood serum had its minimum at 12.6 mU/l and its maximum at 14.5 mU/l. Rising doses of TBCC tended to increase ceruloplasmin activity ($p = 0.06$). Otherwise, no further effects of Cu dose and source could be determined.

c) Superoxide dismutase activity

Superoxide dismutase (SOD) activity (inhibition rate) in the blood serum was not affected by rising Cu doses, independent of Cu source. However, the inhibition rate at the supplementation of TBCC was significantly higher than at $CuSO_4$ (83.0 % vs. 85.3 %, $p = 0.003$). Thus, the supplementation of TBCC appeared to positively affect the SOD activity in serum, compared to $CuSO_4$.

Table 13: Cu concentration [µg/ml], ceruloplasmin activity [mU/l], and superoxide dismutase activity (inhibition rate) [%] in the blood serum, respectively, dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Cu concentration	Ceruloplasmin activity	SOD activity - Inhibition rate
$CuSO_4$	10	0.82	13.1	82.8
	35	0.83	12.6	82.9
	50	0.88	13.3	83.3
TBCC	10	0.83	12.7	84.3
	35	0.81	14.0	85.4
	50	0.86	14.5	86.2
	SEM	0.07	0.73	0.92
P-value	Treatment[1]	0.98	0.28	0.04
	Cu source[2]	0.81	0.16	0.003
	linear $CuSO_4$[3]	0.55	0.93	0.74
	linear TBCC[3]	0.83	0.06	0.12

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.2 Effect of dose and source of copper supplementation on the microbial population in the rumen

The following rumen microorganisms were quantified by qPCR in the rumen fluid: total bacteria, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, *Streptococcus bovis*, archaea, protozoa, and anaerobic fungi. Figure 2 provides an overview of the copy numbers of listed rumen microorganisms during the first three hours after the morning feeding. The amount of the respective microorganisms in the rumen fluid increased continuously during this period except for total bacteria and anaerobic fungi. However, the attention of these results is given to the effect of Cu supplementation on rumen microorganisms within each sampling time rather than the composition of rumen microorganisms in a time course.

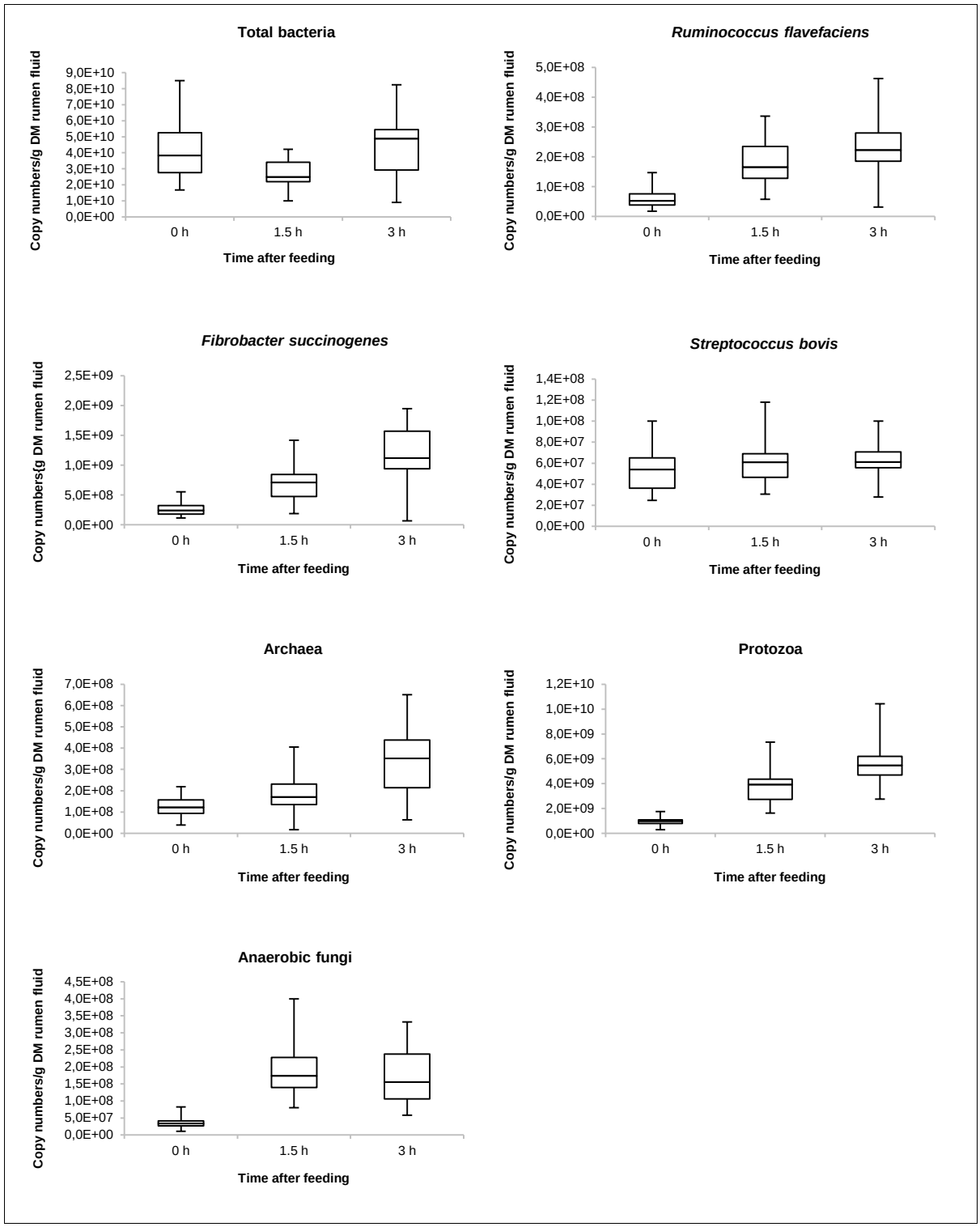

Figure 2: Amount of investigated rumen microorganisms in the rumen fluid [copy numbers/g DM] dependent on sampling time (means across treatments) determined by qPCR analysis

0 h: time of morning feeding; boxplots represent minimum, maximum, and the 25, 50, and 75 % quartiles.

4.2.1 Effect on the microbial population straight before feeding

The concentrations of selected rumen microorganisms (Log_{10} 16S rRNA copy numbers per g DM of rumen fluid; 18S rRNA for protozoa and anaerobic fungi, respectively) right before the morning feeding are shown in Table 14. These results demonstrate the effect of Cu dose and source on the amount of each single microorganism in the rumen fluid.

Additionally, the respective proportions of the selected rumen microorganisms in or relative to total bacteria [%] are presented in Table 15. These results outline possible shifts within the microbial composition in the rumen.

The concentrations of total bacteria, *R. flavefaciens*, *S. bovis*, archaebacteria, and anaerobic fungi were not affected by the supplementation of Cu in different doses and sources. The concentration of *F. succinogenes* was on average lower when $CuSO_4$ was supplemented (log_{10} copy numbers: 8.34 vs. 8.42), however, the figures were statistically not significant ($p = 0.10$). The copy numbers of protozoa were slightly reduced with rising doses of $CuSO_4$ ($p = 0.08$). No significant effect of Cu dose and source on the proportions of all selected microorganisms could be detected. Protozoa had the highest proportion with an average of 2.80 % followed by *F. succinogenes* (0.67 %) and archaebacteria (0.37 %). The lowest proportions were reached by *R. flavefaciens* (0.15 %), *S. bovis* (0.16 %) and anaerobic fungi (0.12 %).

In summary, Cu supplementation differing in dose and source had no consistent effect on the selected microorganisms right before the morning feeding, neither on total counts nor on proportions.

Table 14: Log_{10} 16S rRNA (18S rRNA for protozoa and anaerobic fungi, respectively) copy numbers of selected rumen microorganisms in the rumen fluid [per g DM] right before the morning feeding dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Total bacteria	*Ruminococcus flavefaciens*	*Fibrobacter succinogenes*	*Streptococcus bovis*	Archaea	Protozoa	Anaerobic fungi
$CuSO_4$	10	10.6	7.72	8.35	7.71	8.06	9.08	7.55
	35	10.6	7.72	8.36	7.70	8.07	8.94	7.49
	50	10.5	7.67	8.31	7.67	8.07	8.93	7.47
TBCC	10	10.6	7.75	8.43	7.73	8.12	8.97	7.62
	35	10.6	7.73	8.44	7.70	8.07	8.92	7.50
	50	10.6	7.72	8.40	7.71	8.05	8.99	7.47
	SEM	0.09	0.09	0.07	0.09	0.08	0.07	0.09
P-value	Treatment[1]	0.99	0.99	0.63	1.00	0.99	0.49	0.80
	Cu source[2]	0.79	0.64	0.10	0.74	0.81	0.65	0.70
	linear $CuSO_4$[3]	0.58	0.69	0.67	0.76	0.94	0.08	0.50
	linear TBCC[3]	0.95	0.79	0.79	0.90	0.47	0.92	0.21

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

Table 15: Proportion of selected rumen microorganisms in or relative to total bacteria [%] in the rumen fluid right before the morning feeding dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	*Ruminococcus flavefaciens*	*Fibrobacter succinogenes*	*Streptococcus bovis*	Archaea	Protozoa	Anaerobic fungi
		% in total bacteria	% in total bacteria	% in total bacteria	relative to total bacteria [%]	relative to total bacteria [%]	relative to total bacteria [%]
$CuSO_4$	10	0.13	0.59	0.17	0.34	3.24	0.10
	35	0.15	0.62	0.16	0.36	2.95	0.09
	50	0.14	0.62	0.16	0.41	2.80	0.11
TBCC	10	0.17	0.71	0.17	0.43	2.99	0.13
	35	0.14	0.73	0.14	0.33	2.25	0.10
	50	0.15	0.74	0.16	0.33	2.61	0.08
	SEM	0.03	0.13	0.05	0.11	0.67	0.03
P-value	Treatment[1]	0.92	0.89	1.00	0.97	0.90	0.37
	Cu source[2]	0.49	0.23	0.84	0.93	0.45	0.96
	linear $CuSO_4$[3]	0.85	0.87	0.91	0.66	0.61	0.93
	linear TBCC[3]	0.52	0.86	0.80	0.47	0.59	0.21

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.2.2 Effect on the microbial population 1.5 hours after feeding

Table 16 presents the concentrations of selected rumen microorganisms 1.5 h after the morning feeding and Table 17 the respective proportions in or relative to total bacteria. The different Cu doses and sources showed no significant effects on the concentrations of total bacteria, archaebacteria, protozoa, and anaerobic fungi. The copy numbers of *R. flavefaciens* tended to be reduced by increasing doses of TBCC ($p = 0.08$), but a linear correlation could not be confirmed numerically. The average concentration of *F. succinogenes* was slightly decreased at the $CuSO_4$ treatment, compared to TBCC (log_{10} copy numbers: 8.72 vs. 8.85; $p = 0.09$). A significant negative dose effect could be observed at *S. bovis* ($p = 0.01$), where the concentration declined with increasing doses of $CuSO_4$ (log_{10} copy numbers: 7.83 vs. 7.74 vs. 7.66). The proportions of the selected microorganisms were not affected by the different Cu treatments, except for *F. succinogenes*. In this case, the average proportion was slightly diminished if $CuSO_4$ was supplemented, compared to TBCC (2.30 % vs. 2.81 %; $p = 0.07$).

The concentration of total bacteria declined during the first 1.5 h after feeding. In contrast, the average copy numbers of the single microorganisms increased, respectively. Consequently, the proportions (overall means) of each microorganism increased as well (*R. flavefaciens*: 0.76 % vs. 0.15 %, *F. succinogenes*: 2.56 % vs. 0.67 %, *S. bovis*: 0.23 % vs. 0.16 %, archaebacteria: 0.69 % vs. 0.37 %, protozoa: 15.1 % vs. 2.80 %, anaerobic fungi: 0.73 % vs. 0.12 %).

In total, the supplementation of different Cu doses and sources had no effect on rumen microorganisms and its composition 1.5 h after feeding. However, the concentration of *S. bovis* was negatively affected 1.5 h after feeding by rising doses of $CuSO_4$.

Table 16: Log_{10} 16S rRNA (18S rRNA for protozoa and anaerobic fungi, respectively) copy numbers of selected rumen microorganisms in the rumen fluid [per g DM] 1.5 h after feeding dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Total bacteria	*Ruminococcus flavefaciens*	*Fibrobacter succinogenes*	*Streptococcus bovis*	Archaea	Protozoa	Anaerobic fungi
$CuSO_4$	10	10.4	8.22	8.71	7.83	8.23	9.56	8.24
	35	10.4	8.27	8.72	7.74	8.21	9.56	8.25
	50	10.3	8.16	8.73	7.66	8.11	9.47	8.22
TBCC	10	10.5	8.34	8.94	7.82	8.30	9.62	8.34
	35	10.3	8.16	8.72	7.71	8.14	9.55	8.14
	50	10.4	8.21	8.90	7.82	8.31	9.54	8.23
	SEM	0.07	0.06	0.10	0.05	0.12	0.07	0.07
P-value	Treatment[1]	0.50	0.26	0.32	0.05	0.78	0.73	0.46
	Cu source[2]	0.48	0.68	0.09	0.28	0.49	0.42	0.99
	linear $CuSO_4$[3]	0.44	0.61	0.89	0.01	0.50	0.37	0.87
	linear TBCC[3]	0.55	0.08	0.60	0.82	0.92	0.39	0.16

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

Table 17: Proportion of selected rumen microorganisms in or relative to total bacteria [%] in the rumen fluid 1.5 h after feeding dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	*Ruminococcus flavefaciens*	*Fibrobacter succinogenes*	*Streptococcus bovis*	Archaea	Protozoa	Anaerobic fungi
		% in total bacteria	% in total bacteria	% in total bacteria	relative to total bacteria [%]	relative to total bacteria [%]	relative to total bacteria [%]
$CuSO_4$	10	0.82	2.13	0.27	0.69	15.3	0.72
	35	0.75	2.05	0.21	0.65	15.4	0.71
	50	0.83	2.73	0.22	0.65	14.6	0.80
TBCC	10	0.78	2.90	0.22	0.67	15.3	0.78
	35	0.71	2.61	0.24	0.72	16.8	0.74
	50	0.64	2.92	0.24	0.78	12.9	0.61
	SEM	0.19	0.36	0.03	0.10	2.92	0.12
P-value	Treatment[1]	0.97	0.28	0.58	0.91	0.95	0.88
	Cu source[2]	0.53	0.07	1.00	0.43	0.98	0.72
	linear $CuSO_4$[3]	0.98	0.26	0.11	0.76	0.87	0.68
	linear TBCC[3]	0.58	0.95	0.55	0.41	0.62	0.32

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.2.3 Effect on the microbial population 3 hours after feeding

The concentrations of selected rumen microorganisms 3 h after feeding are shown in Table 18 and the respective proportions in or relative to total bacteria in Table 19. No effect of Cu dose and source could be detected for the concentrations of all selected microorganisms. The proportions of *R. flavefaciens*, archaebacteria, protozoa, and anaerobic fungi were also not affected. In case of *F. succinogenes*, a significant source effect could be observed due to a reduction of the proportion in total bacteria if $CuSO_4$ served as source, compared to TBCC (2.45 % vs. 2.98 %; p = 0.05). The proportion of *S. bovis* showed a slight decrease with rising supplementation of $CuSO_4$ (0.24 % vs. 0.17 % vs. 0.15 %; p = 0.09).

Compared to the previous sampling time (1.5 h), the average concentrations of total bacteria, *F. succinogenes*, archaebacteria, and protozoa increased. Accordingly, the proportions showed the same trend (*F. succinogenes*: 2.72 % vs. 2.56 %, archaebacteria: 0.83 % vs. 0.69 %, protozoa: 16.4 % vs. 15.1 %). The concentration of *R. flavefaciens* increased but the proportion in total bacteria showed a decline (0.59 % vs. 0.76 %). *S. bovis* in total remained approximately at the same level while the proportion decreased (0.18 % vs. 0.23 %). Concentration of anaerobic fungi as well as the proportion relative to total bacteria were lower compared to 1.5 h after feeding (0.47 % vs. 0.73 %).

Concentrations of all selected microorganism were not affected by varying Cu treatments 3 h after feeding. Regarding the proportions in or relative to total bacteria, $CuSO_4$ reduced on average the amount of *F. succinogenes*, compared to TBCC. This negative effect of $CuSO_4$ on *F. succinogenes* was already indicated at 0 h and 1.5 h after feeding.

Table 18: Log_{10} 16S rRNA (18S rRNA for protozoa and anaerobic fungi, respectively) copy numbers of selected rumen microorganisms in the rumen fluid [per g DM] 3 h after feeding dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Total bacteria	*Ruminococcus flavefaciens*	*Fibrobacter succinogenes*	*Streptococcus bovis*	Archaea	Protozoa	Anaerobic fungi
$CuSO_4$	10	10.5	8.27	8.80	7.79	8.37	9.74	8.17
	35	10.6	8.21	8.93	7.77	8.48	9.72	8.12
	50	10.6	8.42	9.02	7.77	8.49	9.67	8.22
TBCC	10	10.6	8.36	9.08	7.83	8.55	9.76	8.24
	35	10.6	8.34	9.06	7.78	8.50	9.79	8.24
	50	10.6	8.27	9.10	7.82	8.56	9.73	8.16
	SEM	0.11	0.09	0.14	0.05	0.10	0.06	0.09
P-value	Treatment[1]	0.93	0.55	0.59	0.91	0.76	0.63	0.84
	Cu source[2]	0.51	0.75	0.14	0.42	0.24	0.20	0.49
	linear $CuSO_4$[3]	0.37	0.29	0.24	0.79	0.38	0.37	0.74
	linear TBCC[3]	0.86	0.48	0.93	0.85	1.00	0.70	0.54

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

Table 19: Proportion of selected rumen microorganisms in or relative to total bacteria [%] in the rumen fluid 3 h after feeding dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	*Ruminococcus flavefaciens*	*Fibrobacter succinogenes*	*Streptococcus bovis*	Archaea	Protozoa	Anaerobic fungi
		% in total bacteria	% in total bacteria	% in total bacteria	relative to total bacteria [%]	relative to total bacteria [%]	relative to total bacteria [%]
$CuSO_4$	10	0.77	2.23	0.24	0.77	18.9	0.61
	35	0.47	2.59	0.17	0.82	19.4	0.42
	50	0.68	2.54	0.15	0.77	13.2	0.48
TBCC	10	0.57	2.84	0.16	0.87	14.8	0.45
	35	0.54	2.87	0.15	0.79	17.4	0.46
	50	0.51	3.24	0.17	0.94	14.3	0.38
	SEM	0.15	0.34	0.04	0.09	4.79	0.12
P-value	Treatment[1]	0.67	0.34	0.53	0.71	0.87	0.76
	Cu source[2]	0.39	0.05	0.40	0.28	0.64	0.43
	linear $CuSO_4$[3]	0.53	0.46	0.09	0.95	0.42	0.34
	linear TBCC[3]	0.78	0.42	0.91	0.69	1.00	0.71

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.3 Effect of dose and source of copper supplementation on ruminal degradation characteristics

A part of this study was to investigate the effect of dose and source of Cu supplementation on ruminal degradation characteristics. Therefore, the TMR and its single components were incubated in the rumen using the *in sacco* technique. Figure 3 provides an overview of the dry matter disappearance of the incubated feedstuffs along the time course of 48 h. About 65 % of TMR, grass silage, maize silage, and soybean meal were degraded during the first 12 h of incubation. The maximum of degradation was reached after 48 h of incubation (between 80 and 95 %). Degradation of wheat meal, however, reached its maximum (about 95 %) already after 9 h of incubation. The differences of the degradation rates between silages and concentrates give first insights in their varying ruminal degradation characteristics, which are described in detail in the following section.

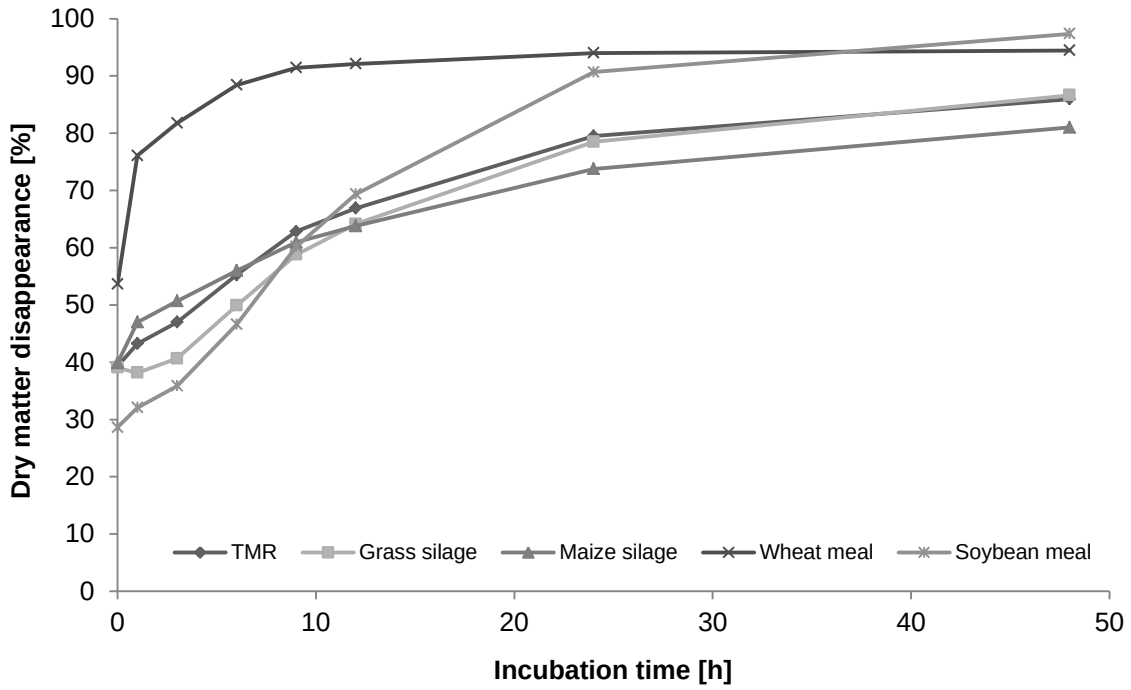

Figure 3: Ruminal dry matter disappearance [%] of incubated feedstuffs (means across treatments)

Following p-values (2-way ANOVA) indicate significant differences between incubation times: TMR ($p < 0.0001$, SEM 0.25), grass silage ($p < 0.0001$, SEM 0.28), maize silage ($p < 0.0001$, SEM 0.23), wheat meal ($p < 0.0001$, SEM 0.19), soybean meal ($p < 0.0001$, SEM 0.37).

4.3.1 Ruminal dry matter degradability of TMR

4.3.1.1 *In sacco* dry matter disappearance

The dry matter disappearance (DMD) of TMR is shown in Table 20. The statistical analysis revealed an enhanced DMD at incubation times of 3 h, 6 h, 9 h and 12 h (1.2 %, 4.3 %, 3.4 %, and 2.6 %) with increasing doses of $CuSO_4$. Thereby, the DMD at 50 mg Cu/kg DM from $CuSO_4$ is noticeably higher than at 10 mg Cu/kg DM and 35 mg Cu/kg DM, especially between 6 h and 12 h (58.1 %, 65.0 %, 68.3 %). In contrast, the rising supplementation of TBCC led to a reduced DMD at 9 h and 48 h of incubation (4.2 % and 0.6 %). A statistically significant source effect could not be observed.

The supplementation of $CuSO_4$ at mild excess stimulated the DMD of TMR between 3 h and 12 h after feeding. Varying doses of TBCC, however, showed no effect until 6 h and reduced the DMD at 9 h and 48 h post feeding.

Table 20: Ruminal dry matter disappearance [%] of TMR dependent on Cu dose and source as well as on incubation time

Cu source	Cu dose [mg/kg DM]	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
$CuSO_4$	10	43.4	46.1^{b}	53.8^{b}	61.6^{b}	65.7	79.8	86.1
	35	43.2	46.8^{ab}	54.5^{b}	61.5^{b}	66.9	79.6	86.0
	50	43.4	47.3^{ab}	58.1^{a}	65.0^{a}	68.3	78.9	86.2
TBCC	10	43.2	47.7^{a}	55.1^{b}	65.1^{a}	66.3	79.2	86.2
	35	42.7	47.1^{ab}	53.7^{b}	62.9^{ab}	67.9	79.4	85.7
	50	43.6	47.0^{ab}	56.1^{b}	60.9^{b}	66.3	80.2	85.6
	SEM	0.28	0.34	0.62	0.81	0.78	0.46	0.19
P-value	Treatment[1]	0.36	0.11	<0.0001	0.001	0.21	0.44	0.21
	Cu source[2]	0.54	0.09	0.34	0.63	0.78	0.66	0.13
	linear $CuSO_4$[3]	0.80	0.04	<0.0001	0.01	0.03	0.26	0.84
	linear TBCC[3]	0.38	0.17	0.46	0.001	0.86	0.13	0.05

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$) within the respective incubation time.

4.3.1.2 Parameters of degradability and effective degradability

Table 21 represents the parameters of degradability (a, b, c, t_0), the totally degradable fraction (TDF) and the effective degradability (ED) of dry matter of TMR. The insoluble, but ruminally degradable fraction (b) declined by 3.3 % with increasing doses of $CuSO_4$. Consequently, the totally degradable fraction (TDF) declined as well due to constant values in the soluble fraction (a). The constant rate of degradation (c) of fraction b showed increased values along with rising doses of $CuSO_4$, but only verified by a statistical trend ($p = 0.09$). Nevertheless, the constant rate of degradation was considerably higher at 50 mg Cu/kg DM, compared to 35 mg Cu/kg DM and 10 mg Cu/kg DM in form of $CuSO_4$, respectively (8.81 %/h vs. 7.56 %/h vs. 7.32 %/h). Additionally, the effective degradability was calculated with regards to rumen passage rates of 2, 5, and 8 %/h (ED2, ED5, ED8). No effect of Cu treatment on ED2 could be observed whereas ED5 and ED8 numerically increased by 1.3 % and 1.7 % combined with rising doses of $CuSO_4$. All in all, rising doses of $CuSO_4$ had a decreasing effect on the insoluble part of ruminally degradable fraction of TMR while the constant rate of degradation as well as the effective degradability were influenced positively. The supplementation of TBCC remained consistently ineffective.

Table 21: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of TMR dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
$CuSO_4$	10	39.4	50.6	7.32	0.70	90.0	77.3	67.1	61.1
	35	39.3	48.9	7.56	0.62	88.3	77.0	67.3	61.4
	50	39.4	47.3	8.81	0.53	86.7	77.2	68.4	62.8
TBCC	10	39.3	48.1	7.82	0.45	87.4	77.1	67.8	62.0
	35	39.3	48.1	7.95	0.78	87.4	76.9	67.4	61.6
	50	39.4	48.6	7.47	0.47	88.0	77.0	67.4	61.6
	SEM	0.02	0.42	0.27	6.17	0.42	0.01	0.01	0.29
P-value	Treatment[1]	0.73	0.20	0.49	0.46	0.21	0.97	0.67	0.52
	Cu source[2]	0.76	0.36	0.74	0.66	0.36	0.52	0.91	0.96
	linear $CuSO_4$[3]	0.95	0.02	0.09	0.39	0.02	0.80	0.15	0.09
	linear TBCC[3]	0.22	0.73	0.72	0.70	0.69	0.83	0.62	0.60

a: soluble fraction, b: insoluble, but ruminally degradable fraction, c: constant rate of degradation of b, t_0: lag time; TDF: totally degradable fraction; 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: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$;

[1] P-value of ANOVA (6 treatments).

[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).

[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.3.2 Ruminal dry matter degradability of grass silage

4.3.2.1 *In sacco* dry matter disappearance

The DMD of grass silage is shown in Table 22. Rising doses of $CuSO_4$ significantly increased the DMD by 3.3 %, 5.1 %, and 2.8 % at 6 h, 9 h, and 12 h after feeding, respectively. The rising supplementation of TBCC had a significantly negative effect on the DMD at incubation times of 9 h and 48 h (4.4 % and 1.2 %). The DMD at 24 h after feeding was slightly increased by rising doses of TBCC (1.6 %). The comparison of the average DMD of both sources resulted in a marginally but significantly higher value at $CuSO_4$, compared to TBCC, after 48 h (86.9 % vs. 86.3 %).

The enhanced supplementation of $CuSO_4$ positively affected the DMD of grass silage between 6 h and 12 h after feeding. In contrast, the increasing supplementation of TBCC remained ineffective until 6 h and reduced the DMD selectively at 9 h and 48 h post feeding.

Table 22: Ruminal dry matter disappearance [%] of grass silage dependent on Cu dose and source as well as on incubation time

Cu source	Cu dose [mg/kg DM]	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
$CuSO_4$	10	38.3	40.5	48.7^{b}	56.3^{b}	62.2	78.5	87.0
	35	38.1	40.3	49.8^{ab}	58.4^{ab}	64.4	78.6	87.0
	50	38.1	40.8	52.0^{a}	61.4^{a}	65.0	77.7	86.8
TBCC	10	38.4	41.3	49.8^{ab}	61.1^{a}	63.5	78.1	87.1
	35	38.0	40.3	48.5^{b}	58.8^{ab}	65.4	78.3	86.0
	50	38.0	40.7	50.5^{ab}	56.7^{b}	64.3	79.7	85.9
	SEM	0.23	0.34	0.76	0.99	0.99	0.56	0.30
P-value	Treatment[1]	0.70	0.36	0.01	<0.001	0.23	0.20	0.01
	Cu source[2]	0.82	0.49	0.32	0.84	0.54	0.32	0.01
	linear $CuSO_4$[3]	0.53	0.65	0.003	<0.001	0.04	0.41	0.61
	linear TBCC[3]	0.16	0.14	0.70	0.001	0.53	0.06	0.003

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$) within the respective incubation time.

4.3.2.2 Parameters of degradability and effective degradability

The parameters of degradability and the effective degradability of dry matter of grass silage are depicted in Table 23. The lag time (t_0) was significantly reduced by 1.01 h with increasing doses of $CuSO_4$. The remaining parameters were not significantly affected, neither by Cu dose nor by Cu source. However, the constant rate of degradation (c) showed a noticeably enhanced value at a dose of 50 mg Cu/kg DM, compared to 35 mg Cu/kg DM and 10 mg Cu/kg DM in form of $CuSO_4$, respectively (8.65 %/h vs. 7.96 %/h vs. 7.52 %/h). The effective degradability was not significantly affected by Cu dose and source but ED5 and ED8 were increased numerically (1.3 % and 1.7 %) with rising doses of $CuSO_4$.

Increasing doses of $CuSO_4$ accelerated the onset of ruminal degradation of grass silage. Apart from that, Cu treatment could not cause remarkable effects on parameters of degradability and effective degradability.

Table 23: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of grass silage dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
$CuSO_4$	10	38.9	50.6	7.52	3.29	89.5	76.0	64.4	57.6
	35	38.5	50.3	7.96	2.64	88.8	76.2	65.2	58.5
	50	38.6	49.1	8.65	2.28	87.7	76.1	65.7	59.3
TBCC	10	38.8	50.4	8.48	2.50	89.2	76.3	65.4	58.8
	35	38.8	47.8	9.80	3.37	86.6	75.4	64.9	58.3
	50	38.5	49.5	8.02	2.66	88.0	75.8	64.9	58.3
	SEM	0.11	0.43	0.37	0.16	0.44	0.01	0.02	0.35
P-value	Treatment[1]	0.84	0.30	0.43	0.19	0.29	0.87	0.85	0.72
	Cu source[2]	0.96	0.33	0.27	0.71	0.35	0.57	0.96	0.98
	linear $CuSO_4$[3]	0.36	0.30	0.33	0.05	0.21	0.81	0.21	0.12
	linear TBCC[3]	0.45	0.33	0.85	0.56	0.26	0.43	0.63	0.62

a: soluble fraction, b: insoluble, but ruminally degradable fraction, c: constant rate of degradation of b, t_0: lag time; TDF: totally degradable fraction; 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: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$;
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.3.3 Ruminal dry matter degradability of maize silage

4.3.3.1 *In sacco* dry matter disappearance

Table 24 represents the DMD of maize silage. At the incubation times of 6 h, 9 h, and 12 h rising doses of $CuSO_4$ resulted in higher DMD (1.9 %, 2.7 %, 2.2 %, respectively). The increased supplementation of TBCC significantly reduced the DMD 3 h (1.2 %) and 9 h (2.3 %) after feeding. A difference between the average DMD of both sources was determined at 12 h of incubation, where the TBCC treatment showed a significantly higher value, compared to $CuSO_4$ (64.6 % vs. 63.0 %).

In summary, $CuSO_4$ was able to induce higher DMD of maize silage between 6 h and 12 h after feeding, especially when supplemented at mild excess. The supplementation of TBCC at increasing doses selectively diminished the DMD at incubation times of 3 h and 9 h.

Table 24: Ruminal dry matter disappearance [%] of maize silage dependent on Cu dose and source as well as on incubation time

Cu source	Cu dose [mg/kg DM]	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
$CuSO_4$	10	47.8	50.3	55.8^{ab}	59.8^{b}	62.3	73.6	81.3
	35	46.5	50.7	54.5^{b}	60.3^{ab}	62.3	73.0	80.8
	50	46.8	50.9	57.7^{a}	62.5^{a}	64.5	73.6	81.0
TBCC	10	47.0	51.6	56.7^{ab}	62.4^{a}	64.3	73.9	80.8
	35	47.0	50.1	54.9^{b}	60.7^{ab}	64.5	73.3	80.8
	50	46.9	50.4	56.4^{ab}	60.1^{ab}	64.9	75.1	81.3
	SEM	0.49	0.49	0.65	0.65	0.74	0.52	0.34
P-value	Treatment[1]	0.52	0.27	0.01	0.01	0.03	0.10	0.82
	Cu source[2]	0.93	0.91	0.86	0.84	0.01	0.10	0.65
	linear $CuSO_4$[3]	0.10	0.38	0.11	0.01	0.06	0.84	0.39
	linear TBCC[3]	0.89	0.05	0.50	0.01	0.54	0.18	0.52

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$) within the respective incubation time.

4.3.3.2 Parameters of degradability and effective degradability

The parameters of degradability and the effective degradability of dry matter of maize silage are shown in Table 25. Increasing supplementation of $CuSO_4$ led to a slight decline of the totally degradable fraction (TDF) by 2.3 %. The constant rate of degradation (c) of fraction b had a notable maximum (8.16 %) at 50 mg/kg $CuSO_4$, which was, however, statistically not significant.

The parameters of degradability and the effective degradability of maize silage were not statistically significant affected by different doses and sources of Cu.

Table 25: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of maize silage dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
$CuSO_4$	10	42.0	41.2	7.00	0.00	83.2	73.2	65.2	60.5
	35	41.8	40.9	6.58	0.05	82.8	72.7	64.5	59.8
	50	41.5	39.3	8.16	0.00	80.9	73.0	65.7	61.2
TBCC	10	41.8	39.4	7.83	0.00	81.1	73.0	65.6	61.1
	35	41.4	40.5	7.22	0.00	81.9	72.8	65.0	60.4
	50	41.4	40.9	7.30	0.00	82.3	73.4	65.6	60.8
	SEM	0.17	0.42	0.24	0.01	0.41	0.01	0.01	0.30
P-value	Treatment[1]	0.81	0.53	0.32	0.44	0.39	0.93	0.72	0.63
	Cu source[2]	0.40	0.73	0.63	0.33	0.48	0.79	0.60	0.63
	linear $CuSO_4$[3]	0.37	0.18	0.18	0.78	0.08	0.67	0.63	0.55
	linear TBCC[3]	0.48	0.23	0.42	1.00	0.36	0.61	0.85	0.68

a: soluble fraction, b: insoluble, but ruminally degradable fraction, c: constant rate of degradation of b, t_0: lag time; TDF: totally degradable fraction; 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: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$;
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.3.4 Ruminal dry matter degradability of wheat meal

4.3.4.1 *In sacco* dry matter disappearance

The DMD of wheat meal is depicted in Table 26. Increasing doses of $CuSO_4$ led to a significant increment of the DMD between 6 h and 12 h after feeding (4.2 %, 2.2 %, 1.2 %, respectively). In contrast to this, the DMD decreased with rising supplementation of TBCC after 3 h (1.5 %) and 9 h (1.4 %) of incubation but increased by 2.0 % after 6 h of incubation. Effects of Cu source could not be observed.

The increasing supplementation of Cu in form of $CuSO_4$ stimulated the DMD continuously between 6 h and 12 h after feeding whereas the increased supplementation of Cu in form of TBCC had no consistent effect on DMD.

Table 26: Ruminal dry matter disappearance [%] of wheat meal dependent on Cu dose and source as well as on incubation time

Cu source	Cu dose [mg/kg DM]	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
$CuSO_4$	10	76.5	81.1	86.4^{c}	90.2^{b}	91.5	94.0	94.4
	35	75.8	81.5	87.7^{bc}	91.6^{ab}	92.1	94.0	94.4
	50	75.5	82.2	90.6^{a}	92.4^{a}	92.7	94.0	94.4
TBCC	10	76.5	82.6	87.7^{bc}	92.1^{a}	92.1	94.1	94.6
	35	76.0	82.0	88.5^{abc}	91.5^{ab}	92.5	94.0	94.5
	50	76.2	81.1	89.7^{ab}	90.7^{ab}	91.6	94.0	94.4
	SEM	0.53	0.62	0.66	0.44	0.39	0.12	0.11
P-value	Treatment[1]	0.71	0.34	<0.001	0.003	0.23	0.96	0.79
	Cu source[2]	0.52	0.43	0.43	0.79	0.94	0.65	0.22
	linear $CuSO_4$[3]	0.15	0.24	<0.0001	<0.001	0.05	0.63	0.98
	linear TBCC[3]	0.69	0.06	0.03	0.03	0.43	0.67	0.39

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$) within the respective incubation time.

4.3.4.2 Parameters of degradability and effective degradability

Table 27 represents the parameters of degradability and the effective degradability of dry matter of wheat meal. Increasing doses of $CuSO_4$ reduced the soluble fraction (a) by 1.1 % and concurrently enhanced the insoluble, but ruminally degradable fraction (b) by 1.7 %. Summed up, the totally degradable fraction (TDF) was slightly increased by 0.7 %.

Additionally, rising doses of $CuSO_4$ revealed higher values for ED2 (0.9 %; p = 0.01), ED5 (1.1 %; p = 0.04) and ED8 (1.1 %; p = 0.08).

All in all, $CuSO_4$ positively affected the degradable fractions as well as the effective degradability with increasing doses. The TBCC treatment remained without any effect.

Table 27: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of wheat meal dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
$CuSO_4$	10	55.2	37.6	44.1	0.00	92.8	90.9	88.5	86.5
	35	54.6	38.3	44.3	0.00	92.9	91.2	88.9	86.9
	50	54.1	39.3	46.5	0.00	93.5	91.8	89.6	87.6
TBCC	10	54.6	38.4	46.8	0.00	93.0	91.4	89.2	87.3
	35	54.4	38.6	46.7	0.00	93.0	91.4	89.2	87.2
	50	54.4	38.5	47.3	0.00	92.9	91.2	89.0	87.0
	SEM	0.12	0.14	1.71	0.00	0.11	0.01	0.01	0.20
P-value	Treatment[1]	0.17	0.02	0.98	.	0.47	0.20	0.37	0.56
	Cu source[2]	0.45	0.78	0.52	.	0.65	0.97	0.67	0.61
	linear $CuSO_4$[3]	0.01	0.001	0.67	.	0.08	0.01	0.04	0.08
	linear TBCC[3]	0.64	0.77	0.93	.	0.90	0.74	0.71	0.71

a: soluble fraction, b: insoluble, but ruminally degradable fraction, c: constant rate of degradation of b, t_0: lag time; TDF: totally degradable fraction,; 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: $ED = a + [(b \times c) / (c+k)] e^{-kt0}$;
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments (p < 0.05).

4.3.5 Ruminal dry matter degradability of soybean meal

4.3.5.1 *In sacco* dry matter disappearance

The DMD of soybean meal is shown in Table 28. Increasing doses of $CuSO_4$ improved the DMD at 6 h (2.4 %) and highly significant at 9 h (7.8 %) after feeding. Higher amounts of TBCC, however, decreased the DMD by 6.0 % after 9 h of incubation. Differences between both sources regarding the average DMD were not observed.

The enhanced supplementation of $CuSO_4$ positively affected the DMD of soybean meal between 6 h and 9 h after feeding. In contrast, the increased supplementation of TBCC remained ineffective except for a selective reduction of DMD at 9 h post feeding.

Table 28: Ruminal dry matter disappearance [%] of soybean meal dependent on Cu dose and source as well as on incubation time

Cu source	Cu dose [mg/kg DM]	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
$CuSO_4$	10	32.0	36.1	46.0^{ab}	55.8^{b}	68.9	91.0	97.3
	35	31.8	35.0	47.1^{ab}	59.5^{b}	68.6	90.0	97.3
	50	32.3	35.6	48.4^{a}	63.6^{a}	69.6	90.4	97.5
TBCC	10	32.5	36.6	46.6^{ab}	64.7^{a}	69.0	91.0	97.5
	35	32.0	36.1	44.4^{b}	58.9^{b}	69.5	89.4	97.4
	50	31.8	35.6	47.1^{ab}	58.7^{b}	70.3	92.2	97.3
	SEM	0.29	0.47	0.92	1.22	1.37	0.77	0.11
P-value	Treatment[1]	0.51	0.22	0.07	<0.0001	0.94	0.17	0.60
	Cu source[2]	0.79	0.16	0.10	0.28	0.61	0.45	0.93
	linear $CuSO_4$[3]	0.47	0.34	0.06	<0.0001	0.58	0.61	0.20
	linear TBCC[3]	0.12	0.13	0.95	<0.001	0.50	0.40	0.19

[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$) within the respective incubation time.

4.3.5.2 Parameters of degradability and effective degradability

Table 29 represents the parameters of degradability and the effective degradability of dry matter of soybean meal. The supplementation of different Cu doses and sources showed no significant effects, neither on parameters of degradability nor on effective degradability.

Table 29: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of soybean meal dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
$CuSO_4$	10	29.5	70.3	8.24	2.38	99.8	83.3	68.3	59.2
	35	29.0	70.8	8.07	1.77	99.8	83.6	68.8	59.7
	50	29.2	69.8	9.28	1.83	99.0	84.0	69.9	61.0
TBCC	10	28.9	70.4	8.88	1.76	99.3	84.2	69.9	60.9
	35	29.5	70.1	8.21	2.07	99.6	83.3	68.5	59.4
	50	29.1	70.9	8.49	2.02	100	84.1	69.4	60.2
	SEM	0.16	0.26	0.28	0.22	0.13	0.01	0.02	0.34
P-value	Treatment[1]	0.78	0.73	0.67	0.93	0.13	0.69	0.45	0.38
	Cu source[2]	0.80	0.71	0.99	0.90	0.67	0.57	0.67	0.73
	linear $CuSO_4$[3]	0.39	0.66	0.28	0.38	0.06	0.34	0.14	0.11
	linear TBCC[3]	0.66	0.61	0.58	0.66	0.12	0.81	0.49	0.39

a: soluble fraction, b: insoluble, but ruminally degradable fraction, c: constant rate of degradation of b, t_0: lag time; TDF: totally degradable fraction; 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: $ED = a + [(b \times c) / (c+k)]\ e^{-kt0}$;
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.4 Effect of dose and source of copper supplementation on rumen physiological parameters

For the investigation of rumen physiological parameters, rumen fluid was sampled in intervals of 1.5 h at seven different times starting with the morning feeding (0 h). Figure 4 shows the concentrations of volatile fatty acids and ammonia-nitrogen in the rumen fluid in a time course between morning and afternoon feeding. The concentrations of acetic acid, propionic acid, butyric acid, and valeric acid increased directly after feeding. In the course of time after feeding, the concentrations decreased to the basal level of 0 h. The concentration of ammonia-nitrogen increased by factor 3 after 1.5 h and reached the starting level after 9 h. The relation between sampling time and concentrations was highly significant for volatile fatty acids and ammonia-nitrogen, respectively, and illustrates the characteristic course of rumen physiological parameters during the day. This study, however, focused on the effect of dose and source of Cu supplementation on rumen physiological parameters at each sampling time, which is presented in the next passage.

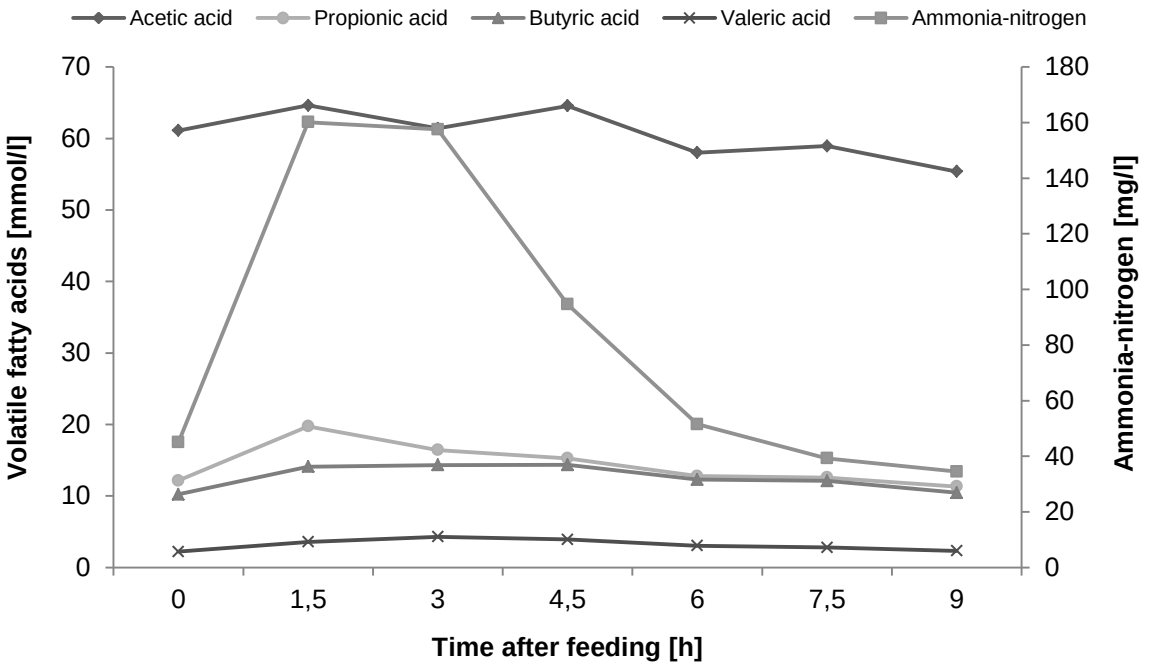

Figure 4: Time course of volatile fatty acid and ammonia-nitrogen concentrations in the rumen fluid (means across treatments)

Following p-values (2-way ANOVA) indicate significant differences between sampling times (0 h: time of morning feeding): acetic acid ($p < 0.0001$, SEM 1.52), propionic acid ($p < 0.0001$, SEM 0.43), butyric acid ($p < 0.0001$, SEM 0.35), valeric acid ($p < 0.0001$, SEM 0.11), ammonia-nitrogen ($p < 0.0001$, SEM 4.17).

4.4.1 pH-value

The pH-values in the rumen fluid are shown in Table 30. The pH-values showed a typical curve regarding the consecutive sampling times. In the morning (0 h), pH-values from 6.93 to 7.01 were measured. Between 1.5 h and 3 h, rumen pH reached its minimum regarding the different Cu treatments (6.61 - 6.82). After that, the pH-values increased to at least 6.99 after 9 h, comparable with the level of the morning feeding.

Rising doses of TBCC tended to decrease rumen pH 1.5 h after feeding. In total, however, Cu dose and source did not affect rumen pH.

Table 30: pH-value in the rumen fluid dependent on Cu dose and source as well as on sampling time

Cu source	Cu dose [mg/kg DM]	Time after feeding						
		0 h	**1.5 h**	**3 h**	**4.5 h**	**6 h**	**7.5 h**	**9 h**
$CuSO_4$	10	7.01	6.77	6.73	6.79	6.79	6.85	7.02
	35	6.99	6.61	6.74	6.72	6.85	6.89	7.04
	50	6.95	6.69	6.76	6.94	6.92	6.93	7.10
TBCC	10	7.00	6.82	6.88	6.92	7.01	7.05	7.15
	35	6.93	6.70	6.85	6.80	6.84	6.92	7.05
	50	6.95	6.68	6.80	6.84	6.79	6.82	6.99
	SEM	0.04	0.03	0.04	0.05	0.05	0.05	0.04
P-value	Treatment[1]	0.62	0.12	0.69	0.60	0.57	0.74	0.84
	Cu source[2]	0.43	0.34	0.13	0.63	0.76	0.70	0.94
	linear $CuSO_4$[3]	0.29	0.17	0.75	0.35	0.36	0.62	0.56
	linear TBCC[3]	0.30	0.06	0.54	0.51	0.11	0.14	0.22

0 h: time of morning feeding
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.4.2 Ammonia-nitrogen

The ammonia-nitrogen (NH_3-N) concentrations in the rumen fluid are represented in Table 31. Supplementation of Cu in different doses and sources had no significant effect on NH_3-N concentrations. However, when comparing both Cu sources the increase after the morning feeding showed differences. The maximum at $CuSO_4$ was continuously reached 3 h after feeding (162 mg/l - 171 mg/l), whereas the maximum for all doses at TBCC was determined 1.5 h after feeding (162 mg/l - 163 mg/l). Afterwards, the NH_3-N concentrations continuously decreased and fell under the basal level of the first measurement in the morning at 9 h post feeding. At this time, the values at $CuSO_4$ treatments were slightly higher than at TBCC treatments ($p < 0.08$).

In summary, the different Cu doses and sources had no statistically significant effect on NH_3-N concentrations in the rumen fluid but the maximum of NH_3-N concentration was numerically delayed at $CuSO_4$ treatments from 1.5 h to 3 h after feeding.

Table 31: Ammonia-nitrogen concentration [mg/l] in the rumen fluid dependent on Cu dose and source as well as on sampling time

Cu source	Cu dose [mg/kg DM]	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
$CuSO_4$	10	43.4	157	162	91.0	51.7	39.6	35.8
	35	48.6	163	167	114	50.3	40.1	36.1
	50	45.2	153	171	87.6	54.5	45.7	38.2
TBCC	10	48.1	163	155	83.8	41.2	37.4	34.5
	35	39.2	162	140	85.4	45.1	31.5	30.7
	50	45.5	163	151	106	66.2	41.2	31.5
	SEM	3.89	14.5	16.7	13.6	7.19	6.56	3.30
P-value	Treatment[1]	0.47	0.99	0.75	0.44	0.16	0.69	0.48
	Cu source[2]	0.61	0.67	0.17	0.58	0.80	0.31	0.08
	linear $CuSO_4$[3]	0.61	0.91	0.68	0.97	0.80	0.51	0.60
	linear TBCC[3]	0.45	0.99	0.80	0.26	0.02	0.77	0.44

0 h: time of morning feeding
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.4.3 Volatile fatty acids

The results of volatile fatty acids (VFA) concentrations in the rumen fluid are listed below. The total VFA concentration (sum of acetic acid, propionic acid, butyric acid, and valeric acid) and the ratio of acetic to propionic acid were calculated.

4.4.3.1 Total volatile fatty acids

Table 32 represents the total volatile fatty acid (VFA) concentration in the rumen fluid. There was no statistically significant effect of different Cu treatments on the total VFA concentration in the rumen fluid between 0 h and 7.5 h. A correlation ($p = 0.04$) of total VFA concentration and Cu doses could be observed within the TBCC treatment 9 h after the morning feeding due to the particularly high value (91.4 mmol/l) at the supplementation of 50 mg Cu/kg DM in form of TBCC. The concentration straight before the morning feeding was 85.7 mmol/l and reached its peak with 102 mmol/l after 1.5 h (overall means). Afterwards, the total VFA concentration decreased below the basal level (0 h) to 79.5 mmol/l after 9 h (overall mean).

Generally, the VFA concentration in the rumen fluid was not affected by varying Cu treatments.

Table 32: Total volatile fatty acid concentration [mmol/l] in the rumen fluid dependent on Cu dose and source as well as on sampling time

Cu source	Cu dose [mg/kg DM]	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
$CuSO_4$	10	80.3	101	102	97.1	88.1	91.9	79.0
	35	84.9	99.7	93.5	106	82.8	81.6	83.0
	50	86.6	106	97.9	95.5	84.3	87.7	72.4
TBCC	10	83.6	101	100	94.1	87.6	82.1	75.4
	35	90.0	102	91.2	95.7	89.0	85.0	75.6
	50	88.8	102	93.6	100	85.2	90.2	91.4
	SEM	5.66	5.06	5.96	6.34	6.51	8.89	5.21
P-value	Treatment[1]	0.80	0.93	0.67	0.71	0.97	0.92	0.10
	Cu source[2]	0.42	0.90	0.51	0.54	0.66	0.85	0.51
	linear $CuSO_4$[3]	0.39	0.47	0.48	1.00	0.61	0.63	0.44
	linear TBCC[3]	0.44	0.77	0.35	0.49	0.82	0.50	0.04

0 h: time of morning feeding
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.4.3.2 Acetic acid

The concentration of acetic acid in the rumen fluid is depicted in Table 33. In the timeframe of 7.5 h after the morning feeding no significant effects of Cu dose and source on the acetic acid concentration could be observed. Before the second feeding (9 h), the statistical analysis resulted a statistical trend ($p = 0.06$) concerning a dose effect within the TBCC treatment arising from the high value (62.9 mmol/l) at 50 mg Cu/kg DM, compared to the remaining values at this time. During the first 4.5 h, the acetic acid concentration stayed on a relative high level between 61.1 mmol/l and 64.6 mmol/l and decreased to a minimum of 55.4 mmol/l after 9 h (overall means).

In total, there was no consistent effect of Cu supplementation on the acetic acid concentration.

Table 33: Acetic acid concentration [mmol/l] in the rumen fluid dependent on Cu dose and source as well as on sampling time

Cu source	Cu dose [mg/kg DM]	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
$CuSO_4$	10	57.4	63.0	64.5	63.8	59.4	62.5	54.8
	35	60.6	63.6	59.8	69.5	55.9	56.0	58.2
	50	62.0	67.7	62.6	63.7	57.1	60.0	50.9
TBCC	10	59.8	64.3	63.8	62.5	59.2	56.5	52.9
	35	64.1	64.2	58.2	62.7	59.8	58.0	52.6
	50	62.8	65.0	59.5	65.2	56.8	60.7	62.9
	SEM	3.98	2.97	3.61	4.09	4.47	6.00	3.62
P-value	Treatment[1]	0.83	0.86	0.70	0.78	0.97	0.95	0.15
	Cu source[2]	0.46	0.90	0.53	0.47	0.73	0.82	0.58
	linear $CuSO_4$[3]	0.37	0.27	0.59	0.88	0.66	0.67	0.53
	linear TBCC[3]	0.51	0.88	0.31	0.64	0.72	0.60	0.06

0 h: time of morning feeding
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.4.3.3 Propionic acid

Table 34 shows the propionic acid concentration in the rumen fluid. Statistically significant effects of different Cu treatments on the propionic acid concentration could be detected 9 h after feeding. The propionic acid concentration was linearly correlated ($p = 0.03$) to Cu doses when TBCC served as source. The comparable high concentration of 13.3 mmol/l at the supplementation of 50 mg Cu/kg DM was decisive for this effect. After feeding, the propionic acid concentration increased rapidly by 38.4 % to 19.8 mmol/l within 1.5 h and declined continuously to the lowest level of 11.3 mmol/l before the second feeding (overall means).

In total, the varying Cu treatments had no consistent effect on the propionic acid concentration in the rumen fluid.

Table 34: Propionic acid concentration [mmol/l] in the rumen fluid dependent on Cu dose and source as well as on sampling time

Cu source	Cu dose [mg/kg DM]	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
$CuSO_4$	10	11.3	20.3	18.2	15.2	13.3	13.6	11.4
	35	12.0	18.8	15.7	16.6	12.1	11.7	11.7
	50	12.2	20.4	16.7	14.5	12.4	12.6	10.1
TBCC	10	11.8	19.1	16.9	14.4	12.8	11.9	10.7
	35	12.9	20.3	15.3	15.1	13.3	12.4	10.8
	50	12.8	19.6	15.8	15.8	12.8	13.3	13.3
	SEM	0.88	1.36	1.39	1.09	0.98	1.38	0.81
P-value	Treatment[1]	0.72	0.92	0.65	0.63	0.92	0.88	0.09
	Cu source[2]	0.33	0.87	0.44	0.69	0.60	0.92	0.41
	linear $CuSO_4$[3]	0.39	0.93	0.34	0.76	0.46	0.53	0.28
	linear TBCC[3]	0.37	0.75	0.49	0.34	0.93	0.44	0.03

0 h: time of morning feeding
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.4.3.4 Butyric acid

Table 35 presents the butyric acid concentration in the rumen fluid. Within the first 7.5 h after feeding no significant effect of different Cu treatments on butyric acid concentration could be detected. After 9 h, the butyric acid concentration correlated ($p = 0.01$) with Cu doses of TBCC due to a significantly increased concentration of 12.3 mmol/l at 50 mg Cu/kg DM. The butyric acid concentration started at a basal level of 10.2 mmol/l prior to an increase up to 14.1 mmol/l within the first 1.5 h (overall means). After keeping this level until 4.5 h, the concentrations dropped almost to the basal level at the end of the time course.

A general effect of different Cu doses and sources on the butyric acid concentration in the rumen fluid could not be observed.

Table 35: Butyric acid concentration [mmol/l] in the rumen fluid dependent on Cu dose and source as well as on sampling time

Cu source	Cu dose [mg/kg DM]	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
$CuSO_4$	10	9.66	14.1	15.4	14.3	12.5	13.0	10.5^{ab}
	35	10.1	13.8	13.9	15.6	11.8	11.2	10.8^{ab}
	50	10.3	14.5	14.4	13.6	11.8	12.2	9.36^{b}
TBCC	10	9.82	13.7	14.8	13.5	12.5	11.3	9.80^{b}
	35	10.7	14.2	13.4	14.1	12.7	11.9	9.97^{b}
	50	10.8	14.3	13.9	14.9	12.4	13.1	12.3^{a}
	SEM	0.71	0.80	0.93	1.10	0.86	1.21	0.66
P-value	Treatment[1]	0.76	0.96	0.60	0.68	0.94	0.73	0.03
	Cu source[2]	0.40	0.90	0.43	0.68	0.46	0.96	0.32
	linear $CuSO_4$[3]	0.51	0.72	0.35	0.75	0.49	0.52	0.26
	linear TBCC[3]	0.27	0.55	0.36	0.37	0.96	0.26	0.01

0 h: time of morning feeding
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.
Different superscripts indicate significant differences between treatments ($p < 0.05$) within the respective sampling time.

4.4.3.5 Valeric acid

The valeric acid concentration in the rumen fluid is depicted in Table 36. In the timeframe of 7.5 h after the morning feeding no statistically significant effect of Cu dose and source on the valeric acid concentration could be observed. Before the second feeding (9 h), statistical analysis revealed a dose effect within the TBCC treatment. At this time, the valeric acid concentration was directly correlated ($p = 0.02$) to the supplemented Cu amount. During the first 3 h after feeding, the concentration increased by 48.3 % to a value of 4.29 mmol/l (overall mean). Afterwards, the concentration declined until the second feeding to 2.31 mmol/l (overall mean), slightly above the starting concentration.

All in all, the valeric acid concentration was not consistently affected by varying Cu treatments.

Table 36: Valeric acid concentration [mmol/l] in the rumen fluid dependent on Cu dose and source as well as on sampling time

Cu source	Cu dose [mg/kg DM]	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
$CuSO_4$	10	1.98	3.44	4.36	3.71	2.95	2.91	2.30
	35	2.28	3.51	4.10	4.37	3.03	2.68	2.43
	50	2.13	3.69	4.24	3.73	2.95	2.90	2.04
TBCC	10	2.15	3.49	4.50	3.66	3.02	2.55	2.07
	35	2.28	3.63	4.17	3.81	3.14	2.74	2.18
	50	2.47	3.68	4.38	4.20	3.16	3.02	2.85
	SEM	0.16	0.21	0.30	0.29	0.32	0.40	0.22
P-value	Treatment[1]	0.30	0.90	0.91	0.32	0.99	0.94	0.07
	Cu source[2]	0.17	0.74	0.60	0.83	0.59	0.84	0.50
	linear $CuSO_4$[3]	0.38	0.39	0.71	0.74	0.98	0.94	0.46
	linear TBCC[3]	0.14	0.48	0.68	0.18	0.72	0.37	0.02

0 h: time of morning feeding
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.4.3.6 Acetic to propionic acid ratio

Table 37 shows the acetic to propionic acid ratio in the rumen fluid. There was no statistically significant effect of Cu dose and source on the acetic to propionic acid ratio within the total time course. The curves of the ratios concerning the different treatments were almost identical. In the morning, the ratio was 5.06 before declining rapidly by 34.4 % to 3.32 during the first 1.5 h (overall means). In the following 7.5 h, the ratio constantly increased to 4.93 and achieved almost the starting value.

Table 37: Acetic to propionic acid ratio in the rumen fluid dependent on Cu dose and source as well as on sampling time

Cu source	Cu dose [mg/kg DM]	Time after feeding						
		0 h	**1.5 h**	**3 h**	**4.5 h**	**6 h**	**7.5 h**	**9 h**
$CuSO_4$	10	5.13	3.13	3.65	4.21	4.54	4.69	4.89
	35	5.09	3.42	3.92	4.26	4.61	4.77	4.99
	50	5.08	3.40	3.83	4.40	4.64	4.83	5.03
TBCC	10	5.08	3.37	3.85	4.36	4.65	4.78	4.98
	35	5.02	3.20	3.79	4.20	4.51	4.70	4.90
	50	4.96	3.37	3.82	4.22	4.49	4.64	4.81
	SEM	0.09	0.15	0.16	0.13	0.08	0.09	0.08
P-value	Treatment[1]	0.72	0.62	0.85	0.77	0.52	0.61	0.34
	Cu source[2]	0.22	0.96	0.85	0.77	0.46	0.38	0.24
	linear $CuSO_4$[3]	0.68	0.16	0.32	0.29	0.34	0.25	0.17
	linear TBCC[3]	0.31	0.88	0.85	0.39	0.12	0.25	0.13

0 h: time of morning feeding
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

4.5 Effect of dose and source of copper supplementation on apparent total tract digestibility

Titanium dioxide (TiO_2) was mixed in the ration as indigestible marker in order to calculate the apparent 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), and acid detergent fibre (ADF). The respective results are presented in Table 38. The supplementation of Cu differing in dose and source showed no statistically significant effect on the apparent total tract digestibility of DM, OM, and all single ingredients. The digestibility remained continuously on the same level within the six treatments, respectively.

Table 38: Apparent total tract nutrient digestibility [%] dependent on Cu dose and source

Cu source	Cu dose [mg/kg DM]	Ingredient								
		DM	OM	CF	CP	TL	NFE	CA	NDF	ADF
$CuSO_4$	10	78.9	81.0	78.6	72.4	66.8	82.8	45.7	75.7	76.0
	35	78.2	80.9	79.2	71.9	65.2	82.5	44.3	76.2	74.8
	50	79.0	80.6	79.5	72.0	65.9	82.0	44.9	75.9	74.6
TBCC	10	78.1	80.2	78.1	71.8	65.6	81.7	44.8	74.2	73.8
	35	78.8	80.4	78.9	72.0	65.7	81.8	46.8	74.8	73.4
	50	78.4	81.3	79.6	72.8	70.4	82.8	47.0	76.3	75.5
	SEM	0.89	0.86	1.26	1.59	2.76	0.79	1.46	1.32	1.54
P-value	Treatment[1]	0.96	0.92	0.95	1.00	0.72	0.80	0.64	0.79	0.76
	Cu source[2]	0.66	0.74	0.82	0.93	0.56	0.60	0.27	0.41	0.43
	linear $CuSO_4$[3]	0.99	0.76	0.58	0.84	0.77	0.47	0.63	0.89	0.45
	linear TBCC[3]	0.75	0.33	0.39	0.67	0.23	0.33	0.23	0.25	0.44

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.
[1] P-value of ANOVA (6 treatments).
[2] P-value of orthogonal contrast between Cu sources ($CuSO_4$ vs. TBCC).
[3] P-value of linear trend along Cu doses within $CuSO_4$ or TBCC, respectively.

5 Discussion

In this study, Cu from $CuSO_4$ and TBCC was used to examine the effect of Cu supplementation in varying doses on Cu metabolism as well as on rumen microbiota and rumen fermentation characteristics. The approach was to reflect moderate and physiologically adequate feeding conditions rather than to simulate an extreme. Therefore, the cows received a diet with moderate concentrations of Mo and S and were neither Cu - depleted nor compromised with Cu toxicity. Cu was supplemented to receive following total dietary Cu concentrations, which can also be found in common feed rations: 10 mg/kg DM (in line with recommendation), 35 mg/kg DM (close to permitted maximum according to feed law), and 50 mg/kg DM (mild excess).

5.1 Copper concentration in ruminal and duodenal contents

Both, $CuSO_4$ and TBCC are inorganic Cu compounds but different in their solubility behaviour. $CuSO_4$ was assumed to be completely soluble and TBCC to be almost insoluble in rumen fluid (Spears *et al.*, 2004). Therefore, the mode of action of both Cu sources in the gastrointestinal tract was examined in order to obtain explanations for possible effects.

5.1.1 Copper concentration in rumen contents

The Cu concentrations in rumen fluid and rumen solid increased linearly with rising Cu doses in feed, independent of Cu source. However, the comparison of Cu sources demonstrated that on average the supplementation of $CuSO_4$ caused higher concentrations in the liquid fraction of rumen fluid (18.9 %) while, when using TBCC, the rumen solid (4.24 %) as well as the particle fraction of rumen fluid (3.98 %) were enriched in Cu. The actual differences were detected after dietary Cu concentrations of 35 mg Cu/kg DM and 50 mg Cu/kg DM. The amount of supplemented Cu at a low Cu level of 10 mg Cu/kg DM was insufficient to see variations of Cu sources regarding the Cu concentrations in the respective fractions.

The differences in Cu recovery can be attributed to different solubilities of $CuSO_4$ and TBCC in the rumen environment. $CuSO_4$ represents a highly water soluble compound whereas TBCC is almost insoluble in water but mostly soluble under acidic conditions (pH < 3.0), which are present in the abomasum (Cromwell *et al.*, 1998; Miles *et al.*, 1998; Spears *et al.*, 2004). Considering that the pH-values measured during this study ranged from 6.61 to 7.15, Cu from $CuSO_4$ was expected to dissolve in the rumen fluid after feed

intake whereas TBCC was assumed to show low solubility. This was confirmed by higher Cu concentrations in rumen solid and the particle fraction of rumen fluid after supplementation of TBCC. Nevertheless, Cu concentrations in the liquid fraction of rumen fluid increased 2.4-fold along with a 5-fold increased supplementation of TBCC. If Cu from TBCC was insoluble, however, it would have been completely precipitated during the ultracentrifugation step and the liquid fraction of rumen fluid would have maintained the same Cu concentration, independent of Cu dose. Consequently, there seems to be a difference between solubility of TBCC in water and the dynamic system of the rumen, which cannot be described solely by the pH-value. Spears *et al.* (2004) assumed that complexation of Cu (from TBCC) by amino acids increases Cu solubility in the abomasum and that further ligands and chelators with similar properties may exist. Such interactions could be conceivable for the rumen environment as well. Although, TBCC seemed to be partially soluble, $CuSO_4$ showed a higher ruminal solubility. This corresponds to the work of Genther and Hansen (2015) in which the solubilities of sulphate trace minerals and hydroxyl trace minerals (Cu, Mn, Zn) in the rumen were examined. Rumen fluid was centrifuged at 28,000 × g for 30 min to obtain a supernatant containing soluble Cu, conforming to the liquid phase of rumen fluid in the current study. $CuSO_4$ showed an increased ruminal solubility, compared to TBCC, but increasing doses of TBCC elevated the Cu concentration in the supernatant as well.

In total, the majority of supplemented Cu in form of $CuSO_4$ as well as TBCC was accumulated in the rumen solid and the particle fraction of rumen fluid. This emerges from a 2-fold higher increase of Cu concentrations in the solid fractions when Cu supplementation was increased, compared to the liquid fraction of rumen fluid. The results of Genther and Hansen (2015) confirm these observations due to the decrease of soluble Cu proportion in whole rumen fluid after elevated supplementation of either $CuSO_4$ or TBCC. Price and Chesters (1985) tested the bioavailability of Cu within rumen digesta of sheep by feeding rats the different fractions (e.g. large particles, microorganisms, soluble material) in order to obtain more information about the distribution of Cu in the rumen. They ascertained that Cu concentrations in the different fractions of solid material were markedly higher, compared to the liquid fraction, and subsequently that about 90 % of total Cu in the rumen were present in solid material. The results of the current study in combination with the findings of the authors mentioned above indicate that Cu mainly associates with solid material after it has been solubilised.

The interaction between Cu, Mo, and S is another important aspect regarding the Cu distribution in rumen contents, described since the 1950's (Dick, 1953). In the rumen environment, microorganisms reduce sulphate to sulfide prior to the formation of

thiomolybdates by reaction of sulfide with molybdate (Dick *et al.*, 1975; Bradley *et al.*, 2011). Following, thiomolybdates which are associated with rumen solid (feed particles, protozoa, and bacteria) form insoluble complexes with Cu. These complexes are completely insoluble during the passage through the gastrointestinal tract, even under the acidic conditions in the abomasum (Allen and Gawthorne, 1987). This is of importance as Cu from Cu-thiomolybdate cannot be absorbed in the intestine and may lead to Cu deficiency in ruminants (Underwood and Suttle, 1999). In this context it is assumed that TBCC is less susceptible to interactions with Mo and S than $CuSO_4$ due to its low solubility in the rumen and may thus be more suitable to prevent an undersupply of Cu in ruminants (Spears *et al.*, 2004).

Current data of Cu concentrations in rumen contents showed no enhanced complex building when $CuSO_4$ was supplemented. Conversely, supplementation of $CuSO_4$ increased the Cu concentration in the liquid fraction of rumen fluid to a higher extent than TBCC. A considerable formation of Cu-thiomolybdates would result in at least equal Cu concentrations of both Cu sources in the liquid fraction of rumen digesta. Goselink (2015) proved in an in vitro study, simulating rumen incubation, the potential of thiomolybdate to irreversibly bind soluble Cu from $CuSO_4$ and TBCC. For that, 30 ml of buffered rumen fluid were admixed with Cu (from $CuSO_4$ and TBCC, respectively), Mo, and S to receive concentrations of 2 µg/ml of Cu, 10 µg/ml of Mo, and 7.5 mg/ml of S. After incubation at 38 °C for 4 h no soluble Cu could be detected in the supernatant of rumen fluid samples, neither of $CuSO_4$ nor of TBCC. At first, this confirms the assumption that TBCC may be solubilised in rumen fluid to a markedly extent due to the complete sequestration by thiomolybdates. Secondly, the concentration of Mo and S was very high and thus able to bind all of the released Cu. Genther and Hansen (2015) fed a corn silage-based diet to five ruminally cannulated steers on *ad libitum* basis and supplemented Cu either as $CuSO_4$ or as TBCC at doses of 5 mg/kg DM and 25 mg/kg DM, respectively. No Mo and S were supplemented and an additional promotion of Cu-thiomolybdate formation was not given. The results clearly showed a significantly stronger increase of the concentration of soluble Cu in the supernatant of rumen fluid after $CuSO_4$ treatment, compared to TBCC treatment. Ward *et al.* (1993) measured a reduced soluble Cu concentration in the supernatant of centrifuged rumen fluid of about 65 % after addition of Mo and S. The total Cu concentration in the diet was 11.2 mg/kg DM and is corresponding to the lowest dose with 10 mg Cu/kg DM in the current study. However, Mo concentration was 10.8 mg/kg DM and S concentration was 3.7 g/kg DM. Especially the extremely high Mo concentration promoted thiomolybdate formation and cannot be compared with moderate Mo concentrations. Similar conditions can be found in Allen and Gawthorne (1987). Cu

concentrations in rumen fluid of sheep were reduced by 32 % to 53 % after supplementing 5 mg Mo/kg DM and 25 mg Mo/kg DM either as tetrathiomolybdate or as ammonium molybdate, respectively. The dietary Cu concentration was at a relative low level of 3.3 mg/kg DM.

In summary, high concentrations of Mo and S are undoubtedly cabable of reducing the amount of soluble Cu in the rumen digesta to a considerable extent due to the formation of thiomolydates. Particularly at low dietary Cu concentrations the pool of soluble Cu can be completely depleted. Furthermore, not only soluble Cu in the liquid fraction of rumen fluid is affected by complex building agents. Cu associated to the solid fraction of rumen fluid, most of all small particles and bacteria, is evidently incorporated into insoluble complexes (Price and Chesters, 1985; Allen and Gawthorne, 1987). Gould and Kendall (2011) concluded that most of the Cu losses in the rumen due to interaction with thiomolybdates take place in the solid phase of rumen fluid. This should be taken into account in prospective evaluation of complex building potential of Cu antagonists in the rumen. However, no serious impairment of potentially available Cu should be expected at moderate concentrations of Mo and S in feedstuffs. Otherwise, the liquid fractions of rumen fluid would be completely depleted, especially at low Cu dose of 10 mg/kg DM (native + supplemented Cu). Gooneratne *et al.* (1989) described that the problem of complex building already appears at native concentrations in feedstuffs, meaning 4 to 10 mg Cu/kg DM, 0 to 5 mg Mo/kg DM, and 1 to 3 g S/kg DM. Nevertheless, at these concentrations the main issue is the lack of dietary Cu. The appropriate supplementation of Cu (10 mg/kg DM) would probably limit considerable Cu losses. The Cu source is assumed not to be decisive but rather the minimum Cu concentration in the diet in order to overcome Cu antagonism.

5.1.2 Composition and copper concentration of duodenal digesta

The composition of the duodenal digesta gives insights into possible effects of Cu supplementation on the preceding ruminal degradation of feedstuffs. In this context, only the bacteria fraction seemed to be affected by Cu supplementation. The amount of bacteria tended to increase with elevated Cu doses from $CuSO_4$ (0.36 mg/g FM vs. 0.42 mg/g FM vs. 0.44 mg/g FM). An improved bacterial growth due to a stimulated feed degradation in the rumen could be a possible explanation. However, the results of the quantification of the microbial population show no improved microbial growth along with increasing doses of $CuSO_4$ (section 4.2). Additionally, the amounts of bacteria at the $CuSO_4$ treatment are comparable to those of the TBCC treatment (0.40 - 0.45 mg/g FM).

For these reasons, the slightly increasing amount of bacteria cannot be traced to differences in ruminal degradation dependent on Cu supplementation.

The Cu concentrations in the single fractions of duodenal digesta increased linearly with elevated amounts of supplemented Cu, independent of Cu source. This corresponds to the results of Cu concentrations in the fractions of the rumen content. Nevertheless, a shift of Cu distribution between rumen and duodenal contents could be detected. The Cu concentration in the liquid fraction decreased during the passage from rumen to duodenum (0.11 - 0.32 µg Cu/ml vs. 0.08 - 0.21 µg Cu/ml). The average Cu concentrations in the overall solid phase of duodenal digesta were located between 24.9 µg/mg DM and 129 µg/mg DM and thus higher than the Cu concentrations in rumen solid (13.5 - 68.7 µg/mg DM) and the particle fraction of rumen fluid (23.2 - 116 µg/mg DM). This is in line with Genther and Hansen (2015) who measured lower soluble Cu concentrations in digesta after simulated acidic digestion than in rumen fluid. Price and Chesters (1985) investigated Cu concentrations in rumen and duodenal fractions of sheep dependent on Mo supplementation. They also observed a decreased amount of soluble Cu in the duodenum if no additional Mo was supplemented. Former studies described that soluble Cu is bound to denaturated microbial protein in the abomasum (Ward and Spears, 1993) or that microbial matter leads to a reduced solubility of Cu (Bremner, 1970). Ivan and Veira (1981) showed a decrease of soluble Cu in the abomasum when dietary protein was increased.

Another subject of interest was the potential of different Cu sources to provide soluble Cu in the duodenum. The liquid phase of duodenal digesta contained Cu which could not be sedimented at 20,000 × g for 30 min. Consequently, this amount of Cu was assumed to be soluble. In contrast to the rumen fluid, there was no observation of a significant source effect on the amount of soluble Cu in the duodenal digesta. Genther and Hansen (2015) also found similar amounts of acidic-soluble Cu from $CuSO_4$ and TBCC. They concluded that TBCC, less soluble in the rumen than $CuSO_4$, was solubilised under acidic conditions leading to equal Cu concentrations in the liquid fraction of duodenal digesta. However, due to decreased soluble Cu concentrations in the duodenum, compared to rumen fluid, this conclusion cannot be approved. Sufficient evidence for solubilised TBCC needs a definite measurement of rising Cu concentrations in the liquid fraction after acidic digestion. Otherwise, solubilisation of TBCC in the abomasum is very likely and cannot be ruled out. Consequently, the majority of soluble Cu, even originating in TBCC, was probably accumulated in the solid phase during the passage through the abomasum.

Due to the physiological importance of soluble and also absorbable Cu in the duodenum, the proportion of precipitable Cu in the liquid phase was determined. This Cu fraction was

assumed to be present in ionic form. A reduction of soluble Cu in the supernatant of duodenal digesta by precipitation could not be observed. Therefore, Cu was presumably not present in ionic form but rather bound to ligands and chelators. Spears *et al.* (2004) demonstrated that the addition of amino acids enhanced the solubility of TBCC at a pH ranging from 2.0 to 5.0.

The bacteria fraction of duodenal digesta was the only fraction with different Cu concentrations dependent on Cu source. The supplementation of $CuSO_4$ resulted on average in 18.7 % higher Cu concentration in bacteria, compared to TBCC. There were several approaches for explanation. Firstly, as described above, soluble Cu was associated with microbial matter in the abomasum (Bremner, 1970), resulting in higher Cu concentrations in the bacteria fraction of duodenal digesta after $CuSO_4$ supplementation. Yet, it must be considered that TBCC is likely to be solubilised in the abomasum as well, contradicting this approach. Secondly, Cu-thiomolybdate accumulates in bacterial matter (Price and Chesters, 1985; Allen and Gawthorne, 1987). However, a noticeable Cu-thiomolybdate formation in the rumen could not be detected in the current study. Thirdly, microbes are only able to use or to interact with soluble minerals (Genther and Hansen, 2015). Subsequently, the high solubility of $CuSO_4$ in the rumen may have enabled rumen bacteria to incorporate more Cu from $CuSO_4$ than from TBCC, which seems to be the most applicable explanation. This is confirmed by Durand and Kawashima (1980) who mentioned that Cu may largely accumulate in microbial or bacterial fractions.

In total, the measured Cu concentrations in the different fractions of duodenal digesta showed no source effect, apart from the bacteria fraction. The proportion of Cu in bacteria was on average only 1.90 %, compared to the total Cu amount in duodenal digesta and thus the relevance with regard to nutritional aspects was limited. Large and small particles contained in sum 91.4 % of total Cu in duodenal digesta whereas the soluble proportion of Cu was on average 8.55 %. In the study of Price and Chesters (1985) 10.5 % of the total Cu in dry matter of duodenal digesta were measured in bacteria, 3.84 % in the supernatant and 85.7 % in the remaining solid material. Furthermore, current data give no indication for higher amounts of soluble Cu in the duodenum when TBCC was supplemented. Independent of Cu source, the majority of Cu was present in the solid material of duodenal digesta. This clearly shows that the evaluation of Cu sources, regarding the potential to provide absorbable Cu in the duodenum, cannot be reduced to the proportion of soluble Cu in the liquid phase.

5.2 Copper digestion and copper status

5.2.1 Amount of apparently digested copper

The amount of apparently digested Cu was strongly increased with rising doses of $CuSO_4$ and TBCC, respectively. This is attributed to the system of Cu homeostasis. The major homeostatic mechanism is biliary excretion which is limited in ruminants, compared to nonruminants (NRC, 2005), leading to high amounts of Cu stored in liver tissue (Underwood, 1977). Therefore, liver Cu concentrations are correlated with bioavailable dietary Cu (McDowell, 1992). This was affirmed by numerous studies which clearly showed increased Cu liver concentrations after Cu supplementation higher than recommendations (Du *et al.*, 1996; Ward and Spears, 1997; Engle and Spears, 2000a; Engle and Spears, 2001; Arthington *et al.*, 2003; Spears *et al.*, 2004; Arthington and Spears, 2007; Hansen *et al.*, 2008).

In contrast to the present results, other studies did not measure a consistently increase of Cu retention. Chase *et al.* (2000) depleted 48 Holstein cows prior to supplementation of 15 and 30 mg Cu/kg DM from $CuSO_4$ or Cu lysine, respectively, in combination with 500 mg Fe/kg DM from $FeSO_4$ in order to challenge Cu antagonism. Cu retention was significantly higher at 30 mg Cu/kg DM than at 15 mg Cu/kg DM and Cu supplemented animals showed higher Cu retention than control animals (not supplemented). After 70 d, however, zero retention was approached due to completely Cu repletion, resulting in excretion of excessive Cu via faeces. Zhang *et al.* (2007) reported higher Cu retention after supplementing goats with 10, 20, and 30 mg Cu/kg DM from $CuSO_4$, compared to control animals, but no differences within increased Cu doses. Another study of Felix *et al.* (2012) also reported a quadratic trend for apparent Cu digestibility after supplementing 100 and 200 mg Cu/kg DM from TBCC to cattle, respectively.

These studies showed that the apparent Cu absorption increases when Cu supplementation is elevated as long as Cu stores are not filled, independent of previous Cu status of the animals. This means that the animals of the present study compensated the increased dietary Cu amounts by enhanced Cu storage in the liver during the experimental time.

For the current investigation, however, the decisive observation was the difference between Cu sources with regard to the extent of increased Cu digestion. The apparently digested amount of Cu from TBCC increased 11-fold from 10 mg Cu/kg DM to 50 mg Cu/kg DM whereas a 16-fold increase was recorded for $CuSO_4$. After a supplementation of 50 mg Cu/kg DM in form of $CuSO_4$ the apparently digested Cu amount

was 35.3 % higher, compared to TBCC. Instead of investigating apparent Cu digestion, several authors used Cu status as well as estimated bioavailabilities to compare Cu absorbability and complex formation of different Cu sources. It was assumed that Cu bioavailability is strongly dependent on absorbable Cu amounts in the intestine and hence dependent on prior formation of insoluble and not absorbable Cu complexes in the rumen. The results of Spears *et al.* (2004) are different to those of the current study. They estimated similar relative bioavailabilities for $CuSO_4$ and TBCC in steers fed low in Mo after supplementation of 50 and 100 mg Cu/day, respectively. This was confirmed by Arthington and Spears (2007), who added 100 mg Cu/day from $CuSO_4$ and TBCC, respectively, to a diet low in Mo and S and by Ward *et al.* (1993), who added 5 mg Cu/day from $CuSO_4$ and Cu lysine, respectively, to diets either high or low in Mo and S. In both studies, Cu status parameters indicated similar Cu availability of Cu sources. Chase *et al.* (2000) also did not observe a difference between $CuSO_4$ and Cu lysine with regard to their ability to replete Cu stores of Cu depleted steers, even though Fe was supplemented to challenge Cu antagonism. In contrast, Hansen *et al.* (2008) reported 140 %, 140 %, and 131 % relative bioavailability of Cu from Cu glycinate, compared with $CuSO_4$ (100 %), based on slope ratios for plasma Cu, plasma ceruloplasmin activity, and liver Cu, respectively. The control diet contained 8.2 mg Cu/kg DM and either 5 or 10 mg Cu/kg DM from both, $CuSO_4$ and Cu glycinate, were supplemented. Additionally, 2 mg Mo/kg DM and 0.15 % S were added to the corn silage-based diet.

For the evaluation of Cu bioavailability, the location of Cu absorption in the intestine has to be considered, too. Cu is reported to be absorbed not only in the duodenum but also in the lower intestine (Crampton *et al.*, 1965). The proportion of soluble Cu of the total Cu amount increased during the passage through the intestine as described by Price and Chesters (1985). The proportion of soluble Cu in the ileum was 10.7 % higher than in the duodenum. The reason for that will be most likely found in the advanced digestion of solid material, resulting in a greater release of soluble Cu. This means that particle associated Cu plays also an important role for Cu absorption in the lower intestine. Another aspect is that Cu absorbability might be negatively affected by the continuously increasing pH of duodenal digesta due to formation of copper hydroxide and basic copper salts (Wapnir and Stiel, 1987; Wapnir, 1998). In this case, the advantage of TBCC, which is supposed to solubilise in the abomasum, would be eliminated.

In summary, present results of Cu digestion indicate higher bioavailability for $CuSO_4$ than for TBCC, meaning that $CuSO_4$ provided greater amounts of absorbable Cu in the intestine at diets moderate in Mo and S. Concentrations of soluble Cu in the duodenum, however, were similar between both Cu sources. In combination, these observations lead

to the hypothesis that a considerable amount of absorbed Cu was allocated in the solid phase of duodenal digesta.

5.2.2 Status parameters of copper in the blood serum

A further approach to verify the amount of absorbed Cu was the examination of Cu status parameters in serum. Cu concentration as well as activity of the cuproenzymes ceruloplasmin and Cu/Zn - superoxide dismutase are commonly measured parameters and were also used in this study. Ceruloplasmin is substantially involved in iron metabolism and carries 95 % of total serum Cu. The Cu dependent enzyme superoxide dismutase plays an important role in detoxification of free radicals (Milne, 1998; Bonham *et al.*, 2002; Tapiero *et al.*, 2003).

To evaluate the results, studies using comparable and relatively moderate dietary Mo and S levels were consulted. Cu supply of animals in the listed literature causes neither severe Cu deficiency nor strong Cu excess.

Increasing doses of either $CuSO_4$ or TBCC had no effect on serum Cu concentrations. This is in line with the results of Ward *et al.* (1993), who observed no increase of plasma Cu after Cu supplementation of 5 mg/kg DM in form of $CuSO_4$ and Cu lysine, although Cu concentration in control diet was low (6.2 mg/kg DM). Ward and Spears (1997) and Du *et al.* (1996) also concluded that an increase of plasma Cu concentrations is not to be expected after Cu supplementation when Cu status is already adequate because of homeostatic mechanisms. In contrast to that, other authors reported greater plasma Cu concentrations when dietary Cu concentrations were increased by adding different Cu sources, even at lower dietary Cu levels compared to the present study (35 mg/kg DM and 50 mg/kg DM) (Engle and Spears, 2000a; Spears *et al.*, 2004; Hansen *et al.*, 2008). Engle and Spears (2000a) also found increased plasma Cu concentrations after Cu supplementation of 20 mg/kg DM from different Cu sources, but the Cu status was previously reduced by adding 10 mg Mo/kg DM. Additionally to the Cu dose, Cu sources revealed no differences in serum Cu concentrations. Similar results were shown by Spears *et al.* (2004) ($CuSO_4$ vs. TBCC), Hansen *et al.* (2008) ($CuSO_4$ vs. Cu glycinate), Engle and Spears (2000a) ($CuSO_4$ vs. Cu citrate vs. Cu proteinate), and Ward *et al.* (1993) ($CuSO_4$ vs. Cu lysine).

Ceruloplasmin activity in serum was not significantly affected by Cu dose apart from a slight increase (1.14-fold) in combination with TBCC supplementation. Differences between Cu sources could not be detected. Ward *et al.* (1993) reported that supplementation of 5 mg Cu/kg DM from $CuSO_4$ and Cu lysine, respectively, to a control

diet low in Cu did not affect ceruloplasmin activity in plasma. Cu supplementation of different sources also showed no effects. Furthermore, Du *et al.* (1996) clearly demonstrated that plasma ceruloplasmin activity is no reliable indicator for the evalutation of Cu status when Cu supply is adequate. Hansen *et al.* (2008), however, detected higher plasma ceruloplasmin activity in steers supplemented with 5 - 10 mg Cu/kg DM than in control steers after 84 and 112 days of treatment. They also demonstrated that supplementation of 10 mg Cu/kg DM from Cu glycinate led to greater ceruloplasmin activity than identical concentrations from $CuSO_4$ after a period of 148 days, but Mo supplementation was elevated to 6 mg/kg DM for the last 28 days of this period. Arthington and Spears (2007) found higher plasma ceruloplasmin concentrations in heifers receiving 100 mg Cu/day from $CuSO_4$ and TBCC, respectively, added to a molasses-based supplement. In contrast to that, they found no differences when Cu was added to a corn-based supplement. Both supplements were moderate in Mo and S concentrations. Furthermore, TBCC tended to change plasma ceruloplasmin concentrations positively from day 0 to 90 of the trial, compared to $CuSO_4$. The study of Spears *et al.* (2004) also contradicts the present results. They observed greater plasma ceruloplasmin activity in steers after supplementation of 50 and 100 mg Cu/day in form of $CuSO_4$ and TBCC than in control steers, supplied with only 4.7 mg/kg DM of dietary Cu. Cu sources showed no different effects.

Cu-/Zn-SOD activity in serum was measured as a third Cu status parameter. Increasing Cu doses did not change SOD activity. Supplementation of Cu from TBCC increased SOD activity by 2.8 %, compared to $CuSO_4$. These findings are partially in contrast to Ward *et al.* (1993), who could not detect any effects of Cu supplementation on erythrocyte SOD activity in steers, neither of Cu dose (control vs. 5 mg/kg DM) nor of Cu source ($CuSO_4$ vs. Cu lysine), although Cu concentration in control diet was low (6.2 mg/kg DM). Ward and Spears (1997) reported that Cu supplementation of 5 mg Cu/kg DM from $CuSO_4$ did not affect SOD activity from red blood cells after a period of 56 days when no Mo was supplemented to a diet relatively low in Cu (6.9 mg/kg DM). This suggests that SOD activity is barely changed over a longer period by Cu supplementation when Cu status is already adequate. The current data of serum SOD activity suggests higher bioavailability and thus higher absorption of Cu from TBCC. This contradicts the results of apparently digested Cu amounts, which provides more information about bioavailability, compared to serum SOD activity.

In summary, Cu supplementation did not noticeably affect Cu status parameters in serum due to homeostasis mechanisms. Cu status parameters in serum, such as serum Cu concentration, serum ceruloplasmin activity, and serum SOD activity, are primarily not

affected by moderate Cu excess. This is confirmed by Du *et al.* (1996), who described that plasma Cu concentration and plasma ceruloplasmin activity are not correlated to liver Cu at excessive Cu supplementation. The review of Milne (1998) underlines that Cu status parameters are if at all affected by severe Cu deficiency along with depleted Cu stores and that these parameters are primarily influenced by factors other than dietary Cu concentration. Therefore, little changes in ceruloplasmin and SOD activity in the current study can most likely not be traced back to varying Cu supply.

5.3 Microbial populations in the rumen

The aim of the present study was to investigate the effect of Cu dose and source on rumen microbiota represented by total bacteria, archaea, protozoa, anaerobic fungi as well as the single bacteria species *Ruminococcus flavefaciens*, *Fibrobacter succinogenes*, and *Streptococcus bovis*. Comparable studies dealing with the influence of Cu on rumen microorganisms are scarce. The following passage describes the functions of different rumen microorganisms and discusses possible effects of a by Cu supplementation impaired microbiome on rumen fermentation.

5.3.1 Ruminococcus flavefaciens

Cu supplementation showed no significant effects on concentrations of *R. flavefaciens* and its proportion in total bacteria between 0 and 3 h after feeding. Even if lesser amounts of *R. flavefaciens* were observed in rumen of steers and sheep than of *F. succinogenes* (Koike and Kobayashi, 2001; Michalet-Doreau *et al.*, 2001; Denman and McSweeney, 2006), it belongs next to *F. succinogenes* and *Ruminococcus albus* to the most important representatives of fibre-degrading bacteria known in rumen (Weimer, 1996; Stewart *et al.*, 1997). A large number of carbohydrate degrading bacteria are able to use numerous monosaccharides and disaccharides as substrate for growth. In contrast, *Ruminococci* and *F. succinogenes* rely on cellulose and its degradation products as energy source (Hungate, 1966). Former *in vitro* studies detected decreased cellulose digestion by adding Cu to incubation solutions, suggesting toxic effects on cellulose-digesting bacteria. First inhibiting effects occurred at very low concentrations of 1.0 µg/ml of incubation solution, but only due to the usage of washed cell suspensions or strained rumen fluid (Hubbert *et al.*, 1958; Martinez and Church, 1970; Ward and Spears, 1993). With regard to concentrations of *R. flavefaciens* in the present study, Cu supplementation up to mild excess could not alter cellulose degradation.

5.3.2 Fibrobacter succinogenes

Current data indicate a source effect of Cu supplementation on *F. succinogenes*. Concentrations tended to be reduced at $CuSO_4$ treatment, compared to TBCC, straight before morning feeding (log_{10} copy numbers: 8.34 vs. 8.42) and 1.5 h after feeding (log_{10} copy numbers: 8.72 vs. 8.85). Additionally, the proportion of *F. succinogenes* in total bacteria was diminished by $CuSO_4$, compared to TBCC, after 1.5 h (2.30 % vs. 2.81 %) and 3 h (2.45 % vs. 2.98 %). Even though statistical analysis showed only trends, except for the proportion after 3 h ($p = 0.05$), a negative effect of $CuSO_4$ on *F. succinogenes* emerged. This corresponds to literature where *Bacteroides succinogenes* compared to other rumen bacteria like *Butyrifibrio fibrosolvens* or *Streptococcus bovis* are described as highly sensitive for Cu toxicity (Forsberg, 1978). Nevertheless, even reduced proportions of *F. succinogenes* in total bacteria are within a range corresponding to literature (0.1 % - 6.6 %) (Stahl *et al.*, 1988; Briesacher *et al.*, 1992; Lin *et al.*, 1994; Krause *et al.*, 1999; Tajima *et al.*, 2001). As mentioned above, *F. succinogenes* undertakes a major part in fibre degradation and severe damages would suggest possible impacts on fibre digestion as a whole. The negative effect of Cu supplementation in the present study, however, was in a comparable range to other studies and thus is not classified as severe.

5.3.3 Streptococcus bovis

S. bovis concentration was significantly reduced by increased Cu supplementation via $CuSO_4$ 1.5 h after feeding (log_{10} copy numbers: 7.83 vs. 7.74 vs. 7.66). After 3 h, the proportion in total bacteria declined with increasing $CuSO_4$ (0.24 % vs. 0.17 % vs. 0.15 %), underlined by a statistical trend. These results were so far unexpected as in a former study *S. bovis* was described as quite insensitive for Cu toxicity (Forsberg, 1978). More than the 20-fold concentration of $CuCl_2$ was necessary to reach growth inhibitory concentrations for *S. bovis*, compared to e.g. *Bacteroides succinogenes* or *Ruminococcus albus*. *S. bovis* is reported to have high amylolytic activity (Cotta, 1988) and rumen dysfunctions along with low ruminal pH may occur in conjunction with enhanced *S. bovis* concentrations (Hungate *et al.*, 1952). This is due to high lactate production by *S. bovis* when there is a high amount of concentrate in feed. In forage rich rations, however, the amount of substrate is limited and growth of *S. bovis* is limited. In this case, the primary fermentation products of *S. bovis* are acetate, formate, and ethanol (Russell and Baldwin, 1979; Russell and Hino, 1985). In the present study proportions of *S. bovis* in total bacteria ranged between 0.14 % and 0.27 %. Stevenson and Weimer (2007) measured proportions of less than 0.03 %. This is contradicting as the TMR in the present study

contained substantially higher amounts of forage suggesting a decreased abundance of *S. bovis*. Reasons for that difference might arise out of methodical approaches. Nevertheless, even low *S. bovis* concentrations in the present study seem to be within a physiological scale. Therefore, the negative effect of Cu supplementation is not assumed to cause a severe damage of *S. bovis* population. Consequences regarding starch degradation at high forage diets are not expected, whereby reductions of starch degradation cannot be ruled out in concentrate rich diets.

5.3.4 Archaea

Cu supplementation did not have any effects at any point of time on archaea population in rumen, neither Cu dose nor Cu source. Former studies reported that archaea contribute 0.5 % to 3.5 % of the added up 16S and 18S rRNA in the rumen (Lin *et al.*, 1997; Sharp *et al.*, 1998; Ziemer *et al.*, 2000). Present results were assessed in relation to total bacteria (only 16S rRNA) and thus slightly overestimated in comparison to literature. Nevertheless, abundance of archaea was comparably small in the present study (0.3 % - 0.94 %). Archaea are strictly anaerobic methanogens which use, next to formate, especially H_2 as energy source along with reduction of CO_2 to CH_4. This mechanism of H_2 removal prevents inhibiting effects of H_2 on hydrogenase activity and oxidation of sugars and improves fermentation rates due to favoured formation of volatile fatty acids (Wolin, 1979; McAllister and Newbold, 2008). Archaea, although abundant in only small quantities in rumen, are very important to maintain rumen function by regulating H_2 as key factor in rumen fermentation (Hungate, 1967). Consequently, reduced archaea could potentially lead to impaired rumen fermentation. However, Cu supplementation did not affect archaea population in this study and negative impacts on rumen fermentation were excluded.

5.3.5 Protozoa

The analysis of protozoa concentrations revealed slightly decreased values straight before feeding with increased supplementation of $CuSO_4$ (log_{10} copy numbers: 9.08 vs. 8.94 vs. 8.93), however, a statistically significant correlation was not observed. The proportion of protozoa relative to total bacteria was not affected. At the remaining times, no effects of Cu supplementation on protozoa could be detected at all. These results are contradictory to literature. Essig *et al.* (1972) defaunated steers by adding 4.4 g of $CuSO_4$ per 100 kg body weight for three weeks and measured a slightly greater protozoa count before the morning feeding for Cu supplemented steers, compared to control steers. Two hours after feeding, however, protozoa count was significantly lower in supplemented steers than in

control steers, indicating a negative effect of $CuSO_4$ on protozoa. Solaiman *et al.* (2007) added 100 mg Cu/day and 200 mg Cu/day in form of $CuSO_4$ to a basal diet containing 14 mg Cu/kg DM, respectively. Protozoa counts also tended to decrease with higher Cu supplementations. Protozoal growth seems to be more sensitive to Cu toxicity at excessive levels than bacterial growth (Durand and Kawashima, 1980). In a former study of Becker and Everett (1930), infusoria, also including ciliates, were successfully removed from lambs by adding a $CuSO_4$ solution. In the present study, ciliate protozoa were measured, representing a group of protozoa highly abundant in rumen (Sylvester *et al.*, 2004). These microorganisms are able to either use soluble sugar or starch or pectin as energy source (Mould and Thomas, 1958; Akkada and Howard, 1961; Bailey and Howard, 1963). Therefore, Kamra (2005) classified ciliate protozoa as soluble sugar utilisers, starch degraders, and lignocellulose hydrolysers. Ciliates use bacterial protein as protein source and are thus largely responsible for bacterial protein turnover (Wallace and McPherson, 1987). Finally, this results in a considerably less efficient use of nitrogen in the rumen (Wallace *et al.*, 2001). Nevertheless, some bacteria species, such as *Methanobacterium*, are less susceptible to lysis by protozoa than others and are attached to protozoal surface (Vogels *et al.*, 1980; Newbold *et al.*, 1996; Ushida and Jouany, 1996). Finlay *et al.* (1994) even reported noticeable amounts of endosymbiotic methanogens in protozoa which may be accountable for up to 37 % of methane emissions and Hegarty (1999) reported reduced rumen methane emissions of about 13 % in the absence of protozoa. On the other side, protozoa also have positive effects on rumen fermentation. They potentially stabilise rumen pH, especially when diets are rich in concentrate, due to their ability of using starch as energy source preventing its fast ruminal degradation (Mathieu *et al.*, 1996; Belzecki and Michalowski, 2001). Nutrient digestibility, nitrogen retention, VFA and ammonia concentrations as well as gain efficiency are diminished in protozoa-free animals (Christiansen *et al.*, 1965; Luther and Perkins, 1967). All in all, a considerable change of protozoa concentration in rumen can lead to an alteration of rumen fermentation characteristics. However, present dietary Cu concentrations, even from $CuSO_4$, did not impair ciliate protozoa and subsequent impacts of Cu supplementation on rumen fermentation are not expected.

5.3.6 Anaerobic fungi

Cu supplementation had at no time any effects on anaerobic fungi concentrations and their proportions relative to total bacteria. Anaerobic fungi are significantly involved in fibre degradation by colonizing lignocellulose (Akin and Rigsby, 1987; Paul *et al.*, 2003). They are able to directly penetrate the cuticle of plant tissues which enables bacteria to use

these sites to attach to protected plant tissues (Akin *et al.*, 1983; Akin and Rigsby, 1987; Ho *et al.*, 1988). Kamra (2005) demonstrated in an *in vitro* study that gas production as well as degradation of fibrous feeds were significantly reduced when anaerobic fungi had been removed from rumen content before. Anaerobic fungi in rumen apparently play a positive role in fibre degradation which might be limited when Cu supplementation has harmful effects. Such effects could not be detected in the present study and fibre degradation is not assumed to be negatively influenced by Cu supplementation up to mild excess.

5.3.7 Total bacteria

Concentrations of total bacteria at each sampling time were not affected by Cu supplementation, independent of Cu dose and source. Forsberg (1978) demonstrated that Cu as a potential toxic trace elements is able to inhibit growth of several functionally important rumen bacteria. However, only little effects on growth rates of rumen bacteria were detected in less than half of highest subinhibitory concentrations. The concentration of Cu^{2+} ions causing 50 % of inhibition of fermentation was determined at a very high concentration of 21 µg/ml of incubation solution. In this context, the chemical form of Cu is decisive for its toxicity. The toxicity of Cu^{+} ions to *Escherichia coli* under anaerobic conditions is comparable to that of Cu^{2+} ions at just one-seventh the concentration (Beswick *et al.*, 1976). Total bacteria make the major proportion of rumen microorganisms. Amounts of 10^{9} - 10^{10} in 1 ml duodenal fluid, reported in literature (Dehority and Orpin, 1997; Koike and Kobayashi, 2001), are corresponding to the scale of the present study. Results show that Cu supplementation did not change the amount of total bacteria in rumen, indicating that rumen fermentation is not impaired by mild Cu excess due to its antimicrobial effects. A possible reason for that is the protective effect of rumen fluid for bacteria (Martinez and Church, 1970; Forsberg, 1978). Bacteria may attach to particulate fractions of rumen fluid and therefore negative effects are limited.

In summary, Cu supplementation from $CuSO_4$ induced some minor and highly selective changes in rumen microbiota but did not considerably change total bacteria. These effects were not observed for TBCC, indicating different modes of actions of both Cu sources. $CuSO_4$ showed significantly higher solubility in rumen and provided higher Cu amounts suggested to be able to interact with rumen microorganisms. Present results of Cu concentrations in the bacteria fraction of duodenal digesta confirm this assumption. Bacteria fraction contained significantly higher Cu amounts when $CuSO_4$ was

supplemented, compared to TBCC. This means that Cu solubility may play an important role regarding antimicrobial effects. Nevertheless, present results clearly demonstrate that Cu supplementation up to mild excess (total dietary Cu concentration: 50 mg/kg DM) does not considerably impair rumen microbiota, independent of Cu source. This is confirmed by Durand and Kawashima (1980) who assumed that depressive effects of trace elements on *in vivo* microbial digestion occur as dietary concentrations reach about 100 mg/kg DM.

5.4 Ruminal degradation characteristics of tested feedstuffs

Ingested feed is degraded by rumen microorganisms. Following the effect of Cu supplementation on rumen microbiota, possible associated changes in ruminal degradation characteristics of tested feedstuffs were examined and the respective results are discussed in this passage.

Partially, supplementation of $CuSO_4$ negatively affected *F. succinogenes* and *S. bovis* populations in rumen fluid, while other microorganisms remained unaffected. In contrast, dry matter degradability, determined by measuring *in sacco* dry matter disappearance (DMD), was positively affected by enhanced supplementation of $CuSO_4$. Overall, DMD was increased in a range from 1.2 % to 7.8 %. This was observed for TMR between 3 h and 12 h, for grass silage, maize silage and wheat meal between 6 h and 12 h, and for soybean meal after 6 h and 9 h of incubation. Supplementation of TBCC also revealed significant effects on DMD, but not consistently. DMD of TMR and grass silage decreased with increasing doses of TBCC after 9 h and 48 h (0.6 % - 4.4 %), DMD of maize silage and wheat meal after 3 h and 9 h (1.2 % - 2.3 %) and DMD of soybean meal after 9 h of incubation (6.0 %). DMD of grass silage, however, was increased after 24 h (1.6 %) and DMD of wheat meal after 6 h of incubation (2.0 %). Additionally, TBCC treatment led to lower DMD for grass silage after 48 h and higher DMD for maize silage after 12 h of incubation, compared to $CuSO_4$ treatment.

Altogether, varying doses of Cu from TBCC affected dry matter degradation only at single points in time and in various ways. Increased doses of Cu from $CuSO_4$, however, stimulated dry matter degradation between 3 h and 12 h after feeding. Lopez-Guisa and Satter (1992) also measured an increased DMD at several incubation times between 1 h and 24 h in heifers after supplementation of 12.2 mg Cu/kg DM from $CuSO_4$ and 0.25 mg Co/kg DM from $CoSO_4$, but not consistently at every point. After 30 h of incubation they could not detect differences in DMD between supplemented and control group, confirming present results. A study of Genther and Hansen (2015), however, revealed contrary results. They fed steers a corn silage based diet (containing 7.4 mg Cu,

30.8 mg Mn, and 32.1 mg Zn per kg DM, respectively) and supplemented Cu, Mn, and Zn either as sulfate or as chloride in two different concentrations (5 mg Cu, 15 mg Mn, and 30 mg Zn, or 25 mg Cu, 60 mg Mn, and 120 mg Zn per kg DM, respectively). Independent of Cu source, DMD was not affected by increasing Cu supplementation. The mean DMD (mean of all incubation times of 6, 12, 24, and 36 h) showed that Cu supplementation tended to reduce DMD and that especially the supplementation of $CuSO_4$ resulted in a significantly lower DMD, compared to control (no supplemented Cu). *In vitro* incubation studies of Engle and Spears (2000b) and Alvarado-Gilis *et al.* (2014) did not detect any effects of Cu supplementation on *in vitro* DMD. In another *in vitro* study, Cu supplementation numerically increased DMD and significantly increased gas production after 24 h and 48 h of incubation (Vázquez-Armijo *et al.*, 2011).

Additionally, parameters of degradability and effective degradability were calculated for tested feedstuffs using current data of DMD. The constant rate of degradation of b (c) tended to be elevated at TMR with increasing doses of $CuSO_4$ and was considerably higher after supplementation of 50 mg Cu/kg DM from $CuSO_4$ at TMR (≥ 1.3 %/h), grass silage (≥ 0.7 %/h), maize silage (≥ 1.6 %/h), wheat meal (≥ 2.2 %/h), and soybean meal (≥ 1.0 %/h), compared to 35 and 10 mg Cu/kg DM from $CuSO_4$. Lopez-Guisa and Satter (1992) reported strongly increased rates of degradation at even lower dietary Cu concentrations. Heifers were supplemented with 12.2 mg Cu/kg DM from $CuSO_4$ in combination with 0.25 mg Co/kg DM from $CoSO_4$ and rates of degradation of alfalfa hay and corn cobs were increased by 6.2 %/h and 2.7 %/h, respectively. Vázquez-Armijo *et al.* (2011) measured in an *in vitro* trial significantly higher rates of gas production when Cu was added to an incubation solution and thus confirming present results, too. Furthermore, effective degradability was positively affected by Cu supplementation in form of $CuSO_4$ as well. ED8 of TMR tended to increase when Cu supplementation was increased ($p = 0.09$). ED5 and ED8 of grass silage were noticeably enhanced with increasing dietary amounts of $CuSO_4$, but not statistically significant. At wheat meal, ED2 and ED5 were significantly higher and ED8 tended to be higher after increased supplementation of $CuSO_4$. In contrast, supplementation of Cu from TBCC had no effects on the constant rate of degradation of b (c) and on effective degradability. This is in line with results of dry matter degradability. DMD was stimulated by $CuSO_4$ treatment whereas TBCC treatment did not reveal plausible effects on DMD.

The insoluble, but ruminally degradable fraction (b) and the totally degradable fraction (TDF) of TMR significantly declined by 3.3 % when Cu supplementation of $CuSO_4$ was increased. The totally degradable fraction (TDF) of maize silage also tended to decline when increasing supplementation of $CuSO_4$. However, the soluble fraction (a) of wheat

meal was significantly reduced after increased supplementation of $CuSO_4$ leading to a gain of the insoluble, but ruminally degradable fraction (b). Together, the totally degradable fraction (TDF) of wheat meal tended to be greater after increased supplementation of $CuSO_4$. In total, current results of degradable fractions (a, b, TDF) are inconsistent, confirmed by literature. Lopez-Guisa and Satter (1992) measured parameters of degradability in heifers and reported a larger rapidly degraded fraction (13 % at corn stalks, 5 % at alfalfa hay) after addition of $CuSO_4$ and $CoSO_4$ to the diet. The potentially degraded fraction of corn cobs, however, was significantly higher in control group, compared to the supplemented group.

The lag time (t_0) for grass silage was significantly reduced by increased supplementation of $CuSO_4$. This is in contrast to the study of Vázquez-Armijo *et al.* (2011). They used 10 ml of rumen fluid of Cu supplemented goats (21.7 mg Cu/kg DM) for *in vitro* incubation of 1.0 g substrate and measured an extended lag time for Cu supplemented animals than for control animals.

Overall, Cu supplementation in form of TBCC did not affect ruminal degradation, independent of Cu dose. Analyses of parameters of degradability and effective degradability revealed no effects of TBCC treatments even though dry matter degradability was partially affected. In contrast, Cu supplementation from $CuSO_4$ at mild excess stimulated ruminal degradation of feedstuffs which was clearly demonstrated by the results of dry matter degradability, rate of degradation, and effective degradability. Obviously, rumen solubility of Cu source was decisive for this effect, which cannot be traced back to changes of microbial concentrations in rumen fluid. The precondition for that would have been a growth promoting effect of Cu supplementation on microbial concentrations. Hence, Cu dependent changes regarding interactions between rumen microorganisms and feed material are assumed to be responsible for the stimulated ruminal degradation. Durand and Kawashima (1980) described that supplementation of trace elements may stimulate different functions of rumen microorganisms, e.g. enzyme function, and that diets deficient in trace elements can lead to impaired rumen fermentation. Nevertheless, Cu is known for its toxic effects on enzyme activity. Faixová and Faix (2002) observed lowered activities of urease and glutamate dehydrogenase after adding Cu to 10 ml of rumen fluid of eight fistulated ewes until a concentration of 5 mmol/l was reached. Goselink (2015) measured slight but significant decreases of amylase activity after addition of $CuSO_4$ to incubation solution. However, activities of alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyltransferase in rumen fluid were not affected by Cu addition (Faixová and Faix, 2002). Therefore, analyses of

specific enzyme activities within rumen contents are necessary to evaluate possible correlations with increased Cu supplementation.

Another considerable aspect is the way rumen microorganisms attach to ingested feed material. Plant tissues are generally protected by a cuticle which resists attachment by microorganisms (Bauchop, 1980; Akin, 1989; McAllister *et al.*, 1990). Consequently, rumen microorganisms use different strategies to overcome these biological barriers (McAllister *et al.*, 1994). A former study of Somers (1973) demonstrated that Mg^{2+} and Ca^{2+} ions showed high affinities for plant cell walls whereas Storry (1961) detected the absorption of Ca^{2+} ions to bacterial cell walls. Lopez-Guisa and Satter (1992) concluded, referring to these studies, that divalent cations may serve as a bridge between surfaces of bacterial and plant cell walls, both negatively charged. Additionally, Durand and Kawashima (1980) described the possible function of Zn^{2+} ions in bacterial cell walls for the adherence to feed fibre. This means, divalent cations are possibly able to accelerate and enhance the break-up of physical barriers of feed particles by rumen microorganisms, reflected in the fermentation lag time (Allen and Mertens, 1988). This would be in line with current results of reduced lag time (t_0) for the fibre rich grass silage in combination with increased supplementation of $CuSO_4$. However, further investigations of this matter are vital to gain better insights in Cu dependent interactions between rumen microorganisms and ingested feed material.

5.5 Rumen physiological parameters

5.5.1 pH-value

Cu supplementation had no effect on rumen pH, independent of Cu dose and source. This is confirmed by several authors who also could not observe any effects of Cu supplementation from $CuSO_4$ and TBCC on pH-values of rumen fluid *in vivo* (Felix et al., 2012; Del Claro et al., 2013; Genther and Hansen, 2015) or *in vitro* (Alvarado-Gilis et al., 2014). Contrary results were only observed by Zhang *et al.* (2007) who measured a significantly decreased pH-value in rumen fluid of goats after supplementation of 10 mg Cu/kg DM from $CuSO_4$. Supplementation of 20 and 30 mg Cu/kg DM, however, did also not alter rumen pH. In the present study as well as in listed *in vivo* studies the diets fed to the animals were moderate in concentrates. For such diets, a considerable drop of pH-values in rumen fluid is not to be expected, even though dry matter degradation is stimulated by increased supplementation of $CuSO_4$.

5.5.2 Ammonia-nitrogen

Cu supplementation, independent of Cu dose and source, had no statistically significant effect on ammonia-nitrogen (NH_3-N) concentrations in the rumen fluid. The increase of NH_3-N concentrations within the first 1.5 h after the morning feeding were similar between both Cu sources. Thereafter, NH_3-N concentrations at $CuSO_4$ treatment further increased to its maximum after 3 h (mean: 167 mg/l). The maximum NH_3-N concentrations at TBCC treatment, however, were already reached 1.5 h after the morning feeding (mean: 163 mg/l). Additionally, NH_3-N concentrations numerically increased with higher amounts of $CuSO_4$ up to 171 mg/l. These observations may indicate a possible correlation with dry matter degradability, which was demonstrably enhanced by increasing $CuSO_4$ supplementation. However, the stimulated dry matter degradability was measured after a minimum of 3 h after feeding. Unfortunately, there is a lack of comparable literature and therefore further investigations to confirm greater NH_3-N concentrations in the rumen fluid due to stimulated dry matter degradability, induced by mild dietary excess of $CuSO_4$, are necessary.

5.5.3 Volatile fatty acids

In the time course from 0 h to 7.5 h after the morning feeding there were no effects of Cu dose and source on total volatile fatty acid (VFA) concentrations as well as on acetic, propionic, butyric, and valeric acid concentrations in rumen fluid. The results of the stimulated dry matter degradation due to increased supplementation of $CuSO_4$ suggest higher amounts of VFA in rumen fluid. Nevertheless, animals were fed on maintenance level and, hence, in a non-intensive manner. Therefore, the increased VFA concentrations in rumen fluid are likely to be compensated by a more efficient absorption of short chain fatty acids from the lumen (Brugger *et al.*, 2016).

However, after 9 h a positive dose effect within the TBCC treatment was determined for total VFA and each single VFA, respectively. Only the acetic to propionic acid ratio remained consistently unaffected regarding all sampling times. This positive dose effect was mainly consisted in greater values at 50 mg Cu/kg DM from TBCC (≥ 19.1 %), compared to 35 and 10 mg Cu/kg DM from TBCC, and is therefore in contrast to results observed for dry matter degradability after TBCC treatments. The results of the in vitro study of Alvarado-Gilis *et al.* (2014) are contradicting as well. They added 10 and 100 mg Cu/kg to an incubation solution and measured higher concentrations of total VFA at the high-Cu treatment, whereas DMD was not affected by enhanced Cu addition. Conversely, acetic, propionic, butyric, and valeric acid concentrations as well as acetic to

propionic acid ratio were not affected by higher Cu addition. Del Claro *et al.* (2013) and Solaiman *et al.* (2007) also measured no effect of Cu supplementation on acetic, propionic, and butyric acid concentrations in rumen fluids of cattle and goats, respectively. Essig *et al.* (1972) reported a negative development of acetic, propionic, and butyric acid concentrations of Cu supplemented steers after feeding. The reason for that was the complete defaunation of steers by adding 4.4 g of $CuSO_4$ per 100 kg body weight. Zhang *et al.* (2007) supplemented 0, 10, 20, or 30 mg Cu/kg DM from $CuSO_4$ to a basal diet of goats containing 7.46 mg Cu/kg DM and maintained inconsistent results as well. Total VFA concentrations were significantly elevated for all Cu supplemented groups but increasing doses revealed no further differences in results. Acetic acid concentrations were higher at 10 and 20 mg Cu/kg DM, compared to control, but supplementation of 30 mg Cu/kg DM remained ineffective. Propionic and butyric acid concentrations were numerically but not significantly higher after Cu supplementation.

Summarised, increased Cu supplementation may affect VFA concentrations in the rumen fluid but consistent results are not available, especially in combination with results of dry matter degradability. As mentioned above, restrictive diets can be a reason for less informative results of VFA concentrations due to compensative absorption mechanisms. A study with animals fed a high-intensive diet could be a useful approach to clarify possible effects of Cu supplementation on VFA concentrations and other physiological parameters in the rumen. However, in non-intensive diets supplemented with Cu up to mild excess, independent of Cu source, VFA concentrations in the rumen fluid seem not to be affected.

5.6 Total tract digestibility

The present study demonstrated that Cu supplementation in form of $CuSO_4$, especially at mild excess, can positively change rumen fermentation due to better dry matter degradation, suggesting higher availability of nutrients for intestinal absorption. Therefore, apparent total tract digestibility was determined for DM, OM, CF, CP, TL, NFE, CA, NDF, and ADF in order to involve the final product of digestion for a complete evaluation of possible effects of Cu supplementation on feed conversion.

However, Cu supplementation differing in dose and source had neither an effect on DM and OM digestibility nor on digestibility of the single ingredients, even though dry matter degradability was significantly increased by enhanced $CuSO_4$ supplementation. In literature, different results are reported. Mondal and Biswas (2007) supplemented 0, 10, 20, and 30 mg Cu/kg DM either from $CuSO_4$ or from Cu proteinate to Cu depleted goat kids. Apparent digestibility of DM, OM, CP, and NfE were not affected by Cu source but

increased linearly with doses up to 30 mg Cu/kg DM, except for CP. Apparent digestibility of CF and CL were improved by Cu proteinate, compared to $CuSO_4$, and similarly by increasing Cu doses. In this case the positive dose effects as well as the source effect on apparent digestibility can be traced back to both the improved physiological status (Cu repletion) of the animals and to positive effects of Cu on rumen fermentation. Lopez-Guisa and Satter (1992) could not measure significant effects of combined Cu and Co supplementation (12.2 mg Cu from $CuSO_4$ and 0.25 mg Co from $CoSO_4$ per kg DM, respectively) on apparent DM digestibility, even though dry matter disappearance was partially higher between 1 h and 24 h of incubation. Felix *et al.* (2012) supplemented cattle up to 200 mg Cu/kg DM from TBCC and observed also no effect on apparent DM digestibility. However, in this study animals were provided with additional sulphur implants, influencing the mode of action of Cu in the rumen.

In three further consecutive studies goats were supplemented with 0, 10, 20, or 30 mg Cu/kg DM from $CuSO_4$, respectively (Zhang *et al.*, 2007; Zhang *et al.*, 2008; Zhang *et al.*, 2009). Apparent digestibility of DM and CP was not affected by Cu supplementation in each study. The first study reported a significantly higher apparent NDF digestibility for groups fed 10 or 20 mg Cu/kg DM, respectively, than for the control group. In the second study, apparent NDF digestibility was higher at 10 mg Cu/kg DM than at 30 mg Cu/kg DM and in the third study it was lower at 30 mg Cu/kg DM, compared to the remaining groups. Apparent ADF digestibility was higher at 10 mg Cu/kg DM than at 30 mg Cu/kg DM in the second and third study. They concluded that supplementation of 10 mg Cu/kg DM might already improve nutrient digestibility, possibly due to enhanced rumen fermentation, and that 30 mg Cu/kg DM has negative effects on NDF digestibility. Data about dry matter degradability are unfortunately not available in order to evaluate whether improved digestibility definitely resulted in enhanced rumen fermentation. Arthington (2005) provided eight steers randomly with intraruminal boluses containing 12.5 mg of Cu oxide and observed a reduced apparent digestibility for CP, NDF, and ADF, compared to non-bolused steers. Total tract OM digestibility, however, was not affected by Cu treatment. At the same time, Cu boluses led to a strongly increased liver Cu concentration, suggesting that boluses released sufficient amounts of Cu to cause toxic effects. In summary, comparisons between the mentioned studies and the present study are difficult due to varying experimental setups, especially considering the Cu status of the animals and missing data of ruminal degradation characteristics. Nevertheless, current results indicate that apparent nutrient digestibility was not affected by Cu supplementation up to mild excess under following preconditions:

Firstly, the Cu status of the animals was in a physiological range, excluding Cu depleted animals or Cu toxicity due to extremely high doses. Secondly, animals were fed restrictively on maintenance level, resulting in slow passage rates and high retention times of ingested feed in the rumen.

Current data of dry matter degradability shows that $CuSO_4$ induced stimulation occurred only in a time frame between 3 h and 12 h of incubation. After 24 h of incubation, no further effects of Cu supplementation on dry matter degradability could be observed. Consequently, promoting effects of $CuSO_4$ supplementation on dry matter degradability are presumably irrelevant with regard to feed conversion when non-intensive diets are fed. Positive effects of Cu supplementation on total tract digestibility are assumed to be possible in the case of high-intensive diets.

6 Conclusion

The results of the Cu concentrations in rumen contents demonstrate that both Cu sources, $CuSO_4$ and TBCC, were solubilised in the rumen environment. In comparison, $CuSO_4$ showed a higher solubility than TBCC. However, solubilised Cu, independent of Cu source, was not irreversibly bound by thiomolybdate to a noticeable extent. This presumably was due to moderate dietary concentrations of Mo and S. Furthermore, the amount of apparently digested Cu was elevated when Cu from $CuSO_4$ was supplemented at mild excess, compared to TBCC. In total, this indicates that $CuSO_4$ provided higher amounts of absorbable Cu in the intestine. Cu from TBCC was presumably not completely solubilised while passing through the abomasum, leading to a loss of absorbable Cu. Consequently, the experimental approach of this study led to the conclusion that former findings for Cu bioavailability cannot be transferred to situations where animals receive adequate and balanced amounts of Cu, S, and Mo.

The quantification of rumen microorganisms revealed a negative effect of Cu from $CuSO_4$ on *F. succinogenes*. Furthermore, the increased supplementation of $CuSO_4$ was capable to reduce the concentration of *S. bovis*. The remaining microorganisms were not affected by Cu supplementation. In summary, Cu supplementation induced only a few minor and highly selective negative changes in the rumen microbiota of cattle. Therefore, a sustainable impairment of microbial populations in the rumen can be ruled out. The present results, however, confirm the assumption that the solubility of Cu source is an important factor for determination of its toxic potential.

Rumen degradability of ingested feedstuffs was stimulated by increased supplementation of $CuSO_4$. This suggests that certain amounts of soluble Cu in the rumen may have beneficial effects on the microbial degradation of ingested feed material, independent of microbial growth. Cu supplementation, however, did not improve the total tract digestibility of ingested feedstuffs. This resulted from the non-intensive ration fed to the animals accompanied by a slow passage rate, which compensated the positive effect of increased doses of $CuSO_4$ on rumen degradation during the first 12 h after feeding.

Cu status parameters in the blood serum (Cu concentration, ceruloplasmin activity, and superoxide dismutase activity) were examined in order to receive a general overview of the Cu status and to affirm results of apparent Cu digestibility. Unfortunately, these Cu status parameters are subject to more influencing factors than just Cu supplementation and absorption, e.g. inflammatory processes. They were often described as not reliable for determination of Cu status, especially at adequate Cu supply. In contrast, liver Cu concentration directly reflects Cu absorption due to liver function being the key regulator

of Cu homeostasis. In the present study, however, no liver biopsies were performed and increased apparent Cu digestibility could not be affirmed by reliable data of Cu status. For future studies, the combined examination of apparent Cu digestibility and liver Cu concentration is recommended. Furthermore, sampling times of blood and liver samples should be reconsidered. Higher sampling frequencies, especially for the initial sampling before the first experimental period, would reveal an improved general idea of the Cu status.

The absolute quantification of rumen microorganisms was conducted by qPCR. This method enabled a direct measurement of changes in microbial concentrations. In comparison, other studies often drew conclusions about antimicrobial effects of supplemented Cu from data of gas production or substrate degradation, not allowing a differentiation between affected microbial growth and affected enzyme activities and other microbial functions. The current study, however, provides for the first time reliable data of absolute quantification of different microorganisms and at the same time of dry matter degradation. This allowed us to show that Cu induced improvement of dry matter degradation is not correlated with altered concentrations of selected microorganisms and that further investigations are needed to gain a deeper insight and additional knowledge on interactions between Cu supplementation and microbial feed degradation in rumen.

7 Literature

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Appendix

Table 39: Cu concentration in rumen fluid [particles: µg/g DM; liquid: µg/ml] and rumen solid [µg/g DM] of the different animals dependent on Cu treatment

Cu treatment	Animal	Rumen fluid		Rumen solid
		Particles	Liquid	
10 mg/kg ($CuSO_4$)	1	20.3	0.132	11.8
10 mg/kg ($CuSO_4$)	2	21.3	0.114	13.3
10 mg/kg ($CuSO_4$)	3	25.5	0.126	15.0
10 mg/kg ($CuSO_4$)	4	18.9	0.128	12.7
10 mg/kg ($CuSO_4$)	5	25.2	0.088	12.7
10 mg/kg ($CuSO_4$)	6	31.0	0.134	15.5
35 mg/kg ($CuSO_4$)	1	79.4	0.249	45.0
35 mg/kg ($CuSO_4$)	2	70.9	0.262	48.0
35 mg/kg ($CuSO_4$)	3	70.9	0.263	47.3
35 mg/kg ($CuSO_4$)	4	75.6	0.215	46.2
35 mg/kg ($CuSO_4$)	5	74.7	0.245	48.8
35 mg/kg ($CuSO_4$)	6	87.8	0.226	48.0
50 mg/kg ($CuSO_4$)	1	108	0.303	61.1
50 mg/kg ($CuSO_4$)	2	130	0.309	70.0
50 mg/kg ($CuSO_4$)	3	107	0.395	65.4
50 mg/kg ($CuSO_4$)	4	90.1	0.237	56.4
50 mg/kg ($CuSO_4$)	5	121	0.285	73.2
50 mg/kg ($CuSO_4$)	6	110	0.382	61.2
10 mg/kg (TBCC)	1	19.9	0.108	10.6
10 mg/kg (TBCC)	2	24.6	0.087	12.9
10 mg/kg (TBCC)	3	26.7	0.126	16.1
10 mg/kg (TBCC)	4	19.9	0.105	12.2
10 mg/kg (TBCC)	5	23.5	0.137	15.4
10 mg/kg (TBCC)	6	24.6	0.119	15.1
35 mg/kg (TBCC)	1	76.2	0.195	44.3
35 mg/kg (TBCC)	2	90.9	0.179	49.2
35 mg/kg (TBCC)	3	83.4	0.181	49.6
35 mg/kg (TBCC)	4	77.9	0.224	43.9
35 mg/kg (TBCC)	5	78.4	0.208	49.6
35 mg/kg (TBCC)	6	76.1	0.209	52.3
50 mg/kg (TBCC)	1	95.4	0.271	57.4
50 mg/kg (TBCC)	2	137	0.239	78.1
50 mg/kg (TBCC)	3	116	0.300	69.3
50 mg/kg (TBCC)	4	113	0.231	64.2
50 mg/kg (TBCC)	5	123	0.220	67.5
50 mg/kg (TBCC)	6	111	0.303	75.3

Table 40: Amounts of dried components (large particles, small particles, and bacteria) and liquid in duodenal digesta [mg/g FM] of the different animals dependent on Cu treatment

Cu treatment	Animal	Large particles	Small particles	Bacteria	Liquid
10 mg/kg ($CuSO_4$)	1	13.5	2.34	0.20	872
10 mg/kg ($CuSO_4$)	2	13.9	5.62	0.34	857
10 mg/kg ($CuSO_4$)	3	16.9	6.16	0.43	839
10 mg/kg ($CuSO_4$)	4	9.30	10.8	0.35	843
10 mg/kg ($CuSO_4$)	5	8.60	8.96	0.48	864
10 mg/kg ($CuSO_4$)	6	11.9	8.68	0.38	844
35 mg/kg ($CuSO_4$)	1	8.71	8.73	0.35	866
35 mg/kg ($CuSO_4$)	2	9.66	9.05	0.46	851
35 mg/kg ($CuSO_4$)	3	10.6	7.28	0.56	867
35 mg/kg ($CuSO_4$)	4	14.1	6.05	0.29	821
35 mg/kg ($CuSO_4$)	5	11.0	8.62	0.48	852
35 mg/kg ($CuSO_4$)	6	11.3	7.70	0.36	847
50 mg/kg ($CuSO_4$)	1	8.72	6.82	0.26	879
50 mg/kg ($CuSO_4$)	2	13.0	10.8	0.44	826
50 mg/kg ($CuSO_4$)	3	11.9	9.79	0.54	851
50 mg/kg ($CuSO_4$)	4	11.5	6.92	0.37	847
50 mg/kg ($CuSO_4$)	5	8.25	7.50	0.39	871
50 mg/kg ($CuSO_4$)	6	13.7	8.84	0.61	794
10 mg/kg (TBCC)	1	10.9	5.52	0.26	877
10 mg/kg (TBCC)	2	16.4	8.16	0.34	821
10 mg/kg (TBCC)	3	5.81	9.25	0.47	891
10 mg/kg (TBCC)	4	11.8	5.65	0.36	809
10 mg/kg (TBCC)	5	10.9	7.45	0.46	862
10 mg/kg (TBCC)	6	15.0	7.79	0.51	820
35 mg/kg (TBCC)	1	7.83	3.57	0.17	903
35 mg/kg (TBCC)	2	9.78	11.1	0.37	844
35 mg/kg (TBCC)	3	13.8	10.5	0.63	832
35 mg/kg (TBCC)	4	11.4	10.3	0.37	833
35 mg/kg (TBCC)	5	10.1	7.00	0.43	876
35 mg/kg (TBCC)	6	16.6	6.80	0.42	819
50 mg/kg (TBCC)	1	12.3	3.96	0.29	876
50 mg/kg (TBCC)	2	11.2	6.05	0.34	865
50 mg/kg (TBCC)	3	9.90	11.0	0.74	859
50 mg/kg (TBCC)	4	7.77	11.8	0.36	844
50 mg/kg (TBCC)	5	10.8	10.2	0.45	849
50 mg/kg (TBCC)	6	13.2	6.71	0.52	845

Table 41: Cu concentration in solid phase [µg/g DM] and liquid phase [µg/ml] of duodenal digesta of the different animals dependent on Cu treatment

Cu treatment	Animal	Solid phase			Liquid phase	
		Large particles	Small particles	Bacteria	Total liquid	Liquid after precipitation
10 mg/kg (CuSO4)	1	19.5	28.4	33.0	0.122	0.128
10 mg/kg (CuSO4)	2	24.1	30.8	27.1	0.065	0.092
10 mg/kg (CuSO4)	3	19.2	32.3	31.6	0.075	0.107
10 mg/kg (CuSO4)	4	21.9	33.1	32.6	0.068	0.083
10 mg/kg (CuSO4)	5	17.7	28.5	27.8	0.059	0.068
10 mg/kg (CuSO4)	6	23.1	37.0	42.4	0.068	0.066
35 mg/kg (CuSO4)	1	68.7	98.6	91.9	0.237	0.246
35 mg/kg (CuSO4)	2	78.3	109	97.8	0.184	0.157
35 mg/kg (CuSO4)	3	77.2	101	74.0	0.118	0.143
35 mg/kg (CuSO4)	4	98.3	115	85.7	0.164	0.169
35 mg/kg (CuSO4)	5	69.3	96.0	82.8	0.149	0.146
35 mg/kg (CuSO4)	6	82.6	130	119	0.124	0.118
50 mg/kg (CuSO4)	1	120	146	109	0.315	0.272
50 mg/kg (CuSO4)	2	96.6	145	120	0.178	0.187
50 mg/kg (CuSO4)	3	87.0	135	103	0.164	0.150
50 mg/kg (CuSO4)	4	123	156	109	0.126	0.132
50 mg/kg (CuSO4)	5	122	157	124	0.201	0.206
50 mg/kg (CuSO4)	6	96.0	152	132	0.161	0.215
10 mg/kg (TBCC)	1	21.2	27.9	27.3	0.070	0.088
10 mg/kg (TBCC)	2	14.6	30.9	35.1	0.062	0.076
10 mg/kg (TBCC)	3	21.2	33.1	29.5	0.085	0.103
10 mg/kg (TBCC)	4	25.8	28.6	37.9	0.103	0.093
10 mg/kg (TBCC)	5	22.0	32.1	36.5	0.068	0.088
10 mg/kg (TBCC)	6	19.8	34.0	29.9	0.070	0.072
35 mg/kg (TBCC)	1	107	110	106	0.133	0.126
35 mg/kg (TBCC)	2	81.9	113	71.8	0.128	0.128
35 mg/kg (TBCC)	3	54.3	103	66.9	0.101	0.100
35 mg/kg (TBCC)	4	75.8	114	86.7	0.247	0.245
35 mg/kg (TBCC)	5	63.2	82.4	58.5	0.356	0.320
35 mg/kg (TBCC)	6	85.7	117	58.8	0.132	0.144
50 mg/kg (TBCC)	1	132	130	87.3	0.239	0.235
50 mg/kg (TBCC)	2	133	155	83.3	0.171	0.183
50 mg/kg (TBCC)	3	72.3	149	99.3	0.128	0.130
50 mg/kg (TBCC)	4	123	172	121	0.301	0.276
50 mg/kg (TBCC)	5	86.3	157	94.5	0.160	0.163
50 mg/kg (TBCC)	6	111	176	83.8	0.251	0.222

Table 42: Cu distribution in the fresh matter of total duodenal digesta (total Cu = Cu content in 1 g FM of duodenal digesta) [µg/g FM] dependent on Cu treatment

Cu treatment	Animal	Total Cu	Large particles	Small particles	Bacteria	Liquid
10 mg/kg ($CuSO_4$)	1	0.44	0.26	0.07	0.007	0.107
10 mg/kg ($CuSO_4$)	2	0.57	0.33	0.17	0.009	0.056
10 mg/kg ($CuSO_4$)	3	0.60	0.33	0.20	0.013	0.063
10 mg/kg ($CuSO_4$)	4	0.63	0.20	0.36	0.011	0.058
10 mg/kg ($CuSO_4$)	5	0.47	0.15	0.26	0.013	0.051
10 mg/kg ($CuSO_4$)	6	0.67	0.28	0.32	0.016	0.057
35 mg/kg ($CuSO_4$)	1	1.70	0.60	0.86	0.032	0.205
35 mg/kg ($CuSO_4$)	2	1.95	0.76	0.99	0.045	0.156
35 mg/kg ($CuSO_4$)	3	1.69	0.82	0.73	0.042	0.102
35 mg/kg ($CuSO_4$)	4	2.24	1.38	0.70	0.025	0.135
35 mg/kg ($CuSO_4$)	5	1.76	0.76	0.83	0.039	0.127
35 mg/kg ($CuSO_4$)	6	2.08	0.94	1.00	0.043	0.105
50 mg/kg ($CuSO_4$)	1	2.35	1.05	0.99	0.029	0.277
50 mg/kg ($CuSO_4$)	2	3.02	1.26	1.57	0.053	0.147
50 mg/kg ($CuSO_4$)	3	2.55	1.04	1.32	0.056	0.139
50 mg/kg ($CuSO_4$)	4	2.64	1.42	1.08	0.041	0.107
50 mg/kg ($CuSO_4$)	5	2.40	1.00	1.17	0.048	0.175
50 mg/kg ($CuSO_4$)	6	2.87	1.31	1.34	0.080	0.128
10 mg/kg (TBCC)	1	0.45	0.23	0.15	0.007	0.062
10 mg/kg (TBCC)	2	0.56	0.24	0.25	0.012	0.051
10 mg/kg (TBCC)	3	0.52	0.12	0.31	0.014	0.076
10 mg/kg (TBCC)	4	0.56	0.30	0.16	0.014	0.083
10 mg/kg (TBCC)	5	0.55	0.24	0.24	0.017	0.059
10 mg/kg (TBCC)	6	0.63	0.30	0.26	0.015	0.057
35 mg/kg (TBCC)	1	1.37	0.84	0.39	0.018	0.121
35 mg/kg (TBCC)	2	2.18	0.80	1.25	0.027	0.108
35 mg/kg (TBCC)	3	1.96	0.75	1.08	0.042	0.084
35 mg/kg (TBCC)	4	2.28	0.86	1.18	0.032	0.205
35 mg/kg (TBCC)	5	1.55	0.64	0.58	0.025	0.312
35 mg/kg (TBCC)	6	2.35	1.43	0.79	0.025	0.108
50 mg/kg (TBCC)	1	2.38	1.63	0.51	0.025	0.210
50 mg/kg (TBCC)	2	2.60	1.49	0.94	0.028	0.147
50 mg/kg (TBCC)	3	2.53	0.72	1.63	0.073	0.110
50 mg/kg (TBCC)	4	3.29	0.96	2.04	0.043	0.254
50 mg/kg (TBCC)	5	2.70	0.93	1.60	0.042	0.135
50 mg/kg (TBCC)	6	2.90	1.47	1.18	0.043	0.212

Table 43: Daily Cu intake [mg/day], Cu concentration in faeces [mg/kg DM], daily faecal Cu excretion [mg/day], apparent digestibility [%], and daily amount of apparently digested Cu [mg/day] of the different animals dependent on Cu treatment

Cu treatment	Animal	Cu intake	Cu concentration in faeces	Faecal Cu excretion	Apparent Cu digestibility	Amount of digested Cu
10 mg/kg ($CuSO_4$)	1	65	37.2	49.8	23.3	15.2
10 mg/kg ($CuSO_4$)	2	65	39.0	62.3	4.11	2.67
10 mg/kg ($CuSO_4$)	3	65	60.1	77.3	-18.9	-12.3
10 mg/kg ($CuSO_4$)	4	65	43.0	56.9	12.4	8.08
10 mg/kg ($CuSO_4$)	5	65	41.5	49.3	24.1	15.7
10 mg/kg ($CuSO_4$)	6	65	48.4	73.0	-12.3	-7.97
35 mg/kg ($CuSO_4$)	1	228	134	203	11.2	25.5
35 mg/kg ($CuSO_4$)	2	228	139	191	11.2	25.5
35 mg/kg ($CuSO_4$)	3	228	146	218	16.0	36.5
35 mg/kg ($CuSO_4$)	4	228	161	199	4.18	9.51
35 mg/kg ($CuSO_4$)	5	228	147	204	12.4	28.3
35 mg/kg ($CuSO_4$)	6	228	155	202	10.2	23.3
50 mg/kg ($CuSO_4$)	1	325	210	262	9.77	31.8
50 mg/kg ($CuSO_4$)	2	325	168	267	13.6	44.1
50 mg/kg ($CuSO_4$)	3	325	163	230	13.7	44.4
50 mg/kg ($CuSO_4$)	4	325	175	270	19.5	63.5
50 mg/kg ($CuSO_4$)	5	325	237	293	29.2	94.7
50 mg/kg ($CuSO_4$)	6	325	206	281	17.0	55.1
10 mg/kg (TBCC)	1	65	38.8	56.0	-18.1	-11.8
10 mg/kg (TBCC)	2	65	40.2	54.8	1.60	1.04
10 mg/kg (TBCC)	3	65	42.9	64.4	10.1	6.57
10 mg/kg (TBCC)	4	65	55.9	76.8	11.7	7.60
10 mg/kg (TBCC)	5	65	40.7	64.0	13.9	9.00
10 mg/kg (TBCC)	6	65	44.2	58.4	15.7	10.2
35 mg/kg (TBCC)	1	228	157	207	10.1	23.1
35 mg/kg (TBCC)	2	228	145	204	23.0	52.4
35 mg/kg (TBCC)	3	228	136	175	5.77	13.1
35 mg/kg (TBCC)	4	228	143	222	5.07	11.5
35 mg/kg (TBCC)	5	228	163	214	14.8	33.6
35 mg/kg (TBCC)	6	228	140	216	8.99	20.4
50 mg/kg (TBCC)	1	325	179	283	12.9	41.8
50 mg/kg (TBCC)	2	325	227	305	6.09	19.8
50 mg/kg (TBCC)	3	325	202	282	13.2	42.8
50 mg/kg (TBCC)	4	325	210	270	16.9	55.0
50 mg/kg (TBCC)	5	325	225	290	19.6	63.8
50 mg/kg (TBCC)	6	325	218	261	7.23	23.5

Table 44: Cu concentration [µg/ml], ceruloplasmin activity [mU/l], and superoxide dismutase activity (inhibition rate) [%] in the blood serum of the different animals dependent on Cu treatment

Cu treatment	Animal	Cu concentration	Ceruloplasmin activity	SOD activity - Inhibition rate
10 mg/kg ($CuSO_4$)	1	1.07	12.7	82.0
10 mg/kg ($CuSO_4$)	2	1.07	13.1	89.2
10 mg/kg ($CuSO_4$)	3	0.59	11.4	83.8
10 mg/kg ($CuSO_4$)	4	0.93	12.8	80.6
10 mg/kg ($CuSO_4$)	5	0.60	13.9	76.7
10 mg/kg ($CuSO_4$)	6	0.68	14.6	84.7
35 mg/kg ($CuSO_4$)	1	0.75	14.4	83.0
35 mg/kg ($CuSO_4$)	2	1.18	12.0	87.6
35 mg/kg ($CuSO_4$)	3	0.92	11.9	83.8
35 mg/kg ($CuSO_4$)	4	0.88	13.0	81.5
35 mg/kg ($CuSO_4$)	5	0.57	10.6	76.7
35 mg/kg ($CuSO_4$)	6	0.70	13.6	85.1
50 mg/kg ($CuSO_4$)	1	0.77	14.1	83.3
50 mg/kg ($CuSO_4$)	2	0.87	15.9	89.0
50 mg/kg ($CuSO_4$)	3	1.25	13.1	84.5
50 mg/kg ($CuSO_4$)	4	1.05	13.3	83.0
50 mg/kg ($CuSO_4$)	5	0.73	11.7	77.1
50 mg/kg ($CuSO_4$)	6	0.63	11.5	82.7
10 mg/kg (TBCC)	1	0.70	12.2	83.2
10 mg/kg (TBCC)	2	0.97	14.6	90.9
10 mg/kg (TBCC)	3	0.81	13.8	84.8
10 mg/kg (TBCC)	4	1.11	12.7	86.8
10 mg/kg (TBCC)	5	0.76	11.9	78.9
10 mg/kg (TBCC)	6	0.62	11.2	81.0
35 mg/kg (TBCC)	1	0.78	13.6	87.4
35 mg/kg (TBCC)	2	0.74	15.2	89.3
35 mg/kg (TBCC)	3	0.68	14.7	89.0
35 mg/kg (TBCC)	4	1.10	17.6	82.1
35 mg/kg (TBCC)	5	0.69	10.6	82.2
35 mg/kg (TBCC)	6	0.89	12.5	82.3
50 mg/kg (TBCC)	1	0.99	13.9	84.7
50 mg/kg (TBCC)	2	0.77	13.6	91.5
50 mg/kg (TBCC)	3	0.76	12.8	86.5
50 mg/kg (TBCC)	4	1.10	19.1	90.9
50 mg/kg (TBCC)	5	0.69	14.4	79.0
50 mg/kg (TBCC)	6	0.82	13.3	84.7

Table 45: Log_{10} 16S rRNA copy numbers of total bacteria in the rumen fluid [per g DM] of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding		
		0 h	1.5 h	3 h
10 mg/kg ($CuSO_4$)	1	10.4	10.6	10.3
10 mg/kg ($CuSO_4$)	2	10.5	10.2	10.0
10 mg/kg ($CuSO_4$)	3	10.5	10.4	10.5
10 mg/kg ($CuSO_4$)	4	10.6	10.4	10.7
10 mg/kg ($CuSO_4$)	5	10.7	10.4	10.7
10 mg/kg ($CuSO_4$)	6	10.9	10.5	10.8
35 mg/kg ($CuSO_4$)	1	10.9	10.4	10.7
35 mg/kg ($CuSO_4$)	2	10.4	10.2	10.0
35 mg/kg ($CuSO_4$)	3	10.5	10.5	10.6
35 mg/kg ($CuSO_4$)	4	10.5	10.3	10.4
35 mg/kg ($CuSO_4$)	5	10.6	10.5	10.8
35 mg/kg ($CuSO_4$)	6	10.7	10.6	10.9
50 mg/kg ($CuSO_4$)	1	10.6	10.4	10.7
50 mg/kg ($CuSO_4$)	2	10.6	10.4	10.8
50 mg/kg ($CuSO_4$)	3	10.4	10.3	10.5
50 mg/kg ($CuSO_4$)	4	10.3	10.5	10.3
50 mg/kg ($CuSO_4$)	5	10.4	10.3	10.6
50 mg/kg ($CuSO_4$)	6	10.9	10.2	10.7
10 mg/kg (TBCC)	1	10.8	10.4	10.7
10 mg/kg (TBCC)	2	10.8	10.6	10.7
10 mg/kg (TBCC)	3	10.8	10.6	10.8
10 mg/kg (TBCC)	4	10.4	10.3	10.3
10 mg/kg (TBCC)	5	10.2	10.5	10.5
10 mg/kg (TBCC)	6	10.6	10.5	10.7
35 mg/kg (TBCC)	1	10.4	10.3	10.4
35 mg/kg (TBCC)	2	10.9	10.3	10.8
35 mg/kg (TBCC)	3	10.7	10.0	10.5
35 mg/kg (TBCC)	4	10.7	10.5	10.7
35 mg/kg (TBCC)	5	10.4	10.4	10.4
35 mg/kg (TBCC)	6	10.6	10.6	10.7
50 mg/kg (TBCC)	1	10.4	10.3	10.3
50 mg/kg (TBCC)	2	10.6	10.6	10.7
50 mg/kg (TBCC)	3	10.7	10.3	10.8
50 mg/kg (TBCC)	4	10.6	10.6	10.7
50 mg/kg (TBCC)	5	10.7	10.4	10.6
50 mg/kg (TBCC)	6	10.6	10.4	10.6

Table 46: Log_{10} 16S rRNA copy numbers of *Ruminococcus flavefaciens* in the rumen fluid [per g DM] of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding		
		0 h	1.5 h	3 h
10 mg/kg ($CuSO_4$)	1	7.36	7.78	7.73
10 mg/kg ($CuSO_4$)	2	7.76	8.43	8.29
10 mg/kg ($CuSO_4$)	3	7.70	8.11	8.28
10 mg/kg ($CuSO_4$)	4	7.69	8.21	8.33
10 mg/kg ($CuSO_4$)	5	7.93	8.44	8.60
10 mg/kg ($CuSO_4$)	6	7.88	8.35	8.36
35 mg/kg ($CuSO_4$)	1	7.89	8.12	8.13
35 mg/kg ($CuSO_4$)	2	7.66	8.20	7.49
35 mg/kg ($CuSO_4$)	3	7.59	8.08	8.35
35 mg/kg ($CuSO_4$)	4	7.73	8.39	8.35
35 mg/kg ($CuSO_4$)	5	7.83	8.49	8.43
35 mg/kg ($CuSO_4$)	6	7.64	8.34	8.49
50 mg/kg ($CuSO_4$)	1	7.59	8.10	8.15
50 mg/kg ($CuSO_4$)	2	7.56	8.04	8.55
50 mg/kg ($CuSO_4$)	3	7.61	7.98	8.34
50 mg/kg ($CuSO_4$)	4	7.43	8.17	8.28
50 mg/kg ($CuSO_4$)	5	7.67	8.18	8.54
50 mg/kg ($CuSO_4$)	6	8.17	8.51	8.67
10 mg/kg (TBCC)	1	7.75	8.07	8.22
10 mg/kg (TBCC)	2	7.82	8.51	8.37
10 mg/kg (TBCC)	3	7.95	8.48	8.47
10 mg/kg (TBCC)	4	7.85	8.32	8.29
10 mg/kg (TBCC)	5	7.66	8.53	8.37
10 mg/kg (TBCC)	6	7.49	8.15	8.43
35 mg/kg (TBCC)	1	7.46	7.97	8.02
35 mg/kg (TBCC)	2	7.91	8.27	8.65
35 mg/kg (TBCC)	3	7.94	8.09	8.22
35 mg/kg (TBCC)	4	7.54	8.13	8.31
35 mg/kg (TBCC)	5	7.67	8.23	8.30
35 mg/kg (TBCC)	6	7.88	8.29	8.52
50 mg/kg (TBCC)	1	7.23	7.76	7.75
50 mg/kg (TBCC)	2	7.79	8.37	8.44
50 mg/kg (TBCC)	3	7.75	8.27	8.33
50 mg/kg (TBCC)	4	7.53	8.12	8.14
50 mg/kg (TBCC)	5	8.03	8.38	8.38
50 mg/kg (TBCC)	6	8.00	8.35	8.55

Table 47: Log_{10} 16S rRNA copy numbers of *Fibrobacter succinogenes* in the rumen fluid [per g DM] of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding		
		0 h	1.5 h	3 h
10 mg/kg ($CuSO_4$)	1	8.41	9.15	8.87
10 mg/kg ($CuSO_4$)	2	8.30	8.34	7.86
10 mg/kg ($CuSO_4$)	3	8.36	8.85	8.94
10 mg/kg ($CuSO_4$)	4	8.13	8.48	8.98
10 mg/kg ($CuSO_4$)	5	8.35	8.71	9.05
10 mg/kg ($CuSO_4$)	6	8.57	8.73	9.11
35 mg/kg ($CuSO_4$)	1	8.74	8.59	9.23
35 mg/kg ($CuSO_4$)	2	8.25	8.33	7.83
35 mg/kg ($CuSO_4$)	3	8.43	8.85	9.22
35 mg/kg ($CuSO_4$)	4	8.27	8.76	8.90
35 mg/kg ($CuSO_4$)	5	8.24	8.87	9.19
35 mg/kg ($CuSO_4$)	6	8.21	8.91	9.21
50 mg/kg ($CuSO_4$)	1	8.30	8.82	9.07
50 mg/kg ($CuSO_4$)	2	8.29	8.33	9.23
50 mg/kg ($CuSO_4$)	3	8.15	8.69	8.88
50 mg/kg ($CuSO_4$)	4	8.09	8.96	8.75
50 mg/kg ($CuSO_4$)	5	8.52	8.93	9.17
50 mg/kg ($CuSO_4$)	6	8.51	8.64	9.03
10 mg/kg (TBCC)	1	8.64	8.86	9.17
10 mg/kg (TBCC)	2	8.66	9.05	9.09
10 mg/kg (TBCC)	3	8.42	8.93	9.16
10 mg/kg (TBCC)	4	8.31	8.85	8.77
10 mg/kg (TBCC)	5	8.06	9.01	9.05
10 mg/kg (TBCC)	6	8.49	8.93	9.23
35 mg/kg (TBCC)	1	8.42	8.83	9.04
35 mg/kg (TBCC)	2	8.50	8.27	9.20
35 mg/kg (TBCC)	3	8.40	8.41	9.01
35 mg/kg (TBCC)	4	8.25	8.83	9.01
35 mg/kg (TBCC)	5	8.40	8.86	8.80
35 mg/kg (TBCC)	6	8.64	9.15	9.29
50 mg/kg (TBCC)	1	8.54	8.92	8.99
50 mg/kg (TBCC)	2	8.55	9.06	9.20
50 mg/kg (TBCC)	3	8.23	8.76	9.14
50 mg/kg (TBCC)	4	8.19	9.13	9.29
50 mg/kg (TBCC)	5	8.35	8.61	8.98
50 mg/kg (TBCC)	6	8.56	8.90	9.00

Table 48: Log_{10} 16S rRNA copy numbers of *Streptococcus bovis* in the rumen fluid [per g DM] of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding		
		0 h	1.5 h	3 h
10 mg/kg ($CuSO_4$)	1	7.93	8.07	7.76
10 mg/kg ($CuSO_4$)	2	7.99	7.82	7.76
10 mg/kg ($CuSO_4$)	3	7.77	7.67	7.76
10 mg/kg ($CuSO_4$)	4	7.43	7.87	7.81
10 mg/kg ($CuSO_4$)	5	7.57	7.80	7.91
10 mg/kg ($CuSO_4$)	6	7.54	7.76	7.74
35 mg/kg ($CuSO_4$)	1	7.79	7.78	7.80
35 mg/kg ($CuSO_4$)	2	7.74	7.59	7.45
35 mg/kg ($CuSO_4$)	3	7.89	7.66	7.78
35 mg/kg ($CuSO_4$)	4	7.93	7.74	7.72
35 mg/kg ($CuSO_4$)	5	7.39	7.86	8.00
35 mg/kg ($CuSO_4$)	6	7.48	7.78	7.88
50 mg/kg ($CuSO_4$)	1	7.54	7.79	7.84
50 mg/kg ($CuSO_4$)	2	7.53	7.70	7.80
50 mg/kg ($CuSO_4$)	3	7.80	7.52	7.78
50 mg/kg ($CuSO_4$)	4	7.80	7.76	7.68
50 mg/kg ($CuSO_4$)	5	7.74	7.49	7.75
50 mg/kg ($CuSO_4$)	6	7.60	7.68	7.79
10 mg/kg (TBCC)	1	7.68	7.92	7.95
10 mg/kg (TBCC)	2	7.62	7.90	7.81
10 mg/kg (TBCC)	3	7.63	7.86	7.79
10 mg/kg (TBCC)	4	7.84	7.64	7.67
10 mg/kg (TBCC)	5	7.73	7.79	7.78
10 mg/kg (TBCC)	6	7.85	7.79	7.95
35 mg/kg (TBCC)	1	7.65	7.63	7.63
35 mg/kg (TBCC)	2	7.59	7.79	7.88
35 mg/kg (TBCC)	3	7.73	7.58	7.74
35 mg/kg (TBCC)	4	7.55	7.84	7.80
35 mg/kg (TBCC)	5	7.74	7.61	7.70
35 mg/kg (TBCC)	6	7.96	7.80	7.90
50 mg/kg (TBCC)	1	7.78	7.82	7.75
50 mg/kg (TBCC)	2	7.93	7.86	7.78
50 mg/kg (TBCC)	3	7.57	7.84	7.85
50 mg/kg (TBCC)	4	7.50	7.96	7.93
50 mg/kg (TBCC)	5	7.50	7.76	7.76
50 mg/kg (TBCC)	6	8.00	7.67	7.86

Table 49: Log_{10} 16S rRNA copy numbers of archaebacteria in the rumen fluid [per g DM] of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding		
		0 h	1.5 h	3 h
10 mg/kg ($CuSO_4$)	1	7.98	8.52	8.18
10 mg/kg ($CuSO_4$)	2	8.34	8.10	7.80
10 mg/kg ($CuSO_4$)	3	8.19	8.37	8.47
10 mg/kg ($CuSO_4$)	4	7.87	8.25	8.68
10 mg/kg ($CuSO_4$)	5	7.86	8.07	8.59
10 mg/kg ($CuSO_4$)	6	8.11	8.09	8.53
35 mg/kg ($CuSO_4$)	1	8.19	8.18	8.54
35 mg/kg ($CuSO_4$)	2	8.20	8.16	8.05
35 mg/kg ($CuSO_4$)	3	8.26	8.39	8.46
35 mg/kg ($CuSO_4$)	4	8.09	8.02	8.31
35 mg/kg ($CuSO_4$)	5	7.86	8.36	8.77
35 mg/kg ($CuSO_4$)	6	7.82	8.14	8.72
50 mg/kg ($CuSO_4$)	1	7.77	7.88	8.56
50 mg/kg ($CuSO_4$)	2	7.96	8.23	8.58
50 mg/kg ($CuSO_4$)	3	8.25	8.17	8.51
50 mg/kg ($CuSO_4$)	4	8.12	8.49	8.34
50 mg/kg ($CuSO_4$)	5	8.22	8.21	8.63
50 mg/kg ($CuSO_4$)	6	8.08	7.70	8.30
10 mg/kg (TBCC)	1	8.00	8.32	8.51
10 mg/kg (TBCC)	2	8.08	8.29	8.56
10 mg/kg (TBCC)	3	8.09	8.33	8.62
10 mg/kg (TBCC)	4	8.21	8.03	8.40
10 mg/kg (TBCC)	5	8.19	8.48	8.61
10 mg/kg (TBCC)	6	8.15	8.35	8.60
35 mg/kg (TBCC)	1	7.91	8.24	8.28
35 mg/kg (TBCC)	2	8.12	8.30	8.73
35 mg/kg (TBCC)	3	7.98	7.23	8.20
35 mg/kg (TBCC)	4	7.89	8.45	8.55
35 mg/kg (TBCC)	5	8.25	8.16	8.51
35 mg/kg (TBCC)	6	8.25	8.48	8.71
50 mg/kg (TBCC)	1	8.06	8.34	8.21
50 mg/kg (TBCC)	2	8.34	8.57	8.67
50 mg/kg (TBCC)	3	8.05	8.20	8.81
50 mg/kg (TBCC)	4	7.60	8.61	8.68
50 mg/kg (TBCC)	5	8.05	8.19	8.69
50 mg/kg (TBCC)	6	8.19	7.95	8.30

Table 50: Log_{10} 18S rRNA copy numbers of protozoa in the rumen fluid [per g DM] of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding		
		0 h	1.5 h	3 h
10 mg/kg ($CuSO_4$)	1	9.06	9.61	9.70
10 mg/kg ($CuSO_4$)	2	8.92	9.58	9.44
10 mg/kg ($CuSO_4$)	3	9.20	9.70	9.80
10 mg/kg ($CuSO_4$)	4	9.21	9.39	9.90
10 mg/kg ($CuSO_4$)	5	8.97	9.66	9.88
10 mg/kg ($CuSO_4$)	6	9.11	9.44	9.70
35 mg/kg ($CuSO_4$)	1	8.91	9.39	9.74
35 mg/kg ($CuSO_4$)	2	8.99	9.63	9.67
35 mg/kg ($CuSO_4$)	3	9.02	9.59	9.76
35 mg/kg ($CuSO_4$)	4	9.25	9.72	9.85
35 mg/kg ($CuSO_4$)	5	8.99	9.59	9.65
35 mg/kg ($CuSO_4$)	6	8.46	9.42	9.64
50 mg/kg ($CuSO_4$)	1	8.79	9.58	9.45
50 mg/kg ($CuSO_4$)	2	8.64	9.22	9.67
50 mg/kg ($CuSO_4$)	3	9.03	9.60	9.71
50 mg/kg ($CuSO_4$)	4	9.01	9.60	9.73
50 mg/kg ($CuSO_4$)	5	8.95	9.61	9.93
50 mg/kg ($CuSO_4$)	6	9.13	9.21	9.51
10 mg/kg (TBCC)	1	8.99	9.58	9.79
10 mg/kg (TBCC)	2	8.71	9.49	9.58
10 mg/kg (TBCC)	3	9.02	9.47	9.81
10 mg/kg (TBCC)	4	9.18	9.72	9.72
10 mg/kg (TBCC)	5	8.87	9.69	9.77
10 mg/kg (TBCC)	6	9.03	9.76	9.92
35 mg/kg (TBCC)	1	8.95	9.62	9.96
35 mg/kg (TBCC)	2	8.82	9.28	9.56
35 mg/kg (TBCC)	3	8.83	9.35	9.72
35 mg/kg (TBCC)	4	9.02	9.67	9.74
35 mg/kg (TBCC)	5	8.91	9.55	9.75
35 mg/kg (TBCC)	6	9.00	9.87	10.02
50 mg/kg (TBCC)	1	9.00	9.43	9.73
50 mg/kg (TBCC)	2	8.84	9.63	9.66
50 mg/kg (TBCC)	3	8.98	9.38	9.76
50 mg/kg (TBCC)	4	8.85	9.52	9.78
50 mg/kg (TBCC)	5	9.14	9.63	9.68
50 mg/kg (TBCC)	6	9.10	9.66	9.75

Table 51: Log_{10} 18S rRNA copy numbers of anaerobic fungi in the rumen fluid [per g DM] of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding		
		0 h	1.5 h	3 h
10 mg/kg ($CuSO_4$)	1	7.46	8.14	8.13
10 mg/kg ($CuSO_4$)	2	7.45	8.22	8.04
10 mg/kg ($CuSO_4$)	3	7.91	8.37	8.50
10 mg/kg ($CuSO_4$)	4	7.43	8.22	7.89
10 mg/kg ($CuSO_4$)	5	7.51	8.26	8.25
10 mg/kg ($CuSO_4$)	6	7.54	8.24	8.22
35 mg/kg ($CuSO_4$)	1	7.92	8.18	8.20
35 mg/kg ($CuSO_4$)	2	7.48	8.29	7.76
35 mg/kg ($CuSO_4$)	3	7.53	8.20	8.45
35 mg/kg ($CuSO_4$)	4	7.57	8.24	8.14
35 mg/kg ($CuSO_4$)	5	7.42	8.35	7.96
35 mg/kg ($CuSO_4$)	6	7.03	8.22	8.19
50 mg/kg ($CuSO_4$)	1	7.47	8.37	7.99
50 mg/kg ($CuSO_4$)	2	7.31	8.06	8.43
50 mg/kg ($CuSO_4$)	3	7.58	8.29	8.42
50 mg/kg ($CuSO_4$)	4	7.47	8.43	8.15
50 mg/kg ($CuSO_4$)	5	7.80	8.26	8.47
50 mg/kg ($CuSO_4$)	6	7.18	7.93	7.88
10 mg/kg (TBCC)	1	7.61	7.97	8.13
10 mg/kg (TBCC)	2	7.43	8.51	8.05
10 mg/kg (TBCC)	3	7.69	8.40	8.38
10 mg/kg (TBCC)	4	7.55	8.13	7.98
10 mg/kg (TBCC)	5	7.73	8.60	8.40
10 mg/kg (TBCC)	6	7.68	8.46	8.52
35 mg/kg (TBCC)	1	7.43	7.95	8.12
35 mg/kg (TBCC)	2	7.21	7.91	8.37
35 mg/kg (TBCC)	3	7.61	8.15	8.28
35 mg/kg (TBCC)	4	7.30	8.01	7.96
35 mg/kg (TBCC)	5	7.63	8.46	8.21
35 mg/kg (TBCC)	6	7.84	8.35	8.52
50 mg/kg (TBCC)	1	7.39	8.05	7.95
50 mg/kg (TBCC)	2	7.63	8.41	8.34
50 mg/kg (TBCC)	3	7.60	8.20	8.15
50 mg/kg (TBCC)	4	7.04	8.26	8.20
50 mg/kg (TBCC)	5	7.61	8.17	7.98
50 mg/kg (TBCC)	6	7.56	8.27	8.36

Table 52: Ruminal dry matter disappearance [%] of TMR of the different animals dependent on Cu treatment and incubation time

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg ($CuSO_4$)	1	41.5	45.0	46.5	46.8	60.6	77.3	86.9
10 mg/kg ($CuSO_4$)	1	40.9	49.1	44.6	49.3	64.8	78.1	86.3
10 mg/kg ($CuSO_4$)	1	40.7	45.9	46.1	54.0	62.4	80.5	86.8
10 mg/kg ($CuSO_4$)	1	42.8	43.9	48.2	.	63.3	80.6	87.3
10 mg/kg ($CuSO_4$)	2	42.8	46.2	48.5	50.2	52.0	76.2	86.0
10 mg/kg ($CuSO_4$)	2	41.3	45.5	48.2	52.9	49.9	74.0	85.7
10 mg/kg ($CuSO_4$)	2	43.5	46.0	51.9	64.3	64.9	77.8	86.1
10 mg/kg ($CuSO_4$)	2	46.1	47.3	51.6	59.6	55.1	75.2	86.6
10 mg/kg ($CuSO_4$)	3	44.0	46.3	59.5	60.9	69.1	82.1	86.4
10 mg/kg ($CuSO_4$)	3	43.3	46.3	56.7	66.7	67.1	81.9	86.3
10 mg/kg ($CuSO_4$)	3	45.5	46.1	54.3	62.1	68.1	81.9	85.9
10 mg/kg ($CuSO_4$)	3	44.7	47.1	57.7	64.5	66.7	80.5	86.0
10 mg/kg ($CuSO_4$)	4	43.2	48.1	57.7	65.0	.	77.5	84.7
10 mg/kg ($CuSO_4$)	4	42.8	46.2	62.1	64.4	70.8	80.3	85.9
10 mg/kg ($CuSO_4$)	4	42.0	46.6	51.1	65.7	71.2	81.9	84.8
10 mg/kg ($CuSO_4$)	4	42.3	46.3	52.7	62.9	71.0	83.7	85.5
10 mg/kg ($CuSO_4$)	5	43.4	48.0	61.8	68.3	71.7	82.4	88.5
10 mg/kg ($CuSO_4$)	5	44.0	.	59.3	64.3	71.1	82.5	87.3
10 mg/kg ($CuSO_4$)	5	45.7	.	60.1	66.7	73.2	80.5	87.2
10 mg/kg ($CuSO_4$)	5	45.6	46.4	.	66.7	71.8	79.6	86.2
10 mg/kg ($CuSO_4$)	6	43.8	45.0	55.7	64.2	63.9	77.5	85.4
10 mg/kg ($CuSO_4$)	6	43.8	46.1	55.6	64.9	67.1	80.7	85.2
10 mg/kg ($CuSO_4$)	6	44.2	42.8	54.9	67.4	69.0	80.8	84.1
10 mg/kg ($CuSO_4$)	6	44.8	43.3	53.4	65.1	67.1	80.8	85.6
35 mg/kg ($CuSO_4$)	1	43.3	49.8	54.3	59.2	66.8	77.7	84.7
35 mg/kg ($CuSO_4$)	1	45.4	44.6	49.2	68.3	67.3	81.1	83.4
35 mg/kg ($CuSO_4$)	1	43.9	43.7	53.1	66.6	69.7	78.7	85.0
35 mg/kg ($CuSO_4$)	1	42.4	43.7	54.5	57.2	64.6	79.8	85.4
35 mg/kg ($CuSO_4$)	2	42.6	43.6	48.9	50.8	62.9	76.6	85.2
35 mg/kg ($CuSO_4$)	2	42.4	43.8	50.0	57.5	59.1	76.9	83.8
35 mg/kg ($CuSO_4$)	2	41.9	48.5	48.5	53.3	65.2	78.4	85.1
35 mg/kg ($CuSO_4$)	2	41.5	44.8	50.6	50.9	66.0	74.1	86.1
35 mg/kg ($CuSO_4$)	3	44.3	45.3	54.2	60.2	68.2	81.0	87.2
35 mg/kg ($CuSO_4$)	3	44.8	48.3	56.2	60.7	73.6	79.8	86.3
35 mg/kg ($CuSO_4$)	3	43.3	46.6	56.0	64.4	68.8	80.9	86.2
35 mg/kg ($CuSO_4$)	3	44.4	46.1	56.6	59.1	71.1	81.0	86.1
35 mg/kg ($CuSO_4$)	4	44.2	46.9	59.1	61.1	75.5	82.0	87.1
35 mg/kg ($CuSO_4$)	4	43.3	47.7	59.1	62.8	70.0	82.1	87.7
35 mg/kg ($CuSO_4$)	4	44.2	49.3	50.4	56.1	.	83.2	86.1
35 mg/kg ($CuSO_4$)	4	43.8	47.8	51.6	60.8	63.6	78.8	88.0
35 mg/kg ($CuSO_4$)	5	41.7	48.7	60.4	70.6	73.0	83.5	86.7

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
35 mg/kg ($CuSO_4$)	5	41.7	46.0	58.3	67.2	73.1	82.1	87.7
35 mg/kg ($CuSO_4$)	5	40.7	50.0	59.8	68.0	71.5	80.9	87.0
35 mg/kg ($CuSO_4$)	5	41.9	50.2	62.4	70.7	71.6	80.7	87.1
35 mg/kg ($CuSO_4$)	6	43.9	48.2	52.5	60.9	57.2	76.2	.
35 mg/kg ($CuSO_4$)	6	42.1	47.1	51.8	60.5	56.0	78.5	86.4
35 mg/kg ($CuSO_4$)	6	45.3	47.1	53.1	62.2	60.8	79.8	84.4
35 mg/kg ($CuSO_4$)	6	43.1	46.2	57.5	67.1	63.8	76.8	84.6
50 mg/kg ($CuSO_4$)	1	42.7	41.7	58.2	67.9	68.9	75.0	86.4
50 mg/kg ($CuSO_4$)	1	44.1	44.7	54.3	64.0	71.3	79.5	87.5
50 mg/kg ($CuSO_4$)	1	43.9	44.8	53.9	59.0	74.2	82.7	87.4
50 mg/kg ($CuSO_4$)	1	44.0	50.6	58.8	67.7	70.4	82.6	87.4
50 mg/kg ($CuSO_4$)	2	43.0	43.2	53.1	63.7	66.1	72.3	85.5
50 mg/kg ($CuSO_4$)	2	43.0	45.4	52.9	60.7	67.7	76.7	84.4
50 mg/kg ($CuSO_4$)	2	44.1	44.8	54.9	52.6	61.1	76.7	84.0
50 mg/kg ($CuSO_4$)	2	42.6	.	56.6	57.0	58.5	68.9	84.5
50 mg/kg ($CuSO_4$)	3	41.6	46.5	61.2	67.0	68.4	80.8	85.3
50 mg/kg ($CuSO_4$)	3	42.2	47.9	59.7	66.2	70.5	80.3	85.1
50 mg/kg ($CuSO_4$)	3	41.4	46.2	60.7	66.9	70.6	81.2	85.3
50 mg/kg ($CuSO_4$)	3	43.8	47.3	62.5	65.8	71.3	81.4	85.3
50 mg/kg ($CuSO_4$)	4	44.5	49.7	61.2	69.4	66.8	81.5	.
50 mg/kg ($CuSO_4$)	4	43.9	51.1	60.4	68.9	67.2	82.0	87.8
50 mg/kg ($CuSO_4$)	4	43.3	48.7	52.7	62.6	57.9	82.8	.
50 mg/kg ($CuSO_4$)	4	44.6	48.8	54.0	63.4	61.7	78.1	86.7
50 mg/kg ($CuSO_4$)	5	43.7	48.6	59.5	66.4	71.1	81.7	87.0
50 mg/kg ($CuSO_4$)	5	44.2	49.0	60.1	66.8	68.4	82.4	86.8
50 mg/kg ($CuSO_4$)	5	44.6	49.2	60.9	76.0	73.4	82.5	87.2
50 mg/kg ($CuSO_4$)	5	43.8	52.3	59.2	66.6	73.2	82.7	88.0
50 mg/kg ($CuSO_4$)	6	42.8	46.6	58.4	66.9	68.0	78.0	86.6
50 mg/kg ($CuSO_4$)	6	42.5	46.2	65.4	68.4	68.2	75.6	85.6
50 mg/kg ($CuSO_4$)	6	43.3	46.3	56.2	62.3	72.1	75.4	85.9
50 mg/kg ($CuSO_4$)	6	43.3	48.7	58.7	63.5	72.7	73.3	86.8
10 mg/kg (TBCC)	1	43.9	46.7	56.2	65.3	66.8	80.3	86.0
10 mg/kg (TBCC)	1	42.3	47.7	55.6	66.3	70.4	81.2	87.0
10 mg/kg (TBCC)	1	41.3	49.9	57.0	61.7	69.8	82.7	87.6
10 mg/kg (TBCC)	1	42.1	45.8	55.4	68.4	68.0	82.9	87.4
10 mg/kg (TBCC)	2	43.4	48.0	50.4	56.9	60.4	79.1	86.9
10 mg/kg (TBCC)	2	43.2	45.4	50.8	63.4	63.6	80.2	85.5
10 mg/kg (TBCC)	2	44.5	47.6	49.1	65.5	66.9	79.8	86.1
10 mg/kg (TBCC)	2	44.3	48.1	50.1	67.4	65.9	79.8	83.1
10 mg/kg (TBCC)	3	43.6	46.8	59.6	66.2	63.8	79.5	85.5
10 mg/kg (TBCC)	3	44.5	46.8	57.8	63.8	66.9	80.6	86.2
10 mg/kg (TBCC)	3	43.8	46.1	57.2	65.8	69.8	80.2	84.3
10 mg/kg (TBCC)	3	42.2	44.9	59.1	66.0	69.9	80.9	86.2

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg (TBCC)	4	41.1	47.1	61.8	63.6	59.9	75.5	84.7
10 mg/kg (TBCC)	4	38.4	47.2	52.0	66.9	58.6	76.3	85.1
10 mg/kg (TBCC)	4	40.9	45.0	56.5	60.2	57.8	76.9	85.6
10 mg/kg (TBCC)	4	42.4	47.5	51.6	57.9	58.3	71.5	87.3
10 mg/kg (TBCC)	5	43.5	.	53.3	67.6	71.3	79.0	86.1
10 mg/kg (TBCC)	5	45.1	49.8	55.2	66.0	69.0	80.1	87.4
10 mg/kg (TBCC)	5	43.1	48.2	55.2	65.0	69.4	77.4	87.5
10 mg/kg (TBCC)	5	42.4	47.5	50.9	65.1	72.6	71.4	86.3
10 mg/kg (TBCC)	6	44.4	49.6	57.0	67.9	64.9	80.3	85.8
10 mg/kg (TBCC)	6	44.8	49.4	55.5	69.1	70.5	81.1	86.3
10 mg/kg (TBCC)	6	45.2	50.7	58.5	70.2	70.7	81.4	87.3
10 mg/kg (TBCC)	6	45.5	50.4	56.3	66.4	65.1	81.9	86.5
35 mg/kg (TBCC)	1	44.3	45.8	50.1	67.1	69.2	80.4	85.3
35 mg/kg (TBCC)	1	44.2	49.0	48.4	65.9	72.6	74.3	85.3
35 mg/kg (TBCC)	1	43.5	46.1	57.4	62.8	68.2	81.2	85.9
35 mg/kg (TBCC)	1	43.3	44.3	54.1	63.4	68.7	82.8	87.2
35 mg/kg (TBCC)	2	39.9	45.9	52.8	56.0	61.6	77.9	84.0
35 mg/kg (TBCC)	2	39.7	46.6	57.9	53.1	60.8	76.1	85.5
35 mg/kg (TBCC)	2	40.3	45.5	47.6	55.3	.	76.8	84.2
35 mg/kg (TBCC)	2	40.1	45.8	49.5	62.0	68.2	73.1	84.7
35 mg/kg (TBCC)	3	44.8	48.1	61.5	68.8	71.5	81.7	86.4
35 mg/kg (TBCC)	3	43.3	49.1	59.0	67.5	70.3	80.5	87.0
35 mg/kg (TBCC)	3	45.7	48.6	62.4	67.9	69.3	80.2	86.7
35 mg/kg (TBCC)	3	43.2	47.8	61.3	67.2	68.4	81.4	86.4
35 mg/kg (TBCC)	4	42.4	48.1	52.6	58.3	63.4	77.8	85.4
35 mg/kg (TBCC)	4	41.9	45.0	53.4	56.4	65.7	78.1	84.2
35 mg/kg (TBCC)	4	42.2	44.3	51.0	54.0	59.1	76.7	85.8
35 mg/kg (TBCC)	4	41.2	45.9	49.4	53.4	63.3	78.3	85.8
35 mg/kg (TBCC)	5	42.5	48.8	53.3	66.1	72.4	81.6	86.0
35 mg/kg (TBCC)	5	43.8	46.1	54.1	66.2	68.0	81.7	86.4
35 mg/kg (TBCC)	5	41.4	48.3	52.0	64.4	69.8	81.7	86.4
35 mg/kg (TBCC)	5	40.0	47.5	52.8	63.2	68.8	81.7	86.2
35 mg/kg (TBCC)	6	44.4	49.7	51.1	68.1	70.5	79.4	86.2
35 mg/kg (TBCC)	6	44.1	46.8	52.0	66.7	68.5	81.0	85.5
35 mg/kg (TBCC)	6	44.6	48.8	53.0	66.5	72.8	79.4	85.8
35 mg/kg (TBCC)	6	44.8	47.3	52.8	68.4	70.6	80.7	85.3
50 mg/kg (TBCC)	1	43.1	44.7	51.1	53.6	69.5	80.1	86.3
50 mg/kg (TBCC)	1	41.9	46.0	52.4	50.6	68.8	80.1	87.3
50 mg/kg (TBCC)	1	42.4	49.4	55.9	58.5	70.1	80.2	87.2
50 mg/kg (TBCC)	1	42.3	45.2	59.3	59.1	70.1	79.1	86.7
50 mg/kg (TBCC)	2	44.8	45.0	58.3	61.7	53.4	78.9	83.4
50 mg/kg (TBCC)	2	49.3	47.0	59.7	62.1	60.3	77.9	84.4
50 mg/kg (TBCC)	2	41.7	44.6	54.7	62.6	55.1	81.2	84.2

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
50 mg/kg (TBCC)	2	43.1	46.4	54.4	56.7	53.6	81.2	84.8
50 mg/kg (TBCC)	3	45.0	51.4	58.9	63.6	72.2	79.7	87.4
50 mg/kg (TBCC)	3	45.0	48.3	57.8	66.8	71.5	.	86.8
50 mg/kg (TBCC)	3	43.6	49.4	60.7	67.5	70.7	80.8	86.5
50 mg/kg (TBCC)	3	44.0	48.9	57.8	69.2	71.5	81.0	86.4
50 mg/kg (TBCC)	4	42.9	45.1	59.9	61.6	68.9	82.8	85.6
50 mg/kg (TBCC)	4	43.8	45.9	58.9	61.8	63.9	80.8	86.8
50 mg/kg (TBCC)	4	43.9	47.1	50.6	55.7	54.6	80.9	84.6
50 mg/kg (TBCC)	4	43.4	46.5	53.1	54.4	54.6	81.9	86.6
50 mg/kg (TBCC)	5	44.6	47.2	54.0	.	70.9	80.4	86.0
50 mg/kg (TBCC)	5	43.6	48.1	57.1	66.1	72.8	79.0	86.0
50 mg/kg (TBCC)	5	44.4	46.5	57.0	65.5	70.6	81.6	85.7
50 mg/kg (TBCC)	5	43.9	45.5	57.1	65.2	69.1	80.1	84.7
50 mg/kg (TBCC)	6	42.7	45.6	56.8	60.4	68.3	80.9	84.7
50 mg/kg (TBCC)	6	43.4	48.0	55.9	60.5	66.5	77.8	85.7
50 mg/kg (TBCC)	6	42.2	48.3	52.4	63.4	72.5	79.2	85.2
50 mg/kg (TBCC)	6	42.3	48.8	52.5	53.9	70.3	80.1	81.5

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

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg ($CuSO_4$)	1	39.7	42.4	45.2	45.5	60.3	76.6	86.9
10 mg/kg ($CuSO_4$)	1	40.1	43.7	45.5	45.3	63.1	74.5	87.3
10 mg/kg ($CuSO_4$)	1	39.4	42.3	45.7	54.0	57.4	78.2	.
10 mg/kg ($CuSO_4$)	1	39.3	41.1	46.0	43.9	57.5	80.5	88.7
10 mg/kg ($CuSO_4$)	2	37.0	38.5	43.2	49.1	46.0	72.7	86.1
10 mg/kg ($CuSO_4$)	2	37.1	38.7	41.5	44.7	52.6	76.4	.
10 mg/kg ($CuSO_4$)	2	36.5	37.6	44.9	51.2	49.3	76.0	87.1
10 mg/kg ($CuSO_4$)	2	37.1	38.7	44.6	52.5	59.7	75.3	85.8
10 mg/kg ($CuSO_4$)	3	39.3	40.7	51.5	56.9	62.3	81.8	87.9
10 mg/kg ($CuSO_4$)	3	39.8	40.6	50.5	63.1	66.1	82.4	88.3
10 mg/kg ($CuSO_4$)	3	39.1	39.3	47.9	60.3	69.4	82.1	86.3
10 mg/kg ($CuSO_4$)	3	39.3	40.4	53.1	60.3	64.0	81.3	86.9
10 mg/kg ($CuSO_4$)	4	39.7	41.7	54.3	62.7	67.7	79.7	88.4
10 mg/kg ($CuSO_4$)	4	38.8	42.2	57.8	58.4	65.5	79.6	88.6
10 mg/kg ($CuSO_4$)	4	38.7	42.0	43.6	61.5	68.0	78.1	.
10 mg/kg ($CuSO_4$)	4	38.6	39.4	47.9	53.3	68.2	78.9	88.5
10 mg/kg ($CuSO_4$)	5	37.5	40.5	53.2	60.1	68.9	77.4	86.9
10 mg/kg ($CuSO_4$)	5	36.8	38.8	53.6	57.9	69.1	79.1	86.7
10 mg/kg ($CuSO_4$)	5	37.8	40.7	51.8	62.4	69.4	78.4	88.0
10 mg/kg ($CuSO_4$)	5	38.7	39.1	53.5	61.0	66.3	77.7	88.3
10 mg/kg ($CuSO_4$)	6	37.5	41.2	48.7	61.3	60.0	79.2	85.6
10 mg/kg ($CuSO_4$)	6	36.7	41.9	50.6	60.4	59.6	79.5	85.0
10 mg/kg ($CuSO_4$)	6	37.7	41.5	46.2	62.7	61.6	79.0	84.1
10 mg/kg ($CuSO_4$)	6	37.8	39.3	48.2	61.9	60.7	79.0	84.9
35 mg/kg ($CuSO_4$)	1	37.1	38.6	53.6	60.3	63.7	80.0	93.7
35 mg/kg ($CuSO_4$)	1	38.0	41.3	52.2	67.0	72.4	78.6	86.5
35 mg/kg ($CuSO_4$)	1	37.0	38.9	49.3	56.8	64.7	79.6	86.9
35 mg/kg ($CuSO_4$)	1	37.1	39.6	51.5	51.3	59.3	78.5	86.5
35 mg/kg ($CuSO_4$)	2	39.3	41.9	47.6	58.3	55.1	77.8	85.9
35 mg/kg ($CuSO_4$)	2	35.4	41.0	46.8	52.6	53.2	75.8	83.8
35 mg/kg ($CuSO_4$)	2	40.0	42.7	43.5	51.7	55.9	76.5	86.9
35 mg/kg ($CuSO_4$)	2	38.5	41.9	44.9	52.8	65.6	76.9	85.8
35 mg/kg ($CuSO_4$)	3	36.3	37.4	49.6	51.0	64.3	76.2	83.8
35 mg/kg ($CuSO_4$)	3	36.9	39.3	49.5	57.6	64.7	77.5	85.1
35 mg/kg ($CuSO_4$)	3	37.8	38.6	46.9	53.4	62.8	78.0	84.1
35 mg/kg ($CuSO_4$)	3	37.7	36.1	48.6	54.1	64.9	78.1	84.0
35 mg/kg ($CuSO_4$)	4	39.3	42.1	.	59.8	73.7	82.9	88.2
35 mg/kg ($CuSO_4$)	4	39.1	41.9	56.5	58.5	74.2	83.4	89.3
35 mg/kg ($CuSO_4$)	4	38.8	41.1	51.9	55.5	67.8	82.2	88.4
35 mg/kg ($CuSO_4$)	4	39.6	41.3	43.0	65.8	66.1	76.6	89.3
35 mg/kg ($CuSO_4$)	5	38.7	41.9	55.5	67.8	71.5	81.8	88.6

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
35 mg/kg ($CuSO_4$)	5	38.8	40.6	53.5	62.7	71.6	82.6	89.5
35 mg/kg ($CuSO_4$)	5	38.7	43.6	54.9	66.3	70.5	81.5	87.8
35 mg/kg ($CuSO_4$)	5	38.4	42.8	56.7	66.7	69.6	80.0	88.6
35 mg/kg ($CuSO_4$)	6	38.1	38.7	46.3	56.7	55.6	74.9	88.5
35 mg/kg ($CuSO_4$)	6	37.6	38.2	46.6	55.7	59.2	78.2	86.9
35 mg/kg ($CuSO_4$)	6	38.0	40.0	47.2	58.7	58.1	78.0	85.8
35 mg/kg ($CuSO_4$)	6	37.7	38.5	50.0	61.7	60.6	71.9	83.9
50 mg/kg ($CuSO_4$)	1	38.0	39.4	48.6	61.7	65.3	78.6	90.1
50 mg/kg ($CuSO_4$)	1	38.0	39.5	47.1	62.4	67.9	79.9	88.9
50 mg/kg ($CuSO_4$)	1	36.1	41.8	47.7	54.4	71.3	79.8	87.6
50 mg/kg ($CuSO_4$)	1	37.8	39.0	53.3	63.7	65.0	79.9	87.9
50 mg/kg ($CuSO_4$)	2	37.2	40.3	48.9	57.4	65.2	71.5	84.9
50 mg/kg ($CuSO_4$)	2	38.2	40.8	49.0	55.7	62.6	77.1	86.7
50 mg/kg ($CuSO_4$)	2	37.8	39.1	48.8	49.4	55.7	71.0	85.8
50 mg/kg ($CuSO_4$)	2	38.1	38.5	52.9	48.5	56.6	67.0	86.3
50 mg/kg ($CuSO_4$)	3	39.4	43.2	58.5	65.1	67.3	80.1	83.4
50 mg/kg ($CuSO_4$)	3	39.0	43.0	52.7	65.3	64.8	78.5	84.5
50 mg/kg ($CuSO_4$)	3	36.8	41.4	56.8	65.9	66.0	79.5	84.3
50 mg/kg ($CuSO_4$)	3	39.4	41.4	56.0	66.0	66.9	78.4	84.3
50 mg/kg ($CuSO_4$)	4	38.0	42.3	53.8	66.0	62.9	79.3	87.6
50 mg/kg ($CuSO_4$)	4	38.0	40.9	51.2	66.4	60.7	77.5	87.4
50 mg/kg ($CuSO_4$)	4	36.8	38.1	43.2	57.4	50.5	80.3	88.7
50 mg/kg ($CuSO_4$)	4	36.9	38.6	47.7	58.1	61.3	79.5	88.0
50 mg/kg ($CuSO_4$)	5	39.2	41.9	59.4	64.4	67.0	82.7	89.4
50 mg/kg ($CuSO_4$)	5	39.4	41.6	54.4	66.4	67.7	79.6	89.3
50 mg/kg ($CuSO_4$)	5	38.3	42.9	55.7	63.6	72.3	82.5	89.0
50 mg/kg ($CuSO_4$)	5	38.6	40.2	52.9	64.6	73.0	82.8	88.6
50 mg/kg ($CuSO_4$)	6	.	41.6	54.5	62.0	62.5	77.8	84.4
50 mg/kg ($CuSO_4$)	6	38.5	41.4	52.3	65.2	68.3	73.5	85.6
50 mg/kg ($CuSO_4$)	6	38.8	40.7	53.2	60.2	68.2	75.5	85.3
50 mg/kg ($CuSO_4$)	6	39.2	41.5	50.5	63.1	70.8	73.3	85.5
10 mg/kg (TBCC)	1	38.3	42.2	49.8	61.7	61.9	78.7	89.5
10 mg/kg (TBCC)	1	38.3	43.2	50.4	65.1	65.8	80.4	88.4
10 mg/kg (TBCC)	1	38.9	41.4	54.6	61.0	68.7	83.3	87.5
10 mg/kg (TBCC)	1	38.8	39.7	51.1	64.6	67.7	83.2	89.3
10 mg/kg (TBCC)	2	37.3	39.7	47.3	48.9	59.0	76.2	88.5
10 mg/kg (TBCC)	2	37.4	40.0	45.7	58.9	63.4	80.9	87.7
10 mg/kg (TBCC)	2	37.8	40.8	42.8	54.7	62.0	78.9	87.8
10 mg/kg (TBCC)	2	37.1	40.3	45.2	59.2	62.1	79.8	85.4
10 mg/kg (TBCC)	3	37.6	41.3	53.3	62.6	65.6	79.6	86.3
10 mg/kg (TBCC)	3	37.9	41.8	53.5	62.0	67.7	81.5	85.6
10 mg/kg (TBCC)	3	38.1	39.7	53.4	57.5	67.0	78.5	84.8
10 mg/kg (TBCC)	3	37.7	39.4	51.4	61.6	68.1	80.2	85.9

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg (TBCC)	4	40.1	43.6	54.8	64.1	53.2	75.1	86.1
10 mg/kg (TBCC)	4	39.5	42.4	56.8	67.0	51.5	71.7	87.5
10 mg/kg (TBCC)	4	39.2	41.1	44.9	56.0	49.8	72.6	88.0
10 mg/kg (TBCC)	4	39.0	41.6	45.7	54.9	54.0	72.4	88.3
10 mg/kg (TBCC)	5	37.2	39.4	47.8	64.2	67.2	77.0	84.6
10 mg/kg (TBCC)	5	38.2	40.0	46.4	61.8	67.7	77.0	86.3
10 mg/kg (TBCC)	5	36.8	41.0	48.3	62.8	69.7	73.8	85.9
10 mg/kg (TBCC)	5	37.7	39.5	44.7	57.9	69.7	74.8	85.4
10 mg/kg (TBCC)	6	40.3	42.7	49.4	62.2	62.2	77.0	87.0
10 mg/kg (TBCC)	6	40.5	42.4	50.4	67.0	66.7	82.2	88.1
10 mg/kg (TBCC)	6	39.5	42.7	54.6	66.0	67.1	80.3	87.5
10 mg/kg (TBCC)	6	39.0	44.4	52.9	65.5	66.2	80.4	87.9
35 mg/kg (TBCC)	1	38.6	39.4	44.7	64.4	69.5	80.0	87.7
35 mg/kg (TBCC)	1	38.1	39.0	45.6	65.5	71.0	81.4	89.2
35 mg/kg (TBCC)	1	39.0	40.2	53.7	63.7	69.9	80.5	88.3
35 mg/kg (TBCC)	1	38.2	39.3	47.8	62.8	64.7	82.9	89.3
35 mg/kg (TBCC)	2	38.4	40.0	58.6	54.4	60.3	78.3	87.0
35 mg/kg (TBCC)	2	38.5	40.4	46.4	46.6	69.8	72.4	85.3
35 mg/kg (TBCC)	2	38.6	41.3	42.9	54.1	67.7	74.4	85.5
35 mg/kg (TBCC)	2	38.0	40.4	42.8	56.7	68.0	72.7	87.6
35 mg/kg (TBCC)	3	38.4	39.8	54.1	60.8	61.9	80.0	86.8
35 mg/kg (TBCC)	3	38.5	40.2	53.5	61.5	65.1	78.7	88.3
35 mg/kg (TBCC)	3	37.9	40.7	53.8	59.4	63.9	79.5	.
35 mg/kg (TBCC)	3	37.4	40.1	53.9	65.1	66.3	78.1	86.7
35 mg/kg (TBCC)	4	37.7	41.5	49.3	51.1	56.9	82.1	85.8
35 mg/kg (TBCC)	4	36.9	39.1	47.2	51.3	64.6	79.2	85.2
35 mg/kg (TBCC)	4	38.3	38.2	45.0	52.0	60.2	77.8	85.0
35 mg/kg (TBCC)	4	37.3	39.7	45.7	47.3	58.8	77.5	85.8
35 mg/kg (TBCC)	5	39.9	41.9	48.1	64.7	70.0	78.1	86.3
35 mg/kg (TBCC)	5	39.4	42.4	51.0	64.4	62.6	79.8	85.5
35 mg/kg (TBCC)	5	39.3	42.5	47.2	58.0	66.8	79.5	86.4
35 mg/kg (TBCC)	5	39.0	42.9	46.8	61.2	64.7	79.1	85.4
35 mg/kg (TBCC)	6	35.7	41.0	44.0	61.2	67.2	77.1	83.5
35 mg/kg (TBCC)	6	36.1	38.5	44.7	57.5	67.0	76.7	82.7
35 mg/kg (TBCC)	6	35.9	40.2	48.4	62.1	67.5	76.6	82.8
35 mg/kg (TBCC)	6	37.0	38.9	47.8	64.7	65.4	77.0	82.9
50 mg/kg (TBCC)	1	38.0	.	48.5	56.7	64.8	79.1	85.2
50 mg/kg (TBCC)	1	34.1	36.6	43.9	46.1	62.9	77.7	85.7
50 mg/kg (TBCC)	1	35.6	37.9	51.1	51.9	65.7	78.3	86.2
50 mg/kg (TBCC)	1	36.4	38.1	49.0	51.0	66.6	79.6	86.1
50 mg/kg (TBCC)	2	38.5	40.2	53.9	58.1	63.7	80.5	87.2
50 mg/kg (TBCC)	2	38.6	40.0	56.1	60.8	54.8	81.2	86.4
50 mg/kg (TBCC)	2	38.6	39.6	50.8	55.4	58.3	83.7	87.0

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
50 mg/kg (TBCC)	2	37.9	39.2	49.6	57.5	52.9	81.8	86.4
50 mg/kg (TBCC)	3	38.0	43.5	52.9	61.5	70.0	80.3	86.1
50 mg/kg (TBCC)	3	40.3	42.6	50.3	63.0	69.8	80.3	86.4
50 mg/kg (TBCC)	3	39.0	44.7	56.3	61.2	69.2	79.1	85.2
50 mg/kg (TBCC)	3	38.3	43.1	52.9	63.8	70.7	.	85.5
50 mg/kg (TBCC)	4	36.9	39.2	55.4	59.5	.	79.5	86.5
50 mg/kg (TBCC)	4	37.6	38.7	52.7	53.8	66.5	80.4	87.9
50 mg/kg (TBCC)	4	37.7	39.4	42.9	50.0	48.8	80.6	87.3
50 mg/kg (TBCC)	4	36.5	40.0	44.4	50.9	60.8	81.3	87.5
50 mg/kg (TBCC)	5	37.6	40.5	50.8	50.9	68.7	79.8	86.1
50 mg/kg (TBCC)	5	38.2	40.0	49.1	63.6	69.6	79.9	85.4
50 mg/kg (TBCC)	5	38.5	40.5	50.4	59.8	69.0	82.1	85.4
50 mg/kg (TBCC)	5	38.2	40.4	52.3	59.5	66.3	80.7	85.7
50 mg/kg (TBCC)	6	39.4	41.2	53.4	58.1	59.4	76.1	83.2
50 mg/kg (TBCC)	6	40.0	43.0	48.8	48.9	64.4	76.3	84.8
50 mg/kg (TBCC)	6	38.7	44.2	49.7	53.4	67.6	76.9	84.0
50 mg/kg (TBCC)	6	39.4	43.0	46.6	64.3	67.5	77.6	83.5

Table 54: Ruminal dry matter disappearance [%] of maize silage of the different animals dependent on Cu treatment and incubation time

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg ($CuSO_4$)	1	43.2	49.0	47.6	49.5	57.2	70.0	81.2
10 mg/kg ($CuSO_4$)	1	45.8	50.2	46.7	50.6	60.2	67.1	79.8
10 mg/kg ($CuSO_4$)	1	45.2	51.3	47.9	57.0	58.9	71.8	81.3
10 mg/kg ($CuSO_4$)	1	44.7	47.8	46.0	50.3	60.2	70.6	82.1
10 mg/kg ($CuSO_4$)	2	48.6	52.0	55.3	54.4	53.5	69.3	80.7
10 mg/kg ($CuSO_4$)	2	46.0	52.8	50.1	51.3	49.5	66.6	81.2
10 mg/kg ($CuSO_4$)	2	46.5	50.6	53.1	55.8	54.8	72.1	80.3
10 mg/kg ($CuSO_4$)	2	45.5	51.8	55.1	58.2	60.2	71.2	81.9
10 mg/kg ($CuSO_4$)	3	46.5	49.7	59.6	62.3	62.0	76.6	80.2
10 mg/kg ($CuSO_4$)	3	49.2	47.6	58.9	61.4	60.9	74.9	81.2
10 mg/kg ($CuSO_4$)	3	46.7	49.0	57.0	61.2	65.7	76.4	79.8
10 mg/kg ($CuSO_4$)	3	46.5	47.9	60.5	61.7	64.5	74.5	80.7
10 mg/kg ($CuSO_4$)	4	48.3	53.2	58.4	61.7	62.5	73.6	79.5
10 mg/kg ($CuSO_4$)	4	48.7	48.0	60.4	59.3	61.9	74.2	81.0
10 mg/kg ($CuSO_4$)	4	47.0	51.5	55.0	60.4	64.7	70.4	78.9
10 mg/kg ($CuSO_4$)	4	49.6	50.2	55.2	57.5	68.0	74.2	78.9
10 mg/kg ($CuSO_4$)	5	50.7	54.7	56.8	63.6	67.3	79.2	82.7
10 mg/kg ($CuSO_4$)	5	48.6	56.1	62.0	65.8	64.9	77.0	83.1
10 mg/kg ($CuSO_4$)	5	49.1	52.5	62.2	68.4	69.2	75.4	83.8
10 mg/kg ($CuSO_4$)	5	48.1	56.1	61.5	66.5	67.8	75.2	83.7
10 mg/kg ($CuSO_4$)	6	51.7	46.7	58.7	64.8	64.5	74.7	81.7
10 mg/kg ($CuSO_4$)	6	49.3	47.5	59.9	63.7	65.2	78.2	83.8
10 mg/kg ($CuSO_4$)	6	50.7	44.5	56.1	63.7	65.9	77.6	82.3
10 mg/kg ($CuSO_4$)	6	50.9	47.4	55.8	66.5	66.3	76.7	82.5
35 mg/kg ($CuSO_4$)	1	45.5	51.5	52.6	61.1	63.7	72.7	86.1
35 mg/kg ($CuSO_4$)	1	48.9	52.4	57.1	62.7	58.3	76.3	81.8
35 mg/kg ($CuSO_4$)	1	47.3	50.5	53.0	54.8	57.9	73.3	84.2
35 mg/kg ($CuSO_4$)	1	45.9	49.7	52.0	58.8	61.8	72.4	82.7
35 mg/kg ($CuSO_4$)	2	44.8	48.4	51.2	55.4	58.0	66.4	79.0
35 mg/kg ($CuSO_4$)	2	45.9	48.5	50.7	58.4	58.8	67.4	77.6
35 mg/kg ($CuSO_4$)	2	44.8	50.8	49.2	58.4	60.5	68.7	76.1
35 mg/kg ($CuSO_4$)	2	46.7	48.9	49.3	56.7	60.6	67.5	79.0
35 mg/kg ($CuSO_4$)	3	48.5	51.2	53.7	53.1	62.7	75.3	80.7
35 mg/kg ($CuSO_4$)	3	47.0	50.9	54.6	57.0	68.0	76.1	80.8
35 mg/kg ($CuSO_4$)	3	59.4	54.0	52.8	59.4	66.0	78.0	80.4
35 mg/kg ($CuSO_4$)	3	49.6	50.6	55.1	59.3	63.4	75.2	80.3
35 mg/kg ($CuSO_4$)	4	46.0	47.8	57.9	60.6	66.3	76.6	81.8
35 mg/kg ($CuSO_4$)	4	47.6	47.5	60.3	61.5	67.7	75.1	80.1
35 mg/kg ($CuSO_4$)	4	46.0	48.6	59.0	55.3	61.0	73.5	79.8
35 mg/kg ($CuSO_4$)	4	46.7	51.4	51.0	62.9	58.5	67.9	80.3
35 mg/kg ($CuSO_4$)	5	44.4	52.5	54.5	66.1	64.4	73.4	79.9

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
35 mg/kg ($CuSO_4$)	5	38.8	47.9	59.0	66.1	69.2	73.7	81.8
35 mg/kg ($CuSO_4$)	5	42.3	53.9	59.8	66.3	65.5	74.4	81.7
35 mg/kg ($CuSO_4$)	5	45.4	51.1	58.5	65.3	67.2	75.4	81.9
35 mg/kg ($CuSO_4$)	6	43.1	53.7	53.6	60.8	54.4	75.9	82.6
35 mg/kg ($CuSO_4$)	6	46.5	.	55.4	59.2	62.1	72.6	81.9
35 mg/kg ($CuSO_4$)	6	45.5	52.0	51.9	64.2	58.4	75.8	81.2
35 mg/kg ($CuSO_4$)	6	48.5	51.7	56.0	62.4	60.5	69.0	78.4
50 mg/kg ($CuSO_4$)	1	48.3	50.6	57.8	63.1	61.4	75.3	82.9
50 mg/kg ($CuSO_4$)	1	46.9	51.3	54.1	62.2	59.7	73.5	83.0
50 mg/kg ($CuSO_4$)	1	47.8	47.9	59.7	59.6	69.8	77.5	81.4
50 mg/kg ($CuSO_4$)	1	44.4	48.7	61.3	64.6	67.4	75.1	83.4
50 mg/kg ($CuSO_4$)	2	47.1	50.2	53.3	62.2	61.2	68.4	76.3
50 mg/kg ($CuSO_4$)	2	47.5	51.6	53.7	57.6	62.5	67.0	75.5
50 mg/kg ($CuSO_4$)	2	46.6	50.6	55.0	59.8	60.4	67.0	79.4
50 mg/kg ($CuSO_4$)	2	45.7	51.0	55.6	56.4	61.3	73.9	82.2
50 mg/kg ($CuSO_4$)	3	45.0	53.5	56.7	63.9	67.1	75.8	81.9
50 mg/kg ($CuSO_4$)	3	43.7	47.2	56.6	65.3	67.1	74.9	80.4
50 mg/kg ($CuSO_4$)	3	47.6	48.6	58.3	63.9	66.5	73.5	81.9
50 mg/kg ($CuSO_4$)	3	45.0	48.6	53.8	64.7	66.5	75.1	80.9
50 mg/kg ($CuSO_4$)	4	46.7	48.3	59.5	66.2	60.2	74.6	81.8
50 mg/kg ($CuSO_4$)	4	46.9	52.5	56.4	65.7	61.6	76.9	81.5
50 mg/kg ($CuSO_4$)	4	46.3	51.3	53.5	59.0	59.9	71.4	81.1
50 mg/kg ($CuSO_4$)	4	46.8	54.1	57.5	61.9	58.9	76.3	80.6
50 mg/kg ($CuSO_4$)	5	46.8	52.7	61.4	62.4	66.6	73.2	80.4
50 mg/kg ($CuSO_4$)	5	49.1	50.8	60.7	59.7	65.6	75.9	81.5
50 mg/kg ($CuSO_4$)	5	47.8	51.6	61.5	64.7	67.8	77.2	80.6
50 mg/kg ($CuSO_4$)	5	45.8	50.0	59.5	60.7	68.9	77.0	82.3
50 mg/kg ($CuSO_4$)	6	46.5	52.2	60.4	.	64.2	71.7	82.8
50 mg/kg ($CuSO_4$)	6	47.2	53.5	57.9	65.3	67.0	70.2	.
50 mg/kg ($CuSO_4$)	6	47.4	53.7	58.8	64.0	65.8	71.7	79.1
50 mg/kg ($CuSO_4$)	6	50.0	51.7	61.7	64.0	69.7	72.9	81.1
10 mg/kg (TBCC)	1	47.5	53.8	54.6	61.6	62.2	74.9	81.5
10 mg/kg (TBCC)	1	44.4	49.9	.	63.7	62.5	76.2	81.4
10 mg/kg (TBCC)	1	43.5	50.3	57.8	60.4	66.2	77.8	81.6
10 mg/kg (TBCC)	1	45.7	50.0	57.7	65.2	65.9	76.1	81.3
10 mg/kg (TBCC)	2	50.0	52.0	57.5	59.6	61.7	72.3	82.0
10 mg/kg (TBCC)	2	47.5	51.4	53.8	62.1	60.8	76.4	81.5
10 mg/kg (TBCC)	2	46.3	52.2	52.6	64.7	65.4	73.6	74.3
10 mg/kg (TBCC)	2	47.2	53.5	54.9	63.5	65.1	73.9	79.7
10 mg/kg (TBCC)	3	48.9	53.0	58.3	65.1	65.2	77.7	83.5
10 mg/kg (TBCC)	3	48.9	51.5	59.4	64.0	66.4	76.1	81.6
10 mg/kg (TBCC)	3	44.8	50.2	59.6	62.1	67.8	76.8	82.6
10 mg/kg (TBCC)	3	49.1	51.0	59.9	65.0	69.0	77.6	83.9

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg (TBCC)	4	41.4	52.2	58.0	58.9	63.8	72.8	77.2
10 mg/kg (TBCC)	4	50.0	47.6	57.1	.	56.1	68.6	79.1
10 mg/kg (TBCC)	4	43.1	47.3	50.8	55.5	55.6	70.0	79.2
10 mg/kg (TBCC)	4	43.2	49.2	53.1	54.9	57.2	68.6	77.7
10 mg/kg (TBCC)	5	47.1	52.1	58.0	62.5	68.1	74.0	80.6
10 mg/kg (TBCC)	5	47.2	51.7	58.4	63.5	67.9	74.3	80.7
10 mg/kg (TBCC)	5	48.1	55.0	57.9	63.1	70.0	70.3	83.0
10 mg/kg (TBCC)	5	47.1	53.9	55.0	62.6	64.8	67.6	80.6
10 mg/kg (TBCC)	6	50.3	51.3	57.6	63.8	63.6	72.1	81.0
10 mg/kg (TBCC)	6	49.3	52.6	57.4	65.1	68.1	71.9	81.6
10 mg/kg (TBCC)	6	49.2	55.0	56.3	63.9	64.9	76.2	81.7
10 mg/kg (TBCC)	6	48.6	52.0	58.4	64.2	63.7	77.6	82.2
35 mg/kg (TBCC)	1	45.5	48.0	51.7	64.0	61.5	69.2	79.6
35 mg/kg (TBCC)	1	49.5	48.1	51.3	63.0	66.1	73.4	80.3
35 mg/kg (TBCC)	1	45.1	47.2	54.7	55.6	64.8	69.9	79.2
35 mg/kg (TBCC)	1	44.7	48.0	55.9	60.2	56.0	77.4	80.5
35 mg/kg (TBCC)	2	41.9	47.1	57.8	51.1	53.9	68.8	79.3
35 mg/kg (TBCC)	2	45.3	51.1	55.1	54.0	61.0	66.6	78.9
35 mg/kg (TBCC)	2	43.2	46.5	51.3	56.7	66.4	68.2	77.9
35 mg/kg (TBCC)	2	44.3	52.8	52.5	56.2	69.1	66.6	77.5
35 mg/kg (TBCC)	3	49.2	53.5	62.1	63.6	65.7	75.9	83.4
35 mg/kg (TBCC)	3	49.7	51.3	60.8	64.3	69.6	76.5	82.7
35 mg/kg (TBCC)	3	48.8	56.4	60.5	64.0	66.8	76.0	83.3
35 mg/kg (TBCC)	3	48.9	53.7	61.9	68.4	68.7	75.0	83.8
35 mg/kg (TBCC)	4	47.6	50.3	53.8	57.6	60.1	77.0	80.5
35 mg/kg (TBCC)	4	44.8	51.7	48.2	54.8	65.8	73.9	81.0
35 mg/kg (TBCC)	4	48.0	50.3	52.2	59.0	57.8	73.4	82.0
35 mg/kg (TBCC)	4	47.3	49.9	54.9	57.7	64.5	73.0	81.8
35 mg/kg (TBCC)	5	47.8	49.4	54.7	61.4	64.1	74.5	80.6
35 mg/kg (TBCC)	5	44.1	51.1	54.0	60.8	63.4	74.5	81.0
35 mg/kg (TBCC)	5	45.5	50.5	50.8	64.0	64.6	76.1	80.9
35 mg/kg (TBCC)	5	45.2	47.9	51.0	65.0	63.9	77.4	80.7
35 mg/kg (TBCC)	6	49.6	.	54.7	63.4	68.2	72.9	81.1
35 mg/kg (TBCC)	6	53.2	50.8	56.2	62.2	74.4	.	81.5
35 mg/kg (TBCC)	6	49.6	50.4	54.8	64.8	64.6	75.3	81.3
35 mg/kg (TBCC)	6	50.2	46.3	55.8	65.7	67.0	75.5	80.4
50 mg/kg (TBCC)	1	51.6	52.4	55.5	57.3	63.5	75.7	83.0
50 mg/kg (TBCC)	1	49.2	49.8	58.7	51.8	67.1	74.9	80.9
50 mg/kg (TBCC)	1	48.2	52.5	58.6	57.3	69.1	77.6	82.8
50 mg/kg (TBCC)	1	46.4	50.8	55.1	59.3	71.6	74.0	82.2
50 mg/kg (TBCC)	2	44.5	45.7	.	55.1	57.4	72.9	75.4
50 mg/kg (TBCC)	2	44.2	44.1	55.1	.	56.0	71.7	.
50 mg/kg (TBCC)	2	42.7	45.9	45.4	54.1	56.4	73.9	75.0

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
50 mg/kg (TBCC)	2	44.6	47.2	55.6	57.0	55.1	70.6	77.5
50 mg/kg (TBCC)	3	48.7	52.8	57.6	62.9	67.8	77.2	82.2
50 mg/kg (TBCC)	3	49.2	53.9	58.7	64.2	67.0	73.0	81.8
50 mg/kg (TBCC)	3	46.5	.	57.5	64.5	66.8	76.6	83.5
50 mg/kg (TBCC)	3	46.9	51.4	58.2	62.8	68.6	76.2	83.0
50 mg/kg (TBCC)	4	45.0	53.5	59.5	60.0	67.2	75.3	81.2
50 mg/kg (TBCC)	4	45.9	48.1	60.4	62.9	68.5	75.5	81.1
50 mg/kg (TBCC)	4	48.1	53.8	55.0	59.9	56.2	73.6	82.7
50 mg/kg (TBCC)	4	46.1	.	63.0	56.0	66.7	75.0	.
50 mg/kg (TBCC)	5	48.1	51.2	57.2	63.6	69.8	76.7	83.9
50 mg/kg (TBCC)	5	48.5	54.2	57.3	62.3	68.1	78.5	85.4
50 mg/kg (TBCC)	5	47.2	50.0	54.5	63.2	67.1	78.7	83.7
50 mg/kg (TBCC)	5	49.6	51.7	59.1	63.1	69.5	77.1	84.3
50 mg/kg (TBCC)	6	46.4	48.3	54.5	59.1	63.1	74.0	80.4
50 mg/kg (TBCC)	6	46.3	49.9	54.8	57.3	62.4	74.7	79.4
50 mg/kg (TBCC)	6	46.4	50.7	50.6	62.7	66.1	74.1	79.0
50 mg/kg (TBCC)	6	45.3	50.4	54.8	65.3	66.6	74.2	80.2

Table 55: Ruminal dry matter disappearance [%] of wheat meal of the different animals dependent on Cu treatment and incubation time

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg ($CuSO_4$)	1	70.2	84.8	78.2	84.7	91.3	94.0	94.5
10 mg/kg ($CuSO_4$)	1	74.6	75.3	81.2	86.9	91.4	94.0	94.6
10 mg/kg ($CuSO_4$)	1	71.6	74.0	76.2	85.4	90.8	94.3	94.7
10 mg/kg ($CuSO_4$)	1	67.1	74.9	73.0	78.3	91.2	94.2	94.9
10 mg/kg ($CuSO_4$)	2	72.4	79.1	82.3	85.5	85.8	94.5	94.0
10 mg/kg ($CuSO_4$)	2	75.2	82.7	79.3	85.9	80.5	94.3	94.4
10 mg/kg ($CuSO_4$)	2	73.4	74.8	82.0	89.4	80.8	94.7	95.1
10 mg/kg ($CuSO_4$)	2	74.1	.	87.8	89.8	89.6	94.5	94.5
10 mg/kg ($CuSO_4$)	3	78.4	82.2	86.3	92.9	93.3	93.6	94.5
10 mg/kg ($CuSO_4$)	3	75.9	82.7	89.1	92.4	92.1	93.4	94.2
10 mg/kg ($CuSO_4$)	3	78.5	83.3	90.1	91.9	93.2	93.6	94.1
10 mg/kg ($CuSO_4$)	3	78.2	82.9	89.4	92.7	93.1	94.2	94.2
10 mg/kg ($CuSO_4$)	4	76.5	86.0	91.1	92.6	92.4	93.5	93.6
10 mg/kg ($CuSO_4$)	4	78.3	81.5	91.8	87.4	93.4	94.1	93.4
10 mg/kg ($CuSO_4$)	4	80.9	82.3	88.5	92.3	92.8	93.5	93.4
10 mg/kg ($CuSO_4$)	4	80.2	83.4	89.0	93.0	92.8	94.2	93.9
10 mg/kg ($CuSO_4$)	5	77.7	85.8	83.3	92.2	93.2	93.9	94.5
10 mg/kg ($CuSO_4$)	5	77.5	79.6	91.6	90.5	98.6	93.9	94.4
10 mg/kg ($CuSO_4$)	5	81.8	84.7	90.4	93.2	.	93.3	94.7
10 mg/kg ($CuSO_4$)	5	80.0	84.5	90.6	92.8	93.7	93.6	94.7
10 mg/kg ($CuSO_4$)	6	77.9	77.7	86.9	93.5	93.3	94.3	95.0
10 mg/kg ($CuSO_4$)	6	75.7	83.6	92.5	93.8	93.9	94.6	94.6
10 mg/kg ($CuSO_4$)	6	79.6	79.1	91.7	93.2	93.6	94.4	94.4
10 mg/kg ($CuSO_4$)	6	81.5	81.1	90.3	93.2	93.6	94.3	94.5
35 mg/kg ($CuSO_4$)	1	75.4	85.2	87.8	.	94.0	94.1	94.9
35 mg/kg ($CuSO_4$)	1	75.8	87.1	90.7	87.8	93.1	94.3	95.3
35 mg/kg ($CuSO_4$)	1	74.6	79.6	87.7	89.1	93.8	94.0	95.0
35 mg/kg ($CuSO_4$)	1	76.1	82.1	92.2	90.8	92.3	94.8	94.9
35 mg/kg ($CuSO_4$)	2	74.7	79.5	84.6	91.4	90.0	94.3	94.5
35 mg/kg ($CuSO_4$)	2	70.3	74.6	83.6	90.6	90.2	93.8	94.6
35 mg/kg ($CuSO_4$)	2	76.1	78.4	81.9	92.4	92.1	94.6	94.6
35 mg/kg ($CuSO_4$)	2	75.6	82.8	86.6	90.3	91.3	94.5	94.1
35 mg/kg ($CuSO_4$)	3	77.4	78.7	89.6	91.0	92.3	94.7	94.6
35 mg/kg ($CuSO_4$)	3	74.0	78.7	87.7	92.4	91.9	94.6	94.8
35 mg/kg ($CuSO_4$)	3	73.6	78.6	88.9	91.8	92.3	94.2	94.6
35 mg/kg ($CuSO_4$)	3	75.7	79.7	85.9	92.3	93.0	96.2	94.6
35 mg/kg ($CuSO_4$)	4	75.5	81.5	92.0	92.3	93.9	93.1	94.0
35 mg/kg ($CuSO_4$)	4	77.5	78.8	90.9	.	94.2	93.4	93.8
35 mg/kg ($CuSO_4$)	4	75.1	78.1	89.1	90.8	92.5	93.4	.
35 mg/kg ($CuSO_4$)	4	80.4	84.9	81.3	93.7	91.8	94.1	93.2
35 mg/kg ($CuSO_4$)	5	75.6	86.0	90.4	93.5	93.1	93.0	94.2

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
35 mg/kg ($CuSO_4$)	5	80.9	80.8	92.1	93.7	93.4	93.5	94.4
35 mg/kg ($CuSO_4$)	5	76.7	87.0	91.5	93.5	87.1	93.8	94.1
35 mg/kg ($CuSO_4$)	5	77.2	86.8	91.7	93.5	92.7	93.7	94.5
35 mg/kg ($CuSO_4$)	6	73.2	78.7	85.8	91.1	92.7	93.5	94.3
35 mg/kg ($CuSO_4$)	6	77.2	.	84.3	89.3	92.2	93.2	93.6
35 mg/kg ($CuSO_4$)	6	75.9	84.9	82.2	92.7	89.6	93.6	94.4
35 mg/kg ($CuSO_4$)	6	74.8	81.3	86.8	91.9	91.7	93.2	94.5
50 mg/kg ($CuSO_4$)	1	74.8	78.0	84.9	91.5	92.6	94.7	94.9
50 mg/kg ($CuSO_4$)	1	72.6	75.0	89.5	91.2	92.7	93.7	94.3
50 mg/kg ($CuSO_4$)	1	77.3	76.0	90.0	86.8	93.0	93.5	94.3
50 mg/kg ($CuSO_4$)	1	77.4	84.2	90.7	92.6	91.3	93.6	95.2
50 mg/kg ($CuSO_4$)	2	75.3	.	91.1	93.3	92.6	93.0	94.3
50 mg/kg ($CuSO_4$)	2	74.3	79.0	90.1	93.5	92.8	94.1	93.9
50 mg/kg ($CuSO_4$)	2	75.4	78.1	91.5	92.0	90.8	93.9	94.1
50 mg/kg ($CuSO_4$)	2	74.0	77.8	91.3	93.6	91.1	94.1	94.6
50 mg/kg ($CuSO_4$)	3	74.2	80.8	92.4	93.2	92.7	94.7	94.9
50 mg/kg ($CuSO_4$)	3	72.8	85.1	92.6	93.4	93.1	94.5	95.1
50 mg/kg ($CuSO_4$)	3	76.1	83.5	92.3	93.1	93.4	94.8	95.0
50 mg/kg ($CuSO_4$)	3	76.8	82.0	92.5	92.9	93.5	94.6	94.9
50 mg/kg ($CuSO_4$)	4	70.8	83.7	92.2	93.0	92.5	94.5	94.8
50 mg/kg ($CuSO_4$)	4	74.4	84.2	91.2	92.7	92.2	94.2	94.6
50 mg/kg ($CuSO_4$)	4	72.8	79.5	88.0	92.6	90.6	94.7	94.5
50 mg/kg ($CuSO_4$)	4	72.2	81.3	87.9	90.8	92.3	94.3	94.7
50 mg/kg ($CuSO_4$)	5	78.7	87.2	89.7	91.2	93.5	93.4	94.0
50 mg/kg ($CuSO_4$)	5	75.9	83.7	90.3	92.6	93.0	94.2	94.3
50 mg/kg ($CuSO_4$)	5	75.0	87.2	90.9	92.2	93.4	93.7	94.6
50 mg/kg ($CuSO_4$)	5	77.8	85.6	90.3	92.0	93.7	94.0	93.7
50 mg/kg ($CuSO_4$)	6	79.1	85.7	93.0	93.8	93.0	93.2	93.9
50 mg/kg ($CuSO_4$)	6	76.9	81.0	91.4	93.3	93.3	93.0	93.8
50 mg/kg ($CuSO_4$)	6	76.2	87.2	91.1	93.2	93.6	93.1	93.1
50 mg/kg ($CuSO_4$)	6	81.1	84.4	88.5	92.9	93.2	93.5	93.6
10 mg/kg (TBCC)	1	79.2	85.6	90.3	93.2	92.4	93.5	93.6
10 mg/kg (TBCC)	1	74.0	79.9	82.5	93.0	92.1	93.7	94.2
10 mg/kg (TBCC)	1	77.2	82.3	91.9	93.6	92.6	93.8	94.1
10 mg/kg (TBCC)	1	77.4	82.2	87.5	93.5	92.5	93.3	94.1
10 mg/kg (TBCC)	2	75.5	82.3	77.7	88.1	89.5	93.1	94.9
10 mg/kg (TBCC)	2	76.6	79.3	82.1	89.1	89.0	93.4	94.7
10 mg/kg (TBCC)	2	72.8	79.8	83.2	90.5	92.8	93.6	94.2
10 mg/kg (TBCC)	2	77.5	79.3	76.6	91.5	92.0	95.3	94.8
10 mg/kg (TBCC)	3	77.6	83.0	91.9	93.4	92.6	94.1	94.9
10 mg/kg (TBCC)	3	79.3	80.9	91.2	93.2	91.7	94.4	94.6
10 mg/kg (TBCC)	3	75.7	81.3	91.9	93.3	94.1	94.5	94.4
10 mg/kg (TBCC)	3	78.1	83.1	91.8	93.7	93.9	94.6	94.5

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg (TBCC)	4	72.4	85.1	87.5	92.7	90.5	94.7	94.4
10 mg/kg (TBCC)	4	77.4	82.8	91.9	93.2	89.0	94.5	94.8
10 mg/kg (TBCC)	4	71.8	82.6	88.5	90.6	90.3	94.4	94.6
10 mg/kg (TBCC)	4	76.0	84.2	86.9	88.9	90.7	94.8	94.0
10 mg/kg (TBCC)	5	75.9	77.5	88.1	92.4	93.9	94.2	95.2
10 mg/kg (TBCC)	5	77.7	82.4	90.9	92.2	93.3	93.7	95.1
10 mg/kg (TBCC)	5	76.0	.	88.1	92.9	92.9	94.2	95.0
10 mg/kg (TBCC)	5	72.3	86.0	85.4	91.4	92.8	94.2	94.7
10 mg/kg (TBCC)	6	78.5	83.3	88.0	91.4	93.2	94.0	94.0
10 mg/kg (TBCC)	6	74.6	83.8	88.0	93.0	93.5	93.8	94.9
10 mg/kg (TBCC)	6	82.2	86.0	91.0	92.6	93.2	94.6	95.2
10 mg/kg (TBCC)	6	79.1	86.8	90.6	92.8	93.1	94.1	94.6
35 mg/kg (TBCC)	1	75.0	77.9	81.8	92.2	93.5	93.9	93.6
35 mg/kg (TBCC)	1	75.3	77.2	82.8	92.5	92.2	94.3	93.8
35 mg/kg (TBCC)	1	72.9	79.8	85.4	92.3	93.3	93.5	94.1
35 mg/kg (TBCC)	1	75.2	74.8	87.0	92.8	89.6	94.5	94.2
35 mg/kg (TBCC)	2	76.8	79.5	91.2	83.5	92.7	93.0	93.6
35 mg/kg (TBCC)	2	73.3	81.7	85.9	94.6	92.4	93.0	93.5
35 mg/kg (TBCC)	2	76.3	79.8	87.5	90.0	93.0	92.7	93.3
35 mg/kg (TBCC)	2	73.6	82.9	86.0	90.6	92.9	92.7	93.1
35 mg/kg (TBCC)	3	77.8	82.7	91.2	92.6	92.3	94.0	95.3
35 mg/kg (TBCC)	3	80.1	85.5	90.9	92.4	91.8	94.1	95.1
35 mg/kg (TBCC)	3	79.0	84.1	92.1	93.0	92.7	93.9	95.1
35 mg/kg (TBCC)	3	78.9	84.7	91.4	92.6	93.0	93.5	95.0
35 mg/kg (TBCC)	4	74.7	81.8	89.1	89.3	91.9	93.9	94.5
35 mg/kg (TBCC)	4	72.5	77.6	84.8	89.7	93.1	94.2	94.6
35 mg/kg (TBCC)	4	73.1	86.4	89.7	92.7	89.6	94.3	94.4
35 mg/kg (TBCC)	4	68.4	80.3	90.8	93.2	93.2	94.3	94.7
35 mg/kg (TBCC)	5	77.2	83.7	89.5	91.0	92.4	94.4	94.8
35 mg/kg (TBCC)	5	78.0	86.0	91.3	93.1	93.2	94.7	94.8
35 mg/kg (TBCC)	5	74.3	85.4	89.2	91.8	93.3	94.9	94.6
35 mg/kg (TBCC)	5	74.7	88.5	90.7	91.4	92.8	93.4	94.9
35 mg/kg (TBCC)	6	81.1	81.8	89.1	91.9	92.6	94.7	94.3
35 mg/kg (TBCC)	6	75.6	81.9	88.3	87.6	93.3	94.2	96.5
35 mg/kg (TBCC)	6	80.6	81.3	90.2	93.0	93.0	94.5	94.8
35 mg/kg (TBCC)	6	79.9	82.9	88.8	92.8	92.7	94.6	94.8
50 mg/kg (TBCC)	1	71.2	.	85.7	86.8	92.2	94.4	94.5
50 mg/kg (TBCC)	1	74.4	74.5	88.9	88.4	90.7	93.8	94.4
50 mg/kg (TBCC)	1	73.7	79.6	90.8	87.1	87.4	94.0	94.7
50 mg/kg (TBCC)	1	72.1	77.8	89.2	89.3	92.7	94.5	94.1
50 mg/kg (TBCC)	2	72.9	76.3	91.7	89.5	88.0	93.5	93.8
50 mg/kg (TBCC)	2	72.7	76.7	91.5	90.5	88.5	93.3	93.1
50 mg/kg (TBCC)	2	75.9	73.3	82.2	91.0	87.1	93.2	94.0

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
50 mg/kg (TBCC)	2	73.4	78.9	90.5	90.7	88.1	94.0	94.1
50 mg/kg (TBCC)	3	79.5	86.3	92.3	93.4	93.3	93.8	93.7
50 mg/kg (TBCC)	3	83.3	84.5	93.1	93.4	93.0	93.2	94.4
50 mg/kg (TBCC)	3	80.7	88.5	92.5	93.3	93.6	93.9	93.6
50 mg/kg (TBCC)	3	81.5	84.7	92.6	93.4	93.1	94.5	93.6
50 mg/kg (TBCC)	4	76.0	76.5	89.4	90.1	94.0	93.5	94.7
50 mg/kg (TBCC)	4	72.2	74.1	89.2	92.3	92.2	93.2	95.4
50 mg/kg (TBCC)	4	72.4	82.9	86.9	86.7	87.0	94.1	94.9
50 mg/kg (TBCC)	4	74.1	76.1	86.5	88.9	91.4	93.9	94.7
50 mg/kg (TBCC)	5	79.1	86.1	92.2	93.3	94.0	94.2	94.9
50 mg/kg (TBCC)	5	78.1	84.5	91.8	93.5	93.7	94.7	94.8
50 mg/kg (TBCC)	5	81.0	85.0	91.3	94.1	94.3	94.7	95.2
50 mg/kg (TBCC)	5	79.2	86.3	90.1	93.5	94.0	94.7	94.8
50 mg/kg (TBCC)	6	77.8	87.0	88.5	87.4	91.6	94.9	94.6
50 mg/kg (TBCC)	6	77.5	81.9	89.9	88.1	92.9	94.2	94.9
50 mg/kg (TBCC)	6	74.2	82.4	89.2	90.7	93.0	94.5	94.7
50 mg/kg (TBCC)	6	76.3	82.3	87.5	92.3	93.0	94.6	94.8

Table 56: Ruminal dry matter disappearance [%] of soybean meal of the different animals dependent on Cu treatment and incubation time

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg ($CuSO_4$)	1	33.2	39.8	45.3	48.5	81.3	91.4	97.9
10 mg/kg ($CuSO_4$)	1	33.4	39.6	47.7	43.3	75.5	91.1	97.6
10 mg/kg ($CuSO_4$)	1	34.0	34.7	42.4	46.4	69.0	91.2	97.8
10 mg/kg ($CuSO_4$)	1	32.2	35.1	41.4	37.8	68.4	93.7	97.9
10 mg/kg ($CuSO_4$)	2	31.8	34.4	37.8	43.9	46.9	70.4	96.5
10 mg/kg ($CuSO_4$)	2	32.2	34.9	35.5	37.5	43.7	81.2	96.2
10 mg/kg ($CuSO_4$)	2	30.1	37.9	38.0	51.7	52.4	.	97.0
10 mg/kg ($CuSO_4$)	2	33.2	36.2	38.6	51.7	61.0	89.1	96.8
10 mg/kg ($CuSO_4$)	3	33.1	34.2	46.6	64.8	60.7	93.5	97.4
10 mg/kg ($CuSO_4$)	3	32.4	37.8	50.4	61.8	73.9	93.8	97.3
10 mg/kg ($CuSO_4$)	3	32.6	35.4	51.0	57.0	71.5	93.4	97.3
10 mg/kg ($CuSO_4$)	3	31.8	34.7	47.4	59.7	73.4	91.9	96.2
10 mg/kg ($CuSO_4$)	4	30.5	35.9	51.1	45.8	67.9	91.5	96.6
10 mg/kg ($CuSO_4$)	4	30.7	35.4	51.3	60.7	73.4	90.4	97.1
10 mg/kg ($CuSO_4$)	4	30.1	34.4	42.1	51.0	.	.	98.8
10 mg/kg ($CuSO_4$)	4	29.4	34.9	40.9	59.2	74.1	94.1	96.5
10 mg/kg ($CuSO_4$)	5	31.3	33.4	47.6	54.9	76.9	92.9	97.9
10 mg/kg ($CuSO_4$)	5	31.8	32.5	52.0	63.6	75.5	89.6	97.6
10 mg/kg ($CuSO_4$)	5	31.1	33.4	48.0	64.1	75.5	93.3	97.6
10 mg/kg ($CuSO_4$)	5	30.1	33.3	48.9	63.7	74.9	93.2	97.8
10 mg/kg ($CuSO_4$)	6	33.1	40.7	49.6	62.3	71.7	93.8	97.7
10 mg/kg ($CuSO_4$)	6	33.3	39.7	52.5	69.7	71.8	94.9	97.2
10 mg/kg ($CuSO_4$)	6	33.0	38.8	49.2	68.6	72.4	94.4	97.0
10 mg/kg ($CuSO_4$)	6	32.9	39.9	47.6	70.3	73.6	94.0	97.2
35 mg/kg ($CuSO_4$)	1	31.6	38.1	52.9	69.3	77.7	93.7	97.7
35 mg/kg ($CuSO_4$)	1	32.6	.	54.9	59.3	74.2	93.4	97.7
35 mg/kg ($CuSO_4$)	1	32.2	34.1	47.6	58.7	64.0	91.7	98.1
35 mg/kg ($CuSO_4$)	1	31.2	36.9	49.4	.	71.8	94.7	97.7
35 mg/kg ($CuSO_4$)	2	33.4	35.8	43.7	50.6	58.3	87.4	96.1
35 mg/kg ($CuSO_4$)	2	34.0	33.3	41.2	51.0	57.8	83.1	95.9
35 mg/kg ($CuSO_4$)	2	32.0	32.6	38.8	48.7	69.4	82.2	96.9
35 mg/kg ($CuSO_4$)	2	31.9	36.2	63.5	53.3	70.5	88.8	97.0
35 mg/kg ($CuSO_4$)	3	33.1	36.2	47.9	60.8	77.3	92.0	97.6
35 mg/kg ($CuSO_4$)	3	33.6	.	44.9	.	75.5	88.6	97.2
35 mg/kg ($CuSO_4$)	3	33.0	36.5	52.2	58.3	75.2	91.6	97.4
35 mg/kg ($CuSO_4$)	3	34.1	35.3	48.0	65.4	74.6	92.1	96.9
35 mg/kg ($CuSO_4$)	4	31.9	35.5	47.3	54.6	70.1	95.8	97.3
35 mg/kg ($CuSO_4$)	4	29.7	34.1	49.2	57.7	75.9	94.4	97.6
35 mg/kg ($CuSO_4$)	4	30.8	34.7	40.8	58.7	63.5	88.2	97.4
35 mg/kg ($CuSO_4$)	4	31.7	36.3	39.3	58.8	69.1	92.5	96.8
35 mg/kg ($CuSO_4$)	5	30.8	35.3	47.1	64.5	77.0	89.3	97.4

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
35 mg/kg ($CuSO_4$)	5	32.3	35.1	50.1	67.7	77.2	92.3	97.4
35 mg/kg ($CuSO_4$)	5	31.0	37.5	51.3	66.5	70.7	89.8	98.4
35 mg/kg ($CuSO_4$)	5	30.5	37.4	51.6	68.3	73.8	90.6	97.5
35 mg/kg ($CuSO_4$)	6	30.4	32.0	40.2	53.2	61.0	89.4	97.6
35 mg/kg ($CuSO_4$)	6	30.6	33.6	42.0	57.6	51.8	82.7	98.1
35 mg/kg ($CuSO_4$)	6	31.2	32.1	43.0	63.6	56.1	88.7	.
35 mg/kg ($CuSO_4$)	6	30.6	31.6	44.3	62.1	55.0	86.1	97.2
50 mg/kg ($CuSO_4$)	1	31.5	31.6	42.4	61.3	79.2	91.3	97.8
50 mg/kg ($CuSO_4$)	1	31.6	31.1	42.7	68.4	67.0	91.8	98.3
50 mg/kg ($CuSO_4$)	1	31.1	31.7	49.8	67.5	73.7	93.5	.
50 mg/kg ($CuSO_4$)	1	31.0	33.3	45.7	58.0	71.0	93.5	97.9
50 mg/kg ($CuSO_4$)	2	32.0	36.1	44.6	59.2	62.9	87.6	97.0
50 mg/kg ($CuSO_4$)	2	31.9	38.3	49.7	62.3	58.3	88.0	97.1
50 mg/kg ($CuSO_4$)	2	30.1	35.3	45.8	51.6	54.5	76.7	97.3
50 mg/kg ($CuSO_4$)	2	32.1	36.8	46.7	52.3	61.4	87.3	97.2
50 mg/kg ($CuSO_4$)	3	32.4	36.9	53.6	61.4	76.6	94.0	97.6
50 mg/kg ($CuSO_4$)	3	33.8	35.8	52.9	64.3	75.4	93.1	97.6
50 mg/kg ($CuSO_4$)	3	32.0	36.8	53.6	68.8	77.5	95.1	97.6
50 mg/kg ($CuSO_4$)	3	33.0	35.5	54.9	69.3	77.6	93.8	97.6
50 mg/kg ($CuSO_4$)	4	33.1	39.6	.	65.4	62.5	92.7	97.3
50 mg/kg ($CuSO_4$)	4	39.3	40.2	52.5	65.2	72.2	92.4	97.3
50 mg/kg ($CuSO_4$)	4	32.9	37.1	46.3	65.0	54.6	91.4	97.5
50 mg/kg ($CuSO_4$)	4	32.5	35.2	45.2	65.6	52.4	93.4	98.7
50 mg/kg ($CuSO_4$)	5	32.6	36.5	50.0	64.5	73.9	91.1	97.3
50 mg/kg ($CuSO_4$)	5	32.9	36.9	49.5	67.7	.	92.2	97.1
50 mg/kg ($CuSO_4$)	5	33.0	36.2	50.5	70.2	80.7	91.9	96.9
50 mg/kg ($CuSO_4$)	5	32.4	36.7	45.6	62.8	79.4	92.3	97.1
50 mg/kg ($CuSO_4$)	6	31.1	33.2	50.2	64.6	67.9	86.4	97.7
50 mg/kg ($CuSO_4$)	6	30.9	35.0	52.6	62.6	71.7	89.6	97.4
50 mg/kg ($CuSO_4$)	6	31.2	34.8	44.4	61.0	77.1	83.2	97.6
50 mg/kg ($CuSO_4$)	6	31.3	34.7	45.0	68.1	73.9	88.0	97.1
10 mg/kg (TBCC)	1	32.5	37.8	45.9	65.2	67.4	92.5	97.5
10 mg/kg (TBCC)	1	32.0	37.5	43.5	65.2	66.8	94.5	98.0
10 mg/kg (TBCC)	1	31.9	35.6	49.8	63.3	65.4	94.3	97.4
10 mg/kg (TBCC)	1	31.3	34.8	43.0	64.0	71.0	94.1	97.3
10 mg/kg (TBCC)	2	30.4	33.4	35.8	50.3	56.1	98.4	98.6
10 mg/kg (TBCC)	2	30.7	33.0	45.9	63.7	61.0	92.4	97.5
10 mg/kg (TBCC)	2	28.8	32.5	39.2	58.0	65.5	91.4	94.9
10 mg/kg (TBCC)	2	31.0	31.9	36.3	64.1	70.8	93.6	97.3
10 mg/kg (TBCC)	3	34.7	36.8	53.2	64.7	74.3	93.8	97.4
10 mg/kg (TBCC)	3	33.5	37.0	60.1	67.1	75.9	94.5	97.9
10 mg/kg (TBCC)	3	33.0	.	53.7	71.3	79.5	93.6	97.7
10 mg/kg (TBCC)	3	33.1	37.2	53.2	73.2	80.6	93.9	97.6

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
10 mg/kg (TBCC)	4	32.1	40.6	47.8	70.1	57.1	87.3	99.1
10 mg/kg (TBCC)	4	33.2	39.4	60.4	60.0	62.9	88.1	97.1
10 mg/kg (TBCC)	4	32.7	37.7	42.6	62.8	55.8	87.6	97.6
10 mg/kg (TBCC)	4	33.0	36.3	41.9	61.1	51.9	91.6	97.7
10 mg/kg (TBCC)	5	33.6	36.5	46.6	66.5	71.8	85.5	97.2
10 mg/kg (TBCC)	5	33.8	36.4	44.0	63.5	73.5	88.4	97.2
10 mg/kg (TBCC)	5	34.4	38.6	44.3	64.3	76.2	79.2	97.1
10 mg/kg (TBCC)	5	33.7	39.0	43.4	63.4	75.9	81.3	97.3
10 mg/kg (TBCC)	6	32.9	37.7	42.0	66.3	74.4	90.3	97.5
10 mg/kg (TBCC)	6	32.9	37.5	50.9	70.7	76.7	89.7	97.0
10 mg/kg (TBCC)	6	31.7	36.8	45.7	65.6	67.3	94.1	97.5
10 mg/kg (TBCC)	6	32.5	38.0	48.4	67.3	78.7	93.4	97.5
35 mg/kg (TBCC)	1	32.1	33.1	39.7	66.3	78.5	83.0	97.1
35 mg/kg (TBCC)	1	31.4	34.6	40.0	62.8	68.2	89.8	96.9
35 mg/kg (TBCC)	1	31.0	36.4	48.9	63.2	73.6	93.3	97.2
35 mg/kg (TBCC)	1	30.6	33.8	48.8	64.5	62.4	93.1	97.5
35 mg/kg (TBCC)	2	29.1	34.6	47.1	45.0	55.1	84.4	97.0
35 mg/kg (TBCC)	2	31.6	34.9	43.0	44.3	70.1	81.0	96.7
35 mg/kg (TBCC)	2	29.7	34.6	38.9	44.6	63.2	84.2	97.3
35 mg/kg (TBCC)	2	29.7	35.8	39.9	47.4	69.1	80.3	97.1
35 mg/kg (TBCC)	3	32.0	34.2	47.6	65.5	66.4	94.5	97.4
35 mg/kg (TBCC)	3	31.9	32.6	47.3	67.9	70.4	92.7	97.9
35 mg/kg (TBCC)	3	31.4	33.2	50.1	67.1	70.7	93.1	97.9
35 mg/kg (TBCC)	3	31.8	33.7	48.1	66.4	69.6	93.4	97.9
35 mg/kg (TBCC)	4	32.0	35.8	47.2	46.3	61.4	.	97.5
35 mg/kg (TBCC)	4	31.5	38.8	44.0	56.9	70.2	90.5	97.4
35 mg/kg (TBCC)	4	31.0	37.5	42.9	45.9	56.8	91.3	97.4
35 mg/kg (TBCC)	4	30.9	34.9	41.2	52.8	69.7	89.1	97.5
35 mg/kg (TBCC)	5	33.7	40.0	47.2	68.3	65.0	93.5	97.2
35 mg/kg (TBCC)	5	32.8	40.2	47.9	71.3	72.9	93.7	97.4
35 mg/kg (TBCC)	5	33.0	37.1	43.4	57.3	74.7	95.1	97.5
35 mg/kg (TBCC)	5	33.2	37.8	47.4	62.2	74.6	94.6	97.8
35 mg/kg (TBCC)	6	34.4	37.7	39.4	61.8	73.8	90.2	97.3
35 mg/kg (TBCC)	6	34.5	39.6	40.3	63.3	79.5	79.6	97.4
35 mg/kg (TBCC)	6	33.3	38.4	.	.	75.4	88.0	97.2
35 mg/kg (TBCC)	6	34.6	37.6	41.1	62.7	78.0	89.0	97.2
50 mg/kg (TBCC)	1	32.6	35.0	45.0	49.1	78.2	92.4	97.2
50 mg/kg (TBCC)	1	35.6	34.1	50.2	54.3	77.0	90.5	97.3
50 mg/kg (TBCC)	1	31.9	37.6	51.7	55.1	80.1	89.8	97.6
50 mg/kg (TBCC)	1	31.5	36.8	50.7	62.8	82.7	92.0	97.5
50 mg/kg (TBCC)	2	31.4	31.9	.	55.5	57.2	89.2	94.7
50 mg/kg (TBCC)	2	31.5	33.1	53.3	62.4	55.5	89.9	96.1
50 mg/kg (TBCC)	2	30.6	32.7	37.5	54.5	58.4	91.6	95.1

Cu treatment	Animal	Time of incubation						
		1.5 h	3 h	6 h	9 h	12 h	24 h	48 h
50 mg/kg (TBCC)	2	31.2	32.3	48.1	60.2	47.5	91.6	96.9
50 mg/kg (TBCC)	3	32.2	37.8	51.1	62.9	71.3	92.6	97.7
50 mg/kg (TBCC)	3	31.8	35.9	47.8	63.2	75.8	91.5	97.5
50 mg/kg (TBCC)	3	31.0	37.6	48.4	63.0	75.5	92.3	97.6
50 mg/kg (TBCC)	3	31.1	37.5	48.8	68.7	73.2	92.6	97.0
50 mg/kg (TBCC)	4	29.8	32.7	45.4	62.9	64.1	89.3	97.7
50 mg/kg (TBCC)	4	29.3	31.5	45.5	63.9	72.5	93.9	97.9
50 mg/kg (TBCC)	4	29.8	33.1	37.5	46.7	45.8	93.5	97.8
50 mg/kg (TBCC)	4	29.8	30.9	42.7	45.7	63.9	95.5	97.6
50 mg/kg (TBCC)	5	32.3	38.0	47.2	67.6	78.9	93.7	97.9
50 mg/kg (TBCC)	5	33.9	36.2	46.4	67.3	80.3	93.7	97.6
50 mg/kg (TBCC)	5	34.0	37.1	48.8	66.7	73.7	93.8	97.8
50 mg/kg (TBCC)	5	32.7	38.2	49.3	65.8	77.8	93.7	97.5
50 mg/kg (TBCC)	6	33.0	39.9	49.2	47.4	69.8	92.1	97.6
50 mg/kg (TBCC)	6	.	36.8	.	54.0	69.5	93.5	97.7
50 mg/kg (TBCC)	6	32.1	38.8	44.9	42.4	78.9	92.2	97.4
50 mg/kg (TBCC)	6	33.1	38.9	46.0	65.5	80.0	92.9	97.7

Table 57: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of TMR of the different animals dependent on Cu treatment

Cu treatment	Animal	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
10 mg/kg ($CuSO_4$)	1	39.3	56.0	4.47	1.33	95.3	77.0	64.1	57.4
10 mg/kg ($CuSO_4$)	2	39.6	57.7	3.55	0.00	97.4	76.6	63.6	57.4
10 mg/kg ($CuSO_4$)	3	39.3	48.5	8.01	0.49	87.8	77.7	68.5	62.7
10 mg/kg ($CuSO_4$)	4	39.3	46.6	9.64	0.98	85.9	77.2	68.5	62.9
10 mg/kg ($CuSO_4$)	5	39.3	47.8	9.84	0.56	87.2	78.6	70.2	64.5
10 mg/kg ($CuSO_4$)	6	39.3	46.9	8.42	0.82	86.2	76.6	67.6	61.8
35 mg/kg ($CuSO_4$)	1	39.3	47.0	7.79	0.77	86.4	76.2	66.9	61.1
35 mg/kg ($CuSO_4$)	2	39.3	51.4	5.03	0.89	90.7	75.4	64.0	57.8
35 mg/kg ($CuSO_4$)	3	39.3	48.7	7.87	0.57	88.0	77.7	68.3	62.4
35 mg/kg ($CuSO_4$)	4	39.3	50.2	7.26	0.45	89.5	78.3	68.4	62.3
35 mg/kg ($CuSO_4$)	5	39.3	47.0	11.7	1.05	86.3	78.6	70.6	65.0
35 mg/kg ($CuSO_4$)	6	39.5	49.2	5.72	0.00	88.7	75.9	65.7	60.0
50 mg/kg ($CuSO_4$)	1	39.3	48.2	8.89	0.86	87.5	78.0	68.8	63.0
50 mg/kg ($CuSO_4$)	2	39.3	47.9	5.75	0.22	87.2	74.7	64.6	59.0
50 mg/kg ($CuSO_4$)	3	39.3	45.7	11.1	0.96	85.0	77.2	69.3	63.8
50 mg/kg ($CuSO_4$)	4	39.6	49.4	7.03	0.00	89.1	78.1	68.5	62.7
50 mg/kg ($CuSO_4$)	5	39.3	47.8	10.4	0.51	87.1	79.0	70.8	65.2
50 mg/kg ($CuSO_4$)	6	39.3	45.0	9.79	0.63	84.3	76.2	68.2	62.9
10 mg/kg (TBCC)	1	39.3	48.6	8.70	0.83	87.9	78.2	68.9	63.0
10 mg/kg (TBCC)	2	39.3	49.1	6.60	0.47	88.4	76.6	66.6	60.7
10 mg/kg (TBCC)	3	39.3	46.7	9.02	0.67	86.1	77.1	68.4	62.8
10 mg/kg (TBCC)	4	39.3	49.2	5.61	0.22	88.5	75.4	65.1	59.3
10 mg/kg (TBCC)	5	39.3	47.2	8.40	0.48	86.5	77.1	68.2	62.6
10 mg/kg (TBCC)	6	39.3	47.9	8.57	0.06	87.2	78.1	69.5	64.0
35 mg/kg (TBCC)	1	39.3	47.7	8.31	0.79	87.0	77.2	67.9	62.1
35 mg/kg (TBCC)	2	39.3	48.1	6.15	1.04	87.4	74.9	64.5	58.6
35 mg/kg (TBCC)	3	39.3	47.0	10.0	0.45	86.3	78.1	70.0	64.5
35 mg/kg (TBCC)	4	39.3	50.5	5.50	0.75	89.8	75.8	64.8	58.7
35 mg/kg (TBCC)	5	39.3	48.2	8.77	1.03	87.5	77.8	68.5	62.6
35 mg/kg (TBCC)	6	39.3	47.2	8.92	0.61	86.5	77.4	68.6	63.0
50 mg/kg (TBCC)	1	39.3	50.2	6.75	0.80	89.6	77.5	67.1	60.9
50 mg/kg (TBCC)	2	39.9	49.2	5.35	0.00	89.1	75.7	65.3	59.6
50 mg/kg (TBCC)	3	39.3	47.3	9.58	0.36	86.6	78.2	69.9	64.4
50 mg/kg (TBCC)	4	39.3	51.4	5.71	0.28	90.7	77.2	66.3	60.2
50 mg/kg (TBCC)	5	39.3	46.8	9.39	0.71	86.1	77.3	68.8	63.2
50 mg/kg (TBCC)	6	39.3	46.6	8.04	0.71	85.9	76.1	67.0	61.4

Table 58: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of grass silage of the different animals dependent on Cu treatment

Cu treatment	Animal	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
10 mg/kg ($CuSO_4$)	1	40.4	51.2	6.32	5.10	91.6	75.5	62.5	55.4
10 mg/kg ($CuSO_4$)	2	38.1	55.2	5.02	4.52	93.3	74.2	60.2	52.9
10 mg/kg ($CuSO_4$)	3	39.2	49.6	8.64	2.83	88.8	77.3	66.5	59.8
10 mg/kg ($CuSO_4$)	4	39.0	50.4	7.90	2.42	89.4	77.3	66.4	59.6
10 mg/kg ($CuSO_4$)	5	38.4	48.5	9.47	2.51	86.9	76.5	66.4	59.9
10 mg/kg ($CuSO_4$)	6	38.2	48.5	7.79	2.37	86.8	75.1	64.5	58.0
35 mg/kg ($CuSO_4$)	1	38.2	51.3	7.81	2.45	89.5	77.1	65.8	59.0
35 mg/kg ($CuSO_4$)	2	38.7	52.8	5.18	2.48	91.5	74.9	62.4	55.7
35 mg/kg ($CuSO_4$)	3	38.0	47.4	8.52	3.30	85.4	74.0	63.3	56.8
35 mg/kg ($CuSO_4$)	4	39.1	50.4	9.00	2.65	89.5	78.2	67.5	60.7
35 mg/kg ($CuSO_4$)	5	38.8	49.0	11.0	2.27	87.8	78.4	68.9	62.5
35 mg/kg ($CuSO_4$)	6	38.5	50.6	6.29	2.71	89.1	74.8	63.1	56.4
50 mg/kg ($CuSO_4$)	1	38.3	50.9	8.61	2.70	89.1	77.4	66.4	59.5
50 mg/kg ($CuSO_4$)	2	38.4	52.6	4.87	1.94	91.0	74.3	62.0	55.5
50 mg/kg ($CuSO_4$)	3	38.9	44.7	11.3	2.07	83.6	75.3	66.8	61.0
50 mg/kg ($CuSO_4$)	4	38.2	52.1	6.79	2.36	90.4	76.6	64.9	58.0
50 mg/kg ($CuSO_4$)	5	39.0	49.6	10.4	2.31	88.6	78.7	68.8	62.3
50 mg/kg ($CuSO_4$)	6	38.9	44.8	10.0	2.27	83.7	74.6	65.6	59.6
10 mg/kg (TBCC)	1	38.8	50.8	8.52	2.33	89.6	78.0	67.3	60.5
10 mg/kg (TBCC)	2	38.2	52.6	6.51	2.92	90.8	76.2	63.9	56.9
10 mg/kg (TBCC)	3	38.4	47.6	9.79	2.47	86.0	76.0	66.3	59.9
10 mg/kg (TBCC)	4	39.0	59.0	3.62	0.89	98.1	76.4	62.7	56.2
10 mg/kg (TBCC)	5	38.8	44.0	13.8	4.29	82.9	74.1	64.9	58.6
10 mg/kg (TBCC)	6	39.4	48.6	8.68	2.10	88.0	77.3	67.2	60.8
35 mg/kg (TBCC)	1	39.0	48.4	12.6	4.09	87.4	77.4	67.2	60.3
35 mg/kg (TBCC)	2	38.7	49.4	6.74	2.65	88.1	74.8	63.6	57.0
35 mg/kg (TBCC)	3	38.6	49.0	8.52	2.24	87.6	76.5	66.2	59.7
35 mg/kg (TBCC)	4	38.7	49.5	7.51	4.30	88.3	74.6	62.7	55.7
35 mg/kg (TBCC)	5	39.2	47.6	8.68	2.51	86.8	76.0	65.9	59.5
35 mg/kg (TBCC)	6	38.3	43.1	14.7	4.44	81.4	73.0	64.1	57.9
50 mg/kg (TBCC)	1	37.5	49.8	8.42	3.65	87.3	74.9	63.5	56.6
50 mg/kg (TBCC)	2	38.7	51.7	6.65	2.44	90.4	76.6	64.8	58.0
50 mg/kg (TBCC)	3	39.0	47.0	10.0	2.10	86.0	76.6	67.2	61.1
50 mg/kg (TBCC)	4	38.1	53.8	6.09	2.81	91.9	76.4	63.8	56.7
50 mg/kg (TBCC)	5	38.6	48.1	9.51	2.76	86.7	76.2	66.0	59.5
50 mg/kg (TBCC)	6	39.2	46.5	7.38	2.18	85.7	74.2	64.1	58.0

Table 59: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of maize silage of the different animals dependent on Cu treatment

Cu treatment	Animal	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
10 mg/kg ($CuSO_4$)	1	41.2	50.9	3.26	0.00	92.1	72.7	61.3	55.9
10 mg/kg ($CuSO_4$)	2	43.3	37.3	5.83	0.00	80.6	71.1	63.4	59.0
10 mg/kg ($CuSO_4$)	3	41.2	40.0	7.92	0.00	81.2	73.1	65.7	61.1
10 mg/kg ($CuSO_4$)	4	42.3	37.7	7.56	0.00	79.9	72.1	64.9	60.6
10 mg/kg ($CuSO_4$)	5	42.5	39.7	9.59	0.00	82.2	75.4	68.6	64.2
10 mg/kg ($CuSO_4$)	6	41.5	41.8	7.83	0.00	83.4	74.8	67.0	62.2
35 mg/kg ($CuSO_4$)	1	42.5	45.5	4.81	0.00	88.0	74.6	64.8	59.6
35 mg/kg ($CuSO_4$)	2	41.8	39.3	4.91	0.00	81.0	69.7	61.2	56.7
35 mg/kg ($CuSO_4$)	3	43.1	40.1	6.24	0.00	83.2	73.5	65.4	60.7
35 mg/kg ($CuSO_4$)	4	41.3	39.9	7.17	0.00	81.2	72.5	64.8	60.2
35 mg/kg ($CuSO_4$)	5	39.9	40.0	10.5	0.32	79.9	73.3	66.5	62.0
35 mg/kg ($CuSO_4$)	6	42.4	40.8	5.85	0.00	83.2	72.8	64.4	59.6
50 mg/kg ($CuSO_4$)	1	41.3	41.8	7.50	0.00	83.1	74.3	66.4	61.5
50 mg/kg ($CuSO_4$)	2	42.4	36.6	6.34	0.00	79.1	70.3	62.9	58.6
50 mg/kg ($CuSO_4$)	3	40.0	41.1	8.92	0.00	81.1	73.6	66.3	61.7
50 mg/kg ($CuSO_4$)	4	42.1	40.2	6.87	0.00	82.3	73.3	65.4	60.7
50 mg/kg ($CuSO_4$)	5	41.4	39.5	9.25	0.00	80.9	73.9	67.0	62.5
50 mg/kg ($CuSO_4$)	6	41.8	36.8	10.1	0.00	78.7	72.5	66.4	62.3
10 mg/kg (TBCC)	1	40.8	41.5	7.90	0.00	82.2	73.8	66.2	61.4
10 mg/kg (TBCC)	2	42.1	37.9	7.74	0.00	80.0	72.2	65.1	60.7
10 mg/kg (TBCC)	3	41.5	41.4	8.70	0.00	82.9	75.1	67.8	63.1
10 mg/kg (TBCC)	4	41.6	39.4	5.37	0.00	81.0	70.3	62.0	57.4
10 mg/kg (TBCC)	5	41.9	37.6	9.03	0.00	79.5	72.7	66.1	61.9
10 mg/kg (TBCC)	6	42.7	38.5	8.24	0.00	81.2	73.7	66.7	62.2
35 mg/kg (TBCC)	1	40.9	40.4	6.60	0.00	81.3	71.9	63.9	59.2
35 mg/kg (TBCC)	2	41.2	38.6	5.74	0.00	79.8	69.8	61.8	57.3
35 mg/kg (TBCC)	3	42.4	39.5	9.57	0.00	81.9	75.1	68.3	63.9
35 mg/kg (TBCC)	4	41.8	43.1	5.40	0.00	84.9	73.3	64.2	59.2
35 mg/kg (TBCC)	5	40.4	41.9	7.34	0.00	82.3	73.3	65.3	60.5
35 mg/kg (TBCC)	6	41.7	39.3	8.64	0.00	81.1	73.7	66.6	62.2
50 mg/kg (TBCC)	1	42.4	41.5	6.73	0.00	83.8	74.3	66.2	61.3
50 mg/kg (TBCC)	2	40.0	39.8	5.52	0.00	79.8	69.2	60.9	56.2
50 mg/kg (TBCC)	3	41.8	40.5	8.59	0.00	82.3	74.7	67.4	62.8
50 mg/kg (TBCC)	4	41.8	40.2	7.57	0.00	82.0	73.6	66.0	61.4
50 mg/kg (TBCC)	5	41.7	43.3	7.85	0.00	84.9	76.1	68.1	63.1
50 mg/kg (TBCC)	6	40.8	40.1	7.52	0.00	80.8	72.4	64.8	60.2

Table 60: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of wheat meal of the different animals dependent on Cu treatment

Cu Treatment	Animal	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
10 mg/kg ($CuSO_4$)	1	57.6	36.3	18.3	0.00	93.9	90.3	86.1	82.9
10 mg/kg ($CuSO_4$)	2	55.6	34.9	35.1	0.00	90.5	88.6	86.1	84.0
10 mg/kg ($CuSO_4$)	3	54.4	38.5	51.4	0.00	92.9	91.4	89.5	87.7
10 mg/kg ($CuSO_4$)	4	54.2	38.3	57.9	0.00	92.5	91.3	89.5	87.9
10 mg/kg ($CuSO_4$)	5	54.4	38.9	55.4	0.00	93.3	91.9	90.0	88.3
10 mg/kg ($CuSO_4$)	6	54.8	39.0	46.8	0.00	93.8	92.2	90.0	88.1
35 mg/kg ($CuSO_4$)	1	54.1	38.8	48.3	0.00	93.0	91.4	89.3	87.4
35 mg/kg ($CuSO_4$)	2	55.4	37.5	33.6	0.00	92.9	90.8	88.0	85.7
35 mg/kg ($CuSO_4$)	3	54.9	38.7	37.2	0.00	93.7	91.7	89.1	86.8
35 mg/kg ($CuSO_4$)	4	54.6	38.2	46.1	0.00	92.8	91.2	89.1	87.2
35 mg/kg ($CuSO_4$)	5	53.9	39.2	57.7	0.00	93.1	91.8	90.0	88.3
35 mg/kg ($CuSO_4$)	6	54.8	37.4	42.9	0.00	92.1	90.5	88.2	86.2
50 mg/kg ($CuSO_4$)	1	54.9	38.2	38.2	0.00	93.1	91.2	88.7	86.5
50 mg/kg ($CuSO_4$)	2	54.4	39.3	39.4	0.00	93.6	91.7	89.2	87.0
50 mg/kg ($CuSO_4$)	3	53.9	40.4	45.9	0.00	94.3	92.6	90.3	88.3
50 mg/kg ($CuSO_4$)	4	53.9	39.7	41.2	0.00	93.6	91.7	89.3	87.1
50 mg/kg ($CuSO_4$)	5	53.8	39.3	56.5	0.00	93.1	91.8	89.9	88.3
50 mg/kg ($CuSO_4$)	6	54.0	39.1	58.0	0.00	93.1	91.8	90.0	88.3
10 mg/kg (TBCC)	1	54.4	38.5	49.0	0.00	92.9	91.3	89.3	87.4
10 mg/kg (TBCC)	2	56.3	35.7	33.2	0.00	92.1	90.0	87.4	85.1
10 mg/kg (TBCC)	3	54.3	39.5	49.5	0.00	93.8	92.3	90.2	88.3
10 mg/kg (TBCC)	4	54.0	38.6	47.8	0.00	92.6	91.0	88.9	87.0
10 mg/kg (TBCC)	5	54.5	39.0	43.2	0.00	93.5	91.7	89.4	87.4
10 mg/kg (TBCC)	6	54.1	39.0	58.1	0.00	93.1	91.8	90.0	88.4
35 mg/kg (TBCC)	1	55.5	37.8	32.2	0.00	93.3	91.1	88.2	85.8
35 mg/kg (TBCC)	2	54.4	37.7	44.0	0.00	92.1	90.5	88.3	86.3
35 mg/kg (TBCC)	3	54.1	39.2	58.1	0.00	93.3	92.0	90.2	88.5
35 mg/kg (TBCC)	4	54.0	39.3	39.2	0.00	93.3	91.4	88.9	86.6
35 mg/kg (TBCC)	5	53.8	39.5	54.0	0.00	93.3	91.9	90.0	88.2
35 mg/kg (TBCC)	6	54.6	38.3	52.8	0.00	92.9	91.5	89.6	87.9
50 mg/kg (TBCC)	1	54.8	37.9	34.0	0.00	92.7	90.6	87.8	85.5
50 mg/kg (TBCC)	2	54.7	37.3	36.3	0.00	92.0	90.0	87.5	85.2
50 mg/kg (TBCC)	3	54.0	39.3	69.7	0.00	93.3	92.2	90.7	89.3
50 mg/kg (TBCC)	4	54.9	38.0	34.3	0.00	92.9	90.8	88.1	85.8
50 mg/kg (TBCC)	5	54.1	39.8	59.4	0.00	93.9	92.6	90.8	89.2
50 mg/kg (TBCC)	6	54.3	38.4	50.1	0.00	92.7	91.2	89.2	87.4

Table 61: Parameters of degradability, totally degradable fraction, and effective degradability of dry matter of soybean meal of the different animals dependent on Cu treatment

Cu treatment	Animal	Parameter of degradability				TDF	Effective degradability		
		a [%]	b [%]	c [%/h]	t_0 [h]	[%]	ED2 [%]	ED5 [%]	ED8 [%]
10 mg/kg ($CuSO_4$)	1	28.6	71.4	6.98	1.47	100	82.5	67.3	58.2
10 mg/kg ($CuSO_4$)	2	32.1	67.9	6.07	5.17	100	78.1	60.8	51.5
10 mg/kg ($CuSO_4$)	3	28.6	71.4	8.19	1.39	100	84.4	70.0	60.9
10 mg/kg ($CuSO_4$)	4	29.4	70.6	8.51	2.44	100	83.8	68.8	59.3
10 mg/kg ($CuSO_4$)	5	29.9	69.1	10.6	2.69	98.9	84.9	70.9	61.6
10 mg/kg ($CuSO_4$)	6	28.6	71.4	9.06	1.10	100	85.8	72.2	63.3
35 mg/kg ($CuSO_4$)	1	28.6	71.4	8.74	1.36	100	85.1	71.0	62.0
35 mg/kg ($CuSO_4$)	2	28.6	71.4	6.11	1.22	100	81.1	65.6	56.7
35 mg/kg ($CuSO_4$)	3	28.6	71.4	8.55	1.30	100	85.0	70.8	61.8
35 mg/kg ($CuSO_4$)	4	29.8	70.2	8.61	2.60	100	83.9	68.8	59.4
35 mg/kg ($CuSO_4$)	5	28.6	70.2	9.59	1.47	98.8	85.0	71.5	62.7
35 mg/kg ($CuSO_4$)	6	29.7	70.3	6.80	2.65	100	81.2	65.2	55.8
50 mg/kg ($CuSO_4$)	1	30.6	67.4	12.8	4.01	98.0	84.4	70.2	60.6
50 mg/kg ($CuSO_4$)	2	28.6	71.4	6.04	1.06	100	81.1	65.7	56.8
50 mg/kg ($CuSO_4$)	3	28.6	71.1	9.93	1.34	99.7	86.3	72.9	64.0
50 mg/kg ($CuSO_4$)	4	28.6	71.4	7.20	0.82	100	83.6	69.0	60.3
50 mg/kg ($CuSO_4$)	5	28.6	70.4	9.80	1.41	99.0	85.5	72.1	63.2
50 mg/kg ($CuSO_4$)	6	29.9	67.2	10.0	2.36	97.0	83.2	69.6	60.7
10 mg/kg (TBCC)	1	28.6	71.4	8.23	1.46	100	84.4	69.9	60.8
10 mg/kg (TBCC)	2	30.5	68.7	10.8	4.70	99.2	83.3	67.6	57.6
10 mg/kg (TBCC)	3	28.6	70.6	10.6	1.25	99.3	86.6	73.7	65.0
10 mg/kg (TBCC)	4	28.6	71.4	6.50	0.76	100	82.4	67.5	58.7
10 mg/kg (TBCC)	5	28.6	69.3	8.06	1.03	97.9	83.0	69.2	60.6
10 mg/kg (TBCC)	6	28.6	71.0	9.12	1.35	99.6	85.3	71.5	62.6
35 mg/kg (TBCC)	1	30.0	68.5	9.54	2.58	98.4	83.7	69.4	60.3
35 mg/kg (TBCC)	2	29.3	70.7	6.08	2.41	100	80.0	63.7	54.5
35 mg/kg (TBCC)	3	30.2	69.2	10.1	2.62	99.4	85.0	70.8	61.5
35 mg/kg (TBCC)	4	30.0	70.0	7.09	2.41	100	82.0	66.4	57.1
35 mg/kg (TBCC)	5	28.6	71.4	8.58	1.21	100	85.1	71.1	62.1
35 mg/kg (TBCC)	6	28.6	71.0	7.87	1.16	99.6	83.9	69.6	60.7
50 mg/kg (TBCC)	1	28.6	71.4	8.56	1.40	100	84.9	70.6	61.6
50 mg/kg (TBCC)	2	29.9	70.1	6.94	2.53	100	81.6	65.8	56.5
50 mg/kg (TBCC)	3	28.6	71.2	9.00	1.40	99.9	85.3	71.3	62.3
50 mg/kg (TBCC)	4	30.1	69.9	9.07	4.13	100	82.8	66.8	56.8
50 mg/kg (TBCC)	5	28.6	71.4	9.61	1.36	100	86.1	72.5	63.6
50 mg/kg (TBCC)	6	28.6	71.4	7.74	1.29	100	83.9	69.3	60.3

Table 62: pH-value in the rumen fluid of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
10 mg/kg ($CuSO_4$)	1	7.01	6.66	6.60	6.44	6.41	6.68	6.90
10 mg/kg ($CuSO_4$)	2	6.81	6.48	6.00	6.68	6.33	6.58	6.94
10 mg/kg ($CuSO_4$)	3	7.04	6.88	7.12	6.93	7.14	7.05	7.10
10 mg/kg ($CuSO_4$)	4	6.94	6.78	6.64	6.28	6.36	6.32	6.70
10 mg/kg ($CuSO_4$)	5	7.22	6.83	6.93	7.11	7.26	7.17	7.12
10 mg/kg ($CuSO_4$)	6	7.05	7.01	7.07	7.27	7.25	7.32	7.38
35 mg/kg ($CuSO_4$)	1	7.01	6.73	6.97	7.05	7.13	7.42	7.49
35 mg/kg ($CuSO_4$)	2	6.95	6.38	6.60	6.27	6.33	6.50	6.74
35 mg/kg ($CuSO_4$)	3	6.94	6.56	6.84	6.89	7.02	7.10	7.36
35 mg/kg ($CuSO_4$)	4	6.88	6.57	6.39	6.49	6.94	6.42	6.94
35 mg/kg ($CuSO_4$)	5	7.03	6.79	6.88	6.78	6.73	6.88	6.66
35 mg/kg ($CuSO_4$)	6	7.11	6.62	6.75	6.84	6.95	7.04	7.06
50 mg/kg ($CuSO_4$)	1	7.11	6.70	6.84	6.91	6.99	7.19	7.30
50 mg/kg ($CuSO_4$)	2	6.83	6.42	6.61	7.11	6.77	7.15	7.10
50 mg/kg ($CuSO_4$)	3	6.93	6.70	6.93	7.15	6.83	6.96	7.11
50 mg/kg ($CuSO_4$)	4	6.88	6.64	6.46	6.82	6.85	6.44	7.13
50 mg/kg ($CuSO_4$)	5	6.97	6.77	7.07	6.88	7.23	7.08	7.17
50 mg/kg ($CuSO_4$)	6	7.00	6.88	6.67	6.79	6.87	6.76	6.81
10 mg/kg (TBCC)	1	7.09	6.80	6.73	6.75	6.88	6.94	7.05
10 mg/kg (TBCC)	2	7.07	6.56	6.75	6.57	7.04	6.89	7.04
10 mg/kg (TBCC)	3	6.98	6.90	7.03	7.34	7.28	7.38	7.36
10 mg/kg (TBCC)	4	6.75	6.62	6.46	6.73	6.50	6.66	6.87
10 mg/kg (TBCC)	5	7.10	7.05	7.09	7.08	7.14	7.11	7.25
10 mg/kg (TBCC)	6	7.00	6.98	7.19	7.07	7.24	7.31	7.33
35 mg/kg (TBCC)	1	6.86	6.55	6.89	6.70	6.81	6.94	7.05
35 mg/kg (TBCC)	2	6.82	6.41	6.49	6.22	6.25	6.44	6.64
35 mg/kg (TBCC)	3	7.13	6.61	6.87	6.96	7.02	7.03	7.13
35 mg/kg (TBCC)	4	6.77	6.73	6.78	6.75	6.81	6.99	7.10
35 mg/kg (TBCC)	5	6.88	6.83	6.85	7.02	6.98	6.90	6.91
35 mg/kg (TBCC)	6	7.13	7.07	7.24	7.17	7.16	7.21	7.45
50 mg/kg (TBCC)	1	6.92	6.66	6.82	6.50	6.44	6.96	7.04
50 mg/kg (TBCC)	2	6.84	6.60	6.60	6.71	6.60	6.67	6.69
50 mg/kg (TBCC)	3	7.06	6.80	6.65	6.85	6.90	6.86	6.85
50 mg/kg (TBCC)	4	6.79	6.16	6.48	6.34	6.58	5.87	6.76
50 mg/kg (TBCC)	5	7.01	6.93	7.17	7.42	7.30	7.43	7.35
50 mg/kg (TBCC)	6	7.07	6.90	7.10	7.23	6.90	7.10	7.25

Table 63: Ammonia-nitrogen concentration [mg/l] in the rumen fluid of the different animals dependent on Cu treatment and on sampling time

Cu treatment	Animal	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
10 mg/kg ($CuSO_4$)	1	34.8	148	128	96.0	30.0	23.1	16.1
10 mg/kg ($CuSO_4$)	2	38.6	202	233	107	84.8	46.7	36.1
10 mg/kg ($CuSO_4$)	3	53.1	112	135	61.6	36.9	34.8	28.2
10 mg/kg ($CuSO_4$)	4	30.4	108	135	114	38.9	23.8	19.1
10 mg/kg ($CuSO_4$)	5	34.0	173	172	58.0	39.3	36.6	60.1
10 mg/kg ($CuSO_4$)	6	69.3	199	168	110	80.1	72.5	55.2
35 mg/kg ($CuSO_4$)	1	55.9	161	155	107	49.0	46.1	31.3
35 mg/kg ($CuSO_4$)	2	40.4	155	196	233	57.0	49.4	24.4
35 mg/kg ($CuSO_4$)	3	38.5	191	158	100	53.6	35.4	31.9
35 mg/kg ($CuSO_4$)	4	47.3	174	218	104	39.1	20.8	29.3
35 mg/kg ($CuSO_4$)	5	46.0	122	75.1	55.2	29.3	44.0	36.4
35 mg/kg ($CuSO_4$)	6	63.7	176	197	84.5	74.0	45.1	63.5
50 mg/kg ($CuSO_4$)	1	48.5	157	183	71.8	35.1	36.2	31.6
50 mg/kg ($CuSO_4$)	2	46.5	204	190	103	77.9	44.7	35.2
50 mg/kg ($CuSO_4$)	3	37.3	119	141	59.0	22.9	25.9	26.7
50 mg/kg ($CuSO_4$)	4	34.6	97.3	226	115	78.2	77.3	27.4
50 mg/kg ($CuSO_4$)	5	48.0	189	159	83.5	40.8	27.9	46.0
50 mg/kg ($CuSO_4$)	6	56.7	154	126	92.6	71.9	62.1	62.6
10 mg/kg (TBCC)	1	52.1	146	101	44.1	20.8	38.5	33.5
10 mg/kg (TBCC)	2	35.3	177	153	84.4	44.1	36.9	27.6
10 mg/kg (TBCC)	3	64.6	165	170	87.3	57.3	41.0	35.3
10 mg/kg (TBCC)	4	28.0	179	238	158	43.2	26.5	29.2
10 mg/kg (TBCC)	5	51.7	148	168	72.6	43.6	38.7	43.6
10 mg/kg (TBCC)	6	57.0	161	97.3	56.6	38.0	42.9	37.8
35 mg/kg (TBCC)	1	27.9	161	165	66.6	23.2	12.4	21.4
35 mg/kg (TBCC)	2	41.1	190	139	101	76.3	44.5	26.2
35 mg/kg (TBCC)	3	31.7	225	135	71.7	45.2	35.6	33.4
35 mg/kg (TBCC)	4	32.4	151	155	121	40.8	20.0	13.7
35 mg/kg (TBCC)	5	53.2	92.4	104	71.4	32.7	30.2	39.2
35 mg/kg (TBCC)	6	49.2	154	143	81.2	52.2	46.0	50.4
50 mg/kg (TBCC)	1	37.1	167	181	137	87.4	25.2	21.0
50 mg/kg (TBCC)	2	52.5	144	183	87.8	70.0	33.3	21.7
50 mg/kg (TBCC)	3	45.1	167	110	67.3	37.3	22.0	30.3
50 mg/kg (TBCC)	4	30.0	141	146	156	70.6	75.6	31.8
50 mg/kg (TBCC)	5	49.6	176	173	87.2	66.7	45.6	35.5
50 mg/kg (TBCC)	6	58.4	181	113	101	65.4	45.6	48.5

Table 64: Total volatile fatty acid concentration [mmol/l] in the rumen fluid of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
10 mg/kg ($CuSO_4$)	1	80.6	85.7	98.9	111	98.8	93.2	90.8
10 mg/kg ($CuSO_4$)	2	80.9	107	143	96.8	110	125	103
10 mg/kg ($CuSO_4$)	3	84.7	128	76.3	81.5	65.3	68.5	71.8
10 mg/kg ($CuSO_4$)	4	86.9	92.9	113	138	113	122	85.9
10 mg/kg ($CuSO_4$)	5	72.8	90.7	93.5	75.8	59.4	79.0	76.1
10 mg/kg ($CuSO_4$)	6	76.1	101	89.9	79.5	82.8	63.4	45.9
35 mg/kg ($CuSO_4$)	1	72.3	102	90.7	92.2	82.2	67.4	88.9
35 mg/kg ($CuSO_4$)	2	86.3	118	123	127	71.4	68.9	74.1
35 mg/kg ($CuSO_4$)	3	79.1	107	96.4	120	91.6	93.8	77.4
35 mg/kg ($CuSO_4$)	4	116	112	98.0	115	86.4	92.8	76.7
35 mg/kg ($CuSO_4$)	5	83.4	80.9	79.0	93.9	85.0	91.0	92.2
35 mg/kg ($CuSO_4$)	6	72.3	79.5	73.7	89.3	80.4	75.6	88.8
50 mg/kg ($CuSO_4$)	1	67.2	92.4	83.6	97.2	87.8	66.6	79.6
50 mg/kg ($CuSO_4$)	2	93.1	117	116	83.3	77.8	78.9	51.0
50 mg/kg ($CuSO_4$)	3	73.0	111	90.8	86.1	74.6	74.7	74.8
50 mg/kg ($CuSO_4$)	4	88.1	118	124	111	115	162	89.6
50 mg/kg ($CuSO_4$)	5	111	118	76.1	87.4	67.5	72.0	70.8
50 mg/kg ($CuSO_4$)	6	87.7	82.2	96.9	108	83.7	71.6	69.0
10 mg/kg (TBCC)	1	71.9	94.4	90.0	88.4	85.6	86.4	77.8
10 mg/kg (TBCC)	2	77.0	101	104	117	77.5	79.3	68.1
10 mg/kg (TBCC)	3	83.9	104	101	79.1	84.6	82.8	85.9
10 mg/kg (TBCC)	4	98.9	116	136	91.2	107	78.8	77.4
10 mg/kg (TBCC)	5	78.3	94.5	88.5	91.4	108	103	79.7
10 mg/kg (TBCC)	6	91.5	93.9	81.6	98.0	62.3	62.7	63.3
35 mg/kg (TBCC)	1	115	102	55.5	99.2	83.6	72.2	78.0
35 mg/kg (TBCC)	2	93.9	89.4	94.5	108	112	101	75.1
35 mg/kg (TBCC)	3	84.7	105	95.7	84.0	70.6	65.6	70.9
35 mg/kg (TBCC)	4	94.0	113	121	109	90.9	100	97.4
35 mg/kg (TBCC)	5	78.7	108	92.4	92.6	84.0	80.0	77.8
35 mg/kg (TBCC)	6	73.9	97.6	88.0	82.2	92.2	91.7	54.2
50 mg/kg (TBCC)	1	63.7	89.2	96.2	112	105	113	107
50 mg/kg (TBCC)	2	104	105	109	86.4	93.8	75.7	94.0
50 mg/kg (TBCC)	3	93.6	92.6	89.4	93.0	78.7	102	82.5
50 mg/kg (TBCC)	4	111	113	91.8	132	89.7	107	89.9
50 mg/kg (TBCC)	5	85.0	108	89.2	100	60.7	74.5	106
50 mg/kg (TBCC)	6	76.1	107	85.9	76.2	83.2	69.1	69.2

Table 65: Acetic acid concentration [mmol/l] in the rumen fluid of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
10 mg/kg ($CuSO_4$)	1	57.9	53.1	62.9	73.4	67.0	63.8	63.2
10 mg/kg ($CuSO_4$)	2	56.3	66.9	86.4	63.2	73.3	84.4	70.5
10 mg/kg ($CuSO_4$)	3	61.6	79.1	49.4	54.6	44.7	48.2	51.4
10 mg/kg ($CuSO_4$)	4	61.6	60.1	70.8	89.3	74.1	81.0	58.7
10 mg/kg ($CuSO_4$)	5	51.8	55.3	59.3	49.8	40.8	53.6	52.5
10 mg/kg ($CuSO_4$)	6	55.4	63.5	58.1	52.7	56.4	43.7	32.5
35 mg/kg ($CuSO_4$)	1	52.1	63.5	59.5	60.7	54.6	46.1	62.7
35 mg/kg ($CuSO_4$)	2	60.8	76.0	74.0	78.4	46.4	45.7	50.7
35 mg/kg ($CuSO_4$)	3	56.8	67.2	62.2	79.9	62.8	65.5	55.2
35 mg/kg ($CuSO_4$)	4	81.4	70.9	61.3	73.7	57.5	62.3	52.7
35 mg/kg ($CuSO_4$)	5	59.8	52.3	52.4	64.0	58.3	63.0	64.6
35 mg/kg ($CuSO_4$)	6	52.6	51.5	49.2	60.5	55.8	53.1	63.0
50 mg/kg ($CuSO_4$)	1	48.0	56.7	52.2	63.7	59.1	45.6	55.6
50 mg/kg ($CuSO_4$)	2	66.0	71.5	72.8	54.7	51.4	53.4	35.4
50 mg/kg ($CuSO_4$)	3	53.2	69.6	59.3	58.4	52.2	53.5	54.6
50 mg/kg ($CuSO_4$)	4	62.2	79.1	76.3	72.8	76.3	.	61.7
50 mg/kg ($CuSO_4$)	5	79.4	74.7	49.4	58.4	46.0	49.7	49.8
50 mg/kg ($CuSO_4$)	6	63.0	54.8	65.4	74.1	57.8	50.0	48.5
10 mg/kg (TBCC)	1	50.9	61.3	58.6	59.2	58.4	59.8	55.1
10 mg/kg (TBCC)	2	54.5	62.6	65.1	76.5	51.8	53.8	46.9
10 mg/kg (TBCC)	3	61.6	65.8	65.2	53.3	58.3	57.2	61.8
10 mg/kg (TBCC)	4	69.1	75.3	83.3	59.2	71.3	53.4	53.6
10 mg/kg (TBCC)	5	55.9	59.7	56.6	60.3	72.3	70.1	54.4
10 mg/kg (TBCC)	6	66.9	61.1	54.2	66.2	43.3	44.4	45.4
35 mg/kg (TBCC)	1	80.3	61.8	34.1	62.7	53.8	47.8	52.5
35 mg/kg (TBCC)	2	66.5	58.6	62.1	72.2	76.4	68.8	52.4
35 mg/kg (TBCC)	3	61.8	65.2	62.0	56.1	49.0	46.2	50.7
35 mg/kg (TBCC)	4	66.4	70.3	74.7	68.4	59.7	67.0	67.1
35 mg/kg (TBCC)	5	56.8	67.1	59.5	62.2	57.5	55.4	55.0
35 mg/kg (TBCC)	6	52.6	62.2	57.1	54.8	62.6	63.0	38.0
50 mg/kg (TBCC)	1	44.7	56.6	59.6	71.5	69.5	75.7	72.3
50 mg/kg (TBCC)	2	72.9	65.5	69.6	56.3	61.9	50.6	63.7
50 mg/kg (TBCC)	3	67.8	61.7	60.3	64.4	55.2	72.0	59.3
50 mg/kg (TBCC)	4	75.7	71.7	55.3	81.5	56.9	67.0	59.6
50 mg/kg (TBCC)	5	61.3	66.2	56.5	66.0	40.3	50.8	73.6
50 mg/kg (TBCC)	6	54.4	68.1	56.0	51.3	56.9	48.3	49.3

Table 66: Propionic acid concentration [mmol/l] in the rumen fluid of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
10 mg/kg ($CuSO_4$)	1	11.8	19.4	18.2	18.3	15.6	14.3	13.8
10 mg/kg ($CuSO_4$)	2	12.9	22.1	29.4	15.6	17.5	19.9	16.3
10 mg/kg ($CuSO_4$)	3	11.1	26.1	12.7	12.4	9.30	9.26	9.41
10 mg/kg ($CuSO_4$)	4	11.9	15.7	17.8	20.9	16.6	17.7	12.2
10 mg/kg ($CuSO_4$)	5	10.0	18.8	15.7	11.6	8.45	11.2	10.6
10 mg/kg ($CuSO_4$)	6	9.92	19.7	15.1	12.4	12.1	8.90	6.27
35 mg/kg ($CuSO_4$)	1	10.3	21.8	15.2	14.6	12.4	9.70	12.4
35 mg/kg ($CuSO_4$)	2	12.4	21.3	23.0	21.9	11.1	10.3	10.9
35 mg/kg ($CuSO_4$)	3	11.1	21.5	16.2	18.6	13.4	13.5	10.6
35 mg/kg ($CuSO_4$)	4	16.6	20.5	16.7	18.0	12.9	14.0	11.1
35 mg/kg ($CuSO_4$)	5	11.9	14.3	11.7	13.3	12.0	12.7	13.0
35 mg/kg ($CuSO_4$)	6	9.50	13.6	11.2	13.1	11.0	10.3	11.9
50 mg/kg ($CuSO_4$)	1	9.74	20.3	14.8	15.4	13.4	9.76	11.4
50 mg/kg ($CuSO_4$)	2	13.6	24.5	20.8	13.4	12.2	11.9	7.52
50 mg/kg ($CuSO_4$)	3	9.51	22.7	15.4	13.3	10.6	10.2	9.88
50 mg/kg ($CuSO_4$)	4	12.6	19.3	23.0	17.1	17.3	24.3	12.8
50 mg/kg ($CuSO_4$)	5	15.9	22.4	12.3	13.2	9.61	10.1	9.79
50 mg/kg ($CuSO_4$)	6	12.1	13.1	13.8	14.5	11.4	9.51	9.40
10 mg/kg (TBCC)	1	10.8	17.3	14.2	13.0	12.4	12.3	11.0
10 mg/kg (TBCC)	2	11.4	20.3	18.3	19.1	12.1	12.3	10.3
10 mg/kg (TBCC)	3	11.0	21.0	16.9	12.0	12.1	12.4	11.5
10 mg/kg (TBCC)	4	14.3	21.0	25.3	14.9	16.2	11.6	11.3
10 mg/kg (TBCC)	5	10.8	18.5	14.1	13.2	15.3	14.1	11.5
10 mg/kg (TBCC)	6	12.5	16.7	12.8	14.4	8.63	8.39	8.31
35 mg/kg (TBCC)	1	17.4	22.4	10.5	17.1	13.6	11.3	11.9
35 mg/kg (TBCC)	2	14.3	16.1	15.1	16.0	16.9	14.9	11.2
35 mg/kg (TBCC)	3	11.1	21.3	15.8	13.0	10.1	9.15	9.60
35 mg/kg (TBCC)	4	13.3	21.9	21.5	18.1	14.0	15.0	14.2
35 mg/kg (TBCC)	5	10.8	22.2	15.4	14.0	11.9	11.0	10.5
35 mg/kg (TBCC)	6	10.4	17.9	13.9	12.3	13.5	13.3	7.51
50 mg/kg (TBCC)	1	9.63	18.5	18.4	19.6	17.3	18.0	16.6
50 mg/kg (TBCC)	2	15.8	21.4	18.0	13.5	14.5	11.6	14.4
50 mg/kg (TBCC)	3	12.3	14.8	12.8	12.8	10.7	13.8	11.1
50 mg/kg (TBCC)	4	16.8	20.3	16.6	22.3	14.1	16.6	13.3
50 mg/kg (TBCC)	5	11.7	23.0	15.1	15.3	8.77	10.4	14.7
50 mg/kg (TBCC)	6	10.5	19.5	13.8	11.3	11.6	9.43	9.32

Table 67: Butyric acid concentration [mmol/l] in the rumen fluid of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
10 mg/kg ($CuSO_4$)	1	8.84	10.5	13.6	15.1	13.0	12.2	11.2
10 mg/kg ($CuSO_4$)	2	9.48	14.3	21.4	13.5	14.3	16.0	12.9
10 mg/kg ($CuSO_4$)	3	10.1	18.0	11.0	11.7	9.32	9.17	9.14
10 mg/kg ($CuSO_4$)	4	11.2	13.7	18.9	22.1	18.0	19.6	12.7
10 mg/kg ($CuSO_4$)	5	9.12	13.4	14.4	11.7	8.43	11.7	10.8
10 mg/kg ($CuSO_4$)	6	9.29	14.5	13.2	11.8	12.2	9.25	6.18
35 mg/kg ($CuSO_4$)	1	7.98	13.5	12.3	12.9	12.0	9.27	11.3
35 mg/kg ($CuSO_4$)	2	10.7	16.6	21.0	20.9	10.9	10.3	10.3
35 mg/kg ($CuSO_4$)	3	9.34	14.4	13.5	16.7	12.5	12.3	9.56
35 mg/kg ($CuSO_4$)	4	14.9	16.2	15.4	17.9	12.8	13.3	10.4
35 mg/kg ($CuSO_4$)	5	9.43	10.9	11.1	12.9	11.6	12.2	11.8
35 mg/kg ($CuSO_4$)	6	8.25	11.1	10.3	12.6	10.9	10.0	11.4
50 mg/kg ($CuSO_4$)	1	7.61	12.4	12.9	14.0	12.2	9.01	10.0
50 mg/kg ($CuSO_4$)	2	11.1	17.5	17.7	12.1	11.3	11.2	6.75
50 mg/kg ($CuSO_4$)	3	8.71	14.8	12.3	11.4	9.72	9.24	8.63
50 mg/kg ($CuSO_4$)	4	10.8	14.9	18.6	15.7	15.9	23.5	12.1
50 mg/kg ($CuSO_4$)	5	12.7	16.4	11.1	12.8	9.79	10.2	9.33
50 mg/kg ($CuSO_4$)	6	10.6	11.3	14.0	15.5	12.0	10.1	9.34
10 mg/kg (TBCC)	1	7.99	12.5	12.9	12.5	11.7	11.4	9.65
10 mg/kg (TBCC)	2	8.87	14.3	15.7	16.5	10.9	10.6	8.70
10 mg/kg (TBCC)	3	9.48	14.1	14.4	10.9	11.6	10.8	10.5
10 mg/kg (TBCC)	4	12.9	15.9	21.3	13.3	15.6	11.2	10.4
10 mg/kg (TBCC)	5	9.35	12.6	13.0	13.7	16.5	15.1	11.3
10 mg/kg (TBCC)	6	10.3	12.6	11.7	14.4	8.71	8.45	8.18
35 mg/kg (TBCC)	1	14.1	14.4	8.53	15.7	13.2	10.9	11.1
35 mg/kg (TBCC)	2	10.4	11.8	12.9	14.8	14.6	13.3	9.17
35 mg/kg (TBCC)	3	10.0	14.8	14.1	12.2	9.70	8.70	8.98
35 mg/kg (TBCC)	4	11.8	16.5	19.2	17.5	13.8	14.6	13.2
35 mg/kg (TBCC)	5	9.13	14.6	13.2	12.9	11.7	11.0	10.2
35 mg/kg (TBCC)	6	8.99	13.4	12.5	11.7	13.0	12.5	7.17
50 mg/kg (TBCC)	1	7.67	11.3	13.5	16.0	14.4	15.7	14.2
50 mg/kg (TBCC)	2	11.9	14.4	16.0	12.5	13.2	10.4	12.2
50 mg/kg (TBCC)	3	11.2	12.9	12.5	12.6	10.6	13.3	10.1
50 mg/kg (TBCC)	4	14.6	16.6	15.4	22.2	15.0	19.2	13.8
50 mg/kg (TBCC)	5	10.0	15.2	13.4	15.1	9.48	11.0	15.0
50 mg/kg (TBCC)	6	9.42	15.2	12.4	10.8	12.0	9.25	8.75

Table 68: Valeric acid concentration [mmol/l] in the rumen fluid of the different animals dependent on Cu treatment and sampling time

Cu treatment	Animal	Time after feeding						
		0 h	1.5 h	3 h	4.5 h	6 h	7.5 h	9 h
10 mg/kg ($CuSO_4$)	1	2.10	2.71	4.20	4.19	3.21	2.88	2.58
10 mg/kg ($CuSO_4$)	2	2.28	3.37	6.05	4.48	4.62	4.65	3.69
10 mg/kg ($CuSO_4$)	3	1.93	4.34	3.18	2.79	2.00	1.85	1.89
10 mg/kg ($CuSO_4$)	4	2.22	3.44	5.13	5.55	3.88	3.99	2.39
10 mg/kg ($CuSO_4$)	5	1.89	3.19	4.10	2.76	1.78	2.51	2.23
10 mg/kg ($CuSO_4$)	6	1.49	3.61	3.46	2.50	2.23	1.56	1.00
35 mg/kg ($CuSO_4$)	1	1.96	3.09	3.70	3.96	3.26	2.33	2.50
35 mg/kg ($CuSO_4$)	2	2.44	3.67	5.16	5.79	2.97	2.53	2.23
35 mg/kg ($CuSO_4$)	3	1.89	3.61	4.50	4.68	2.99	2.56	1.96
35 mg/kg ($CuSO_4$)	4	3.27	4.16	4.66	4.93	3.20	3.22	2.46
35 mg/kg ($CuSO_4$)	5	2.22	3.33	3.72	3.76	3.06	3.09	2.85
35 mg/kg ($CuSO_4$)	6	1.90	3.22	2.89	3.10	2.67	2.33	2.59
50 mg/kg ($CuSO_4$)	1	1.79	3.01	3.75	4.04	3.20	2.25	2.54
50 mg/kg ($CuSO_4$)	2	2.36	3.66	5.02	3.15	2.86	2.39	1.37
50 mg/kg ($CuSO_4$)	3	1.56	3.52	3.78	2.98	2.10	1.85	1.61
50 mg/kg ($CuSO_4$)	4	2.44	4.28	5.83	5.44	4.90	6.81	3.06
50 mg/kg ($CuSO_4$)	5	2.76	4.66	3.34	3.07	2.05	2.04	1.90
50 mg/kg ($CuSO_4$)	6	1.88	3.03	3.73	3.69	2.59	2.08	1.78
10 mg/kg (TBCC)	1	2.15	3.32	4.22	3.75	3.10	2.81	2.08
10 mg/kg (TBCC)	2	2.18	3.44	4.53	4.42	2.64	2.56	2.14
10 mg/kg (TBCC)	3	1.83	3.40	4.46	2.90	2.63	2.39	2.07
10 mg/kg (TBCC)	4	2.61	3.50	6.22	3.71	3.90	2.58	2.14
10 mg/kg (TBCC)	5	2.25	3.70	4.72	4.18	4.26	3.49	2.53
10 mg/kg (TBCC)	6	1.85	3.61	2.86	3.02	1.60	1.44	1.44
35 mg/kg (TBCC)	1	2.80	3.13	2.45	3.80	2.84	2.15	2.49
35 mg/kg (TBCC)	2	2.73	2.84	4.38	4.63	4.38	3.69	2.37
35 mg/kg (TBCC)	3	1.94	3.28	3.78	2.75	1.87	1.60	1.63
35 mg/kg (TBCC)	4	2.39	4.16	5.54	4.87	3.54	3.50	2.92
35 mg/kg (TBCC)	5	2.01	4.16	4.36	3.47	2.98	2.62	2.17
35 mg/kg (TBCC)	6	1.83	4.19	4.51	3.33	3.21	2.89	1.53
50 mg/kg (TBCC)	1	1.70	2.84	4.69	5.06	3.92	3.86	3.53
50 mg/kg (TBCC)	2	3.37	3.98	5.57	4.16	4.13	3.07	3.65
50 mg/kg (TBCC)	3	2.38	3.20	3.75	3.10	2.28	2.69	2.01
50 mg/kg (TBCC)	4	3.52	4.07	4.44	6.17	3.76	4.24	3.24
50 mg/kg (TBCC)	5	1.99	3.41	4.15	3.91	2.13	2.23	2.88
50 mg/kg (TBCC)	6	1.88	4.60	3.69	2.81	2.76	2.03	1.81

Table 69: Apparent total tract nutrient digestibility [%] of the different animals dependent on Cu treatment

Cu treatment	Animal	Ingredient								
		DM	OM	CF	CP	TL	NFE	CA	NDF	ADF
10 mg/kg ($CuSO_4$)	1	79.4	81.5	77.4	70.6	65.5	84.4	47.5	77.8	77.0
10 mg/kg ($CuSO_4$)	2	75.4	77.7	73.5	67.8	52.4	80.3	43.6	71.7	70.1
10 mg/kg ($CuSO_4$)	3	80.2	82.0	78.1	75.7	74.0	83.6	50.9	75.7	73.5
10 mg/kg ($CuSO_4$)	4	79.6	81.7	79.1	74.1	70.4	83.4	45.1	76.7	78.6
10 mg/kg ($CuSO_4$)	5	81.7	83.7	82.9	79.1	77.7	84.4	48.5	80.2	81.6
10 mg/kg ($CuSO_4$)	6	76.8	79.4	80.4	67.1	60.9	80.6	38.4	72.3	75.3
35 mg/kg ($CuSO_4$)	1	79.9	78.6	77.1	69.0	63.6	80.3	46.6	74.0	71.0
35 mg/kg ($CuSO_4$)	2	74.0	81.1	77.1	70.9	64.0	83.8	44.7	76.2	74.0
35 mg/kg ($CuSO_4$)	3	78.9	79.3	77.1	69.8	63.8	81.1	46.0	74.1	72.6
35 mg/kg ($CuSO_4$)	4	77.0	83.1	81.9	74.4	74.8	84.4	47.0	78.0	76.7
35 mg/kg ($CuSO_4$)	5	81.0	81.0	79.1	71.9	61.2	82.9	39.7	75.0	76.7
35 mg/kg ($CuSO_4$)	6	78.7	82.1	82.9	75.4	64.1	82.7	42.0	79.7	78.1
50 mg/kg ($CuSO_4$)	1	81.0	82.8	83.6	76.6	70.3	83.2	47.2	80.3	80.4
50 mg/kg ($CuSO_4$)	2	79.0	77.7	75.8	67.5	62.4	79.5	44.6	71.0	70.4
50 mg/kg ($CuSO_4$)	3	78.6	80.3	76.2	71.5	63.0	82.7	46.3	75.3	73.5
50 mg/kg ($CuSO_4$)	4	80.8	78.8	76.9	69.4	60.6	80.4	42.3	74.2	70.2
50 mg/kg ($CuSO_4$)	5	78.2	83.0	83.9	74.3	72.1	83.7	47.8	78.4	76.1
50 mg/kg ($CuSO_4$)	6	76.3	81.3	80.5	73.0	67.2	82.6	41.2	76.3	77.0
10 mg/kg (TBCC)	1	78.9	80.1	77.4	71.4	59.9	82.2	38.9	71.6	72.9
10 mg/kg (TBCC)	2	75.8	81.0	80.0	75.7	72.1	81.9	44.9	76.6	77.0
10 mg/kg (TBCC)	3	79.7	78.5	77.0	71.1	65.6	79.7	52.5	71.4	72.7
10 mg/kg (TBCC)	4	77.3	81.2	77.8	71.4	69.6	83.5	44.2	76.8	70.8
10 mg/kg (TBCC)	5	77.8	78.2	75.5	68.6	59.2	80.2	43.6	72.4	72.0
10 mg/kg (TBCC)	6	79.0	81.8	81.2	72.8	67.2	83.0	44.9	76.4	77.1
35 mg/kg (TBCC)	1	78.3	81.6	80.5	72.1	71.9	83.0	48.1	75.4	75.1
35 mg/kg (TBCC)	2	80.2	80.6	77.8	72.4	63.1	82.5	41.2	73.0	74.6
35 mg/kg (TBCC)	3	79.8	81.8	81.2	78.6	68.6	82.0	53.1	77.7	78.0
35 mg/kg (TBCC)	4	76.3	77.8	76.5	68.1	63.7	79.4	48.7	70.9	68.0
35 mg/kg (TBCC)	5	78.3	82.0	79.7	72.0	70.4	84.0	45.9	78.2	72.9
35 mg/kg (TBCC)	6	79.7	78.8	77.8	69.1	56.2	80.2	43.7	73.6	71.7
50 mg/kg (TBCC)	1	75.7	78.0	73.8	66.9	66.1	80.7	44.1	72.6	70.3
50 mg/kg (TBCC)	2	79.3	81.6	80.0	72.4	75.2	83.0	42.3	76.9	75.8
50 mg/kg (TBCC)	3	78.5	80.4	75.9	74.1	66.0	82.5	47.1	73.2	74.5
50 mg/kg (TBCC)	4	80.3	82.1	82.9	76.9	78.2	82.1	49.2	78.5	77.9
50 mg/kg (TBCC)	5	81.5	82.1	82.8	72.8	68.6	83.0	49.8	76.5	78.7
50 mg/kg (TBCC)	6	74.9	83.6	82.0	73.6	68.0	85.6	49.6	80.2	75.9