

Aus dem Institut für Tierernährung und Stoffwechselphysiologie  
der Christian-Albrechts-Universität zu Kiel

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**Postruminal digestion of abomasally infused corn starch with or without  
exogenous amylase administration in cattle**

**Dissertation**

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**ABBREVIATIONS**

<b>ADF</b>	Acid detergent fiber expressed inclusive of residual ash
<b>ADF<sub>org</sub></b>	Acid detergent fiber expressed exclusive of residual ash
<b>ADL</b>	Acid detergent lignin
<b>ATP</b>	Adenosine triphosphate
<b>ATTD<sub>DM</sub></b>	Apparent total tract disappearance of dry matter
<b>ATTD<sub>N</sub></b>	Apparent total tract disappearance of nitrogen
<b>ATTD<sub>OM</sub></b>	Apparent total tract disappearance of organic matter
<b>ATTD<sub>Starch</sub></b>	Apparent total tract disappearance of starch
<b>BW</b>	Body weight
<b>CA</b>	Crude ash
<b>CC</b>	Cracked corn
<b>CP</b>	Crude protein
<b>CSH</b>	Corn starch hydrolysate
<b>DE</b>	Digestible energy
<b>DGC</b>	Dry-ground corn
<b>DM</b>	Dry matter
<b>DMI</b>	Dry matter intake
<b>DRC</b>	Dry-rolled corn
<b>GC</b>	Ground corn
<b>GE</b>	Gross energy

<b>HMGC</b>	High-moisture ground corn
<b>HMRC</b>	High-moisture rolled corn
<b>HPLC</b>	High-pressure liquid chromatography
<b>LSM</b>	Least square mean
<b>MCP</b>	Microbial crude protein
<b>ME</b>	Metabolizable energy
<b>N</b>	Nitrogen
<b>NaCl</b>	Sodium chloride
<b>NCS</b>	Native corn starch
<b>NDF</b>	Neutral detergent fiber inclusive of residual ash
<b>NDF<sub>org</sub></b>	Neutral detergent fiber exclusive of residual ash
<b>OM</b>	Organic matter
<b>PB</b>	Purine bases
<b>PDV</b>	Portal-drained viscera
<b>RIA</b>	Radioimmunoassay
<b>SEM</b>	Standard error of mean
<b>SFC</b>	Steam-flaked corn
<b>SGLT1</b>	Sodium-dependent glucose transporter 1
<b>TiO<sub>2</sub></b>	Titanium dioxide
<b>U</b>	Units
<b>VFA</b>	Volatile fatty acids



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# 1 General Introduction

## 1.1 STARCH DIGESTION IN CATTLE

Feeding diets containing high proportion of cereal grain is a common way to meet the enhanced energy requirements of lactating dairy cows and high producing beef cattle, although their digestive tract is primarily adapted to the digestion of roughages. The main component of cereal grain is starch which contributes up to 80% of dry matter (DM) depending on grain type (Giuberti et al., 2014). Thus, the extent and efficiency of starch digestion is of great interest in research of ruminant nutrition. The digestibility of grain starch in the total digestive tract is usually quite high with values ranging between 90% and 100% of intake (Huntington, 1997). However, the extent and site of starch digestion depend on several factors like grain type, grain processing, harvest maturity, moisture content and particle size (Ferraretto et al., 2013).

The first site of starch digestion is the rumen, where starch is fermented under anaerobic conditions by ruminal microbes yielding a protein-rich microbial matter and volatile fatty acids (VFA), mainly acetate, butyrate and propionate. The microbial matter is digested in the small intestine, contributing to the animals' protein supply, while the VFA are absorbed across the rumen epithelium and enter the portal blood system. As the major precursor substance of hepatic gluconeogenesis, propionate contributes to the animals' glucose and energy supply or to lactose synthesis in lactating dairy cows (Veenhuizen et al., 1988). In contrast, acetate and butyrate are precursor substances for the synthesis of milk fat and are efficiently utilized by fattening animals (Russell et al., 1992). Feeding high amounts of starch or other rapidly fermented carbohydrates is associated with an accumulation of VFA and low ruminal pH, promoting ruminal acidosis and increasing the risk of production related metabolic disorders (Owens et al., 1998).

The rate and extent of ruminal starch fermentation depend on several factors including grain type, grain processing, diet composition, level of feed intake and the degree of adaptation of

ruminal microflora as reviewed by Kotarski et al. (1992). Starch, as a macromolecule stored within a granular structure, consists of two fractions: amylose and amylopectin, with the former being more easily fermentable than the latter (Huntington et al., 2006). The ratio of these fractions varies between species and varieties of cereal grain leading to different rates and extents of ruminal fermentation (Giuberti et al., 2014). For example, 50% of starch ingested from dry-rolled sorghum escape ruminal fermentation while starch of dry-rolled wheat is fermented in the rumen to an extent of 88% (Huntington, 1997).

The extent of ruminal starch fermentation can be further altered by mechanical or chemical alteration of cereal grains prior to feeding. In grain kernels, most of the starch is present in the peripheral and corneous endosperm, where the starch granules are embedded in protein matrices, which are a physiochemical impediment to starch digestion in ruminants (Giuberti et al., 2014). Furthermore, whole grain kernels are resistant to bacterial attachment, resulting in a poor fermentability (Huntington, 1997). Therefore, cereal grains are typically processed prior to feeding, for example by rolling or cracking. While starch of whole corn grains escapes ruminal fermentation in amounts up to 41%, only 31% of cracked corn starch and only 22% of ground corn starch are digested postruminally (Owens et al., 1986). In a similar manner, treating cereal grains with sodium hydroxide, formaldehyde or anhydrous ammonia (Mandell et al., 1988; Robinson and Kennelly, 1989; Fluharty and Loerch, 1989; Schmidt et al., 2006) as well as steeping cereal grains in organic acids (Iqbal et al., 2009) is supposed to increase starch flow to the small intestine. A literature overview of the extent and site of starch digestion in beef steers and dairy cows fed differently processed corn starch varying in particle size, moisture content or steaming time is presented in Table 1.

**Table 1.** Extent and site of starch digestion in beef steers and dairy cows fed differently processed corn starch

Reference	Animals	Treatment	Starch flow (kg/d)					Starch disappearance						
			Intake	Duodenum	Ileum	Feces	Rumen	Small intestine	Large intestine	Total tract				
							% <sup>1</sup>	kg/d	% <sup>1</sup>	kg/d	% <sup>1</sup>	kg/d	%	
a)	Steers	GC	1.00	0.36	0.03	0.01	64	0.64	93	0.33	54	0.01	99	0.99
			1.95	0.54	0.08	0.02	73	1.42	85	0.46	78	0.06	99	1.93
			2.44	0.78	0.17	0.04	67	1.59	78	0.61	76	0.13	98	2.39
b)	Steers	GC	2.68	0.98	0.36	0.06	63	1.65	64	0.62	83	0.30	98	2.63
			1.70	0.45	0.23	0.09	78	1.25	49	0.22	51	0.14	94	1.60
			3.22	0.94	0.50	0.23	77	2.30	45	0.41	57	0.27	93	2.98
c)	Steers	CC	2.70	0.91	0.29	0.89	66	1.79	68	0.61	nd	nd	93	2.51
		CC/Amylase	2.70	0.97	0.30	0.19	64	1.73	69	0.67	nd	nd	93	2.51
		CC/Bicarbonate	2.65	1.00	0.22	0.19	61	1.65	74	0.78	nd	nd	93	2.47
d)	Steers	DRC	2.71	0.86	0.40	0.24	68	1.85	54	0.47	nd	nd	91	2.47
		SFC <sub>34min</sub>	2.71	0.41	0.03	0.02	85	2.30	94	0.38	nd	nd	99	2.69
		SFC <sub>47min</sub>	2.71	0.57	0.02	0.02	79	2.14	96	0.54	nd	nd	99	2.69
e)	Dairy cows	SFC <sub>67min</sub>	2.71	0.38	0.02	0.01	86	2.33	96	0.37	nd	nd	100	2.70
		DGC	7.93	3.11	2.70	0.88	61	4.82	9	0.41	67	1.82	89	7.06
		DRC	7.94	2.48	2.86	1.99	69	5.46	-	-	30	0.87	76	6.03
f)	Dairy cows	HMGC	8.80	1.14	0.47	0.15	87	7.66	59	0.67	68	0.32	98	8.65
		HMRC	8.30	1.58	0.57	0.38	81	6.72	57	1.00	35	0.20	96	7.92
		GC <sub>0.7mm</sub>	4.33	1.80	0.54	0.39	59	2.53	67	1.26	31	0.20	91	3.94
		GC <sub>1.8mm</sub>	4.33	2.17	0.81	0.61	50	2.16	61	1.36	19	0.17	86	3.72
		GC <sub>3.7mm</sub>	4.27	2.75	1.44	1.32	35	1.52	47	1.30	9	0.14	69	2.95

a: Karr et al. (1966); b: Russell et al. (1981); c: Remillard et al. (1990); d: Zinn (1990); e: Knowlton et al. (1998b); f: Rémond et al. (2004).

GC = ground corn; CC = cracked corn; DRC = dry-rolled corn; SFC = steam-flaked corn; SFC = dry-ground corn; DGC = dry-ground corn; HMGC = high-moisture ground corn;

HMRC = high-moisture rolled corn.

<sup>1</sup>Expressed as percentage of starch flow to respective segment.

On average, between 5 and 20% of dietary starch escapes ruminal fermentation (Huntington, 1997). In the small intestine starch molecules are exposed to enzymatic hydrolysis. The  $\alpha$ -1,4-glycosidic bonds of starch molecules are broken down by pancreatic amylase resulting in di- and trisaccharides of glucose and limit dextrins (Mills et al., 1999). Glucose is liberated by intestinal disaccharidases (e.g. maltase and isomaltase) and absorbed across the brush border membrane of the small intestine (Harmon, 1992). In comparison to ruminal fermentation of starch, which is associated to energy losses due to methane production and heat of fermentation, starch hydrolysis and absorption of glucose in the small intestine is assumed to be energetically more efficient (Owens et al., 1986; Huntington, 1997). Therefore, shifting starch digestion from the rumen to the small intestine is assumed to increase the energy supply to lactating dairy cows and high producing beef cattle. This consideration led to a growing interest to investigate the capacity for starch digestion in the small intestine of ruminants.

Values for the disappearance of corn starch in the small intestine of dairy cows and beef steers (expressed as percentage of intestinal starch flow) range between 9% for dry-ground corn (Knowlton et al., 1998b) and 100% for abomasally infused starch hydrolysate (Branco et al., 1999). Experiments with duodenal infusions of corn and wheat starch demonstrated that starches with lower rate and extent of ruminal fermentation are also digested in a lesser extent in the small intestine (Matthé, 2001). Also, small intestinal disappearance of starch from dry-rolled corn was demonstrated to be clearly lower than of steam-flaked corn (54 vs. 94%) by Zinn (1990). Furthermore, experiments with an increasing flow of corn starch to the small intestine indicated a decreasing efficiency of postruminal starch digestion with increasing intestinal supplies (Karr et al., 1966; Russell et al., 1981a; Kreikemeier et al., 1991; Matthé, 2001). The amount of starch disappearing from the small intestine increased with increasing flow to this segment, but starch disappearance as percentage of the intestinal flow decreased. Possible limiting factors discussed in literature include the capacity for glucose absorption, the time for starch hydrolysis, the access of enzymes to starch granules as well as the activity of



hydrolyzing enzymes as dependent on a sufficient secretion, adequate working conditions (pH and temperature) and the absence of enzymes inhibitors (Owens et al., 1986; Huntington, 1997).

An incomplete digestion of starch in the small intestine is inevitable accompanied by an enhanced fermentation of starch in the hindgut. While the arising VFA from hindgut fermentation can be absorbed, the resulting microbial matter is excreted with the feces, which might override any gains in energetic efficiency of postruminal starch digestion. Therefore, an extensive starch digestion in the small intestine needs to be ensured in order to improve the energy supply to ruminants by shifting starch digestion from the rumen to the small intestine.

Different attempts to improve starch digestion in the small intestine of ruminants can be found in literature. For example, an increased duodenal protein supply by abomasal casein infusion was demonstrated to increase starch disappearance from the small intestine (Richards et al., 2002). It can be speculated that this benefit resulted from an increased secretion of pancreatic amylase due to increased duodenal protein supply as demonstrated by Swanson et al. (2004). In line with this, supplementing hydrolyzing enzymes is also suggested to increase starch digestion in the small intestine. The effect of a dietary supplementation of amylase has already been evaluated in several experiments with lactating dairy cows (Wenning, 2008; Gencoglu et al., 2010; Ferraretto et al., 2011; Weiss et al., 2011; McCarthy et al., 2013; Noziere et al., 2014; Vargas-Rodriguez et al., 2014). Amylase supplementation was demonstrated to increase the efficiency of feed conversion to milk (Gencoglu et al., 2010; Ferraretto et al., 2011) as well as ruminal starch digestibility in lactating dairy cows (Noziere et al., 2014), but starch digestibility in the total tract was not affected in these experiments. However, amylase was added to total mixed rations, making it difficult to differentiate the site and extent of the enzyme effect in these experiments.

Remillard et al. (1990) infused an exogenous amylase into the jejunum of beef steers and reported no effect on postruminal digestion of corn starch at an intestinal flow of 1 kg/d. Yet,

animals were adapted to starch digestion and endogenous amylase secretion might have been sufficient for postruminal digestion of starch at an intestinal flow of this amount. Published experiments investigating the effect of an abomasally infused amylase in animals not adapted to starch digestion or at intestinal starch flows greater than 1 kg/d were not found.

### 1.2 OBJECTIVES AND HYPOTHESES

The aim of this thesis is to evaluate the effect of an abomasally infused amylase on postruminal digestion of corn starch in heifers. The experiments reported herein are targeted to determine whether pancreatic amylase limits starch digestion in the small intestine of heifers adapted or not adapted to starch digestion. Furthermore, the maximum capacity for postruminal starch digestion in heifers should be assessed by abomasal infusion of increasing amounts of corn starch. Differences in the extent of starch disappearance from the small intestine are expected to be reflected by differences in the flow of starch to the hindgut, and therefore the extent of starch fermentation in the hindgut and fecal starch excretion. Correspondingly, differences in the extent of starch fermentation in the hindgut are expected to be reflected by differences in the fecal excretion of microbial crude protein (MCP) and VFA.

The aim of our first experiment is to investigate the effect of an abomasally infused amylase on postruminal digestion of moderate amounts of corn starch (~ 1.0 kg/d) in heifers, which were not adapted to starch digestion. Furthermore, the effect of postruminal starch digestion on glucose, insulin and glucagon concentrations in peripheral blood is examined. It is hypothesized that starch digestion in the small intestine is limited by an insufficient activity of pancreatic amylase and might therefore be improved by exogenous administration. An increased excretion of VFA and MCP with feces due to an enhanced fermentation of starch in the hindgut is expected, when the amount of starch exceeds the capacity for starch hydrolysis or glucose absorption in the small intestine. Furthermore, a higher excretion of VFA and MCP with feces

is expected in animals not infused with amylase, if starch digestion in the small intestine is limited by pancreatic amylase.

In a second experiment we investigate the effect of an abomasally infused amylase on postruminal starch digestion in heifers when the intestinal starch flow exceeds the amount of 1.0 kg/d by a stepwise increase up to 2.0 kg/d. It is hypothesized that starch disappearance from the small intestine increases, but the efficiency of starch digestion decreases with increasing flow of starch to the small intestine as observed in earlier studies. Furthermore, the maximum capacity for postruminal starch digestion in animals adapted to starch digestion should be determined. It is hypothesized that heifers receiving an exogenous amylase might be able to digest greater quantities of starch in the small intestine.

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## Manuscript I

### **Postruminal digestion of starch infused into the abomasum of heifers with or without exogenous amylase administration<sup>1</sup>**

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## 2.1 ABSTRACT

The effect of an exogenous amylase on postruminal digestion of starch infused into the abomasum of cattle was studied. Four rumen-cannulated heifers were fed 5.5 kg DM/d of a diet without starch, and assigned randomly to a crossover design. The experiment consisted of 2 periods lasting 23 d each with 10 d for adaptation to the diet followed by 13 d of abomasal infusion and sample collection. During the first 3 d of each infusion phase, isotonic saline solution was infused (1 liter/h) for measurement of baseline values in feces, followed by daily infusions of 880 g DM corn starch (1 kg/10 liters of water) without or with the addition of 2% of amylase. Titanium dioxide (10 g/d) was ruminally administered for estimation of fecal excretion. Digestion of starch in small intestine was calculated as the difference between the amounts of infused starch, disappeared from hindgut and fecal excretion. The apparent disappearance of starch from the hindgut was estimated based on the increment of microbial nitrogen (N) excretion due to starch infusion (1 g microbial N/100 g fermented starch) compared to baseline values. The concentration of purine bases in feces was used to estimate excretion of microbial N. Microbial N excretion increased with starch infusion ( $P < 0.05$ ) but was not influenced by amylase ( $P = 0.81$ ). Starch disappearance from the small intestine was not improved by amylase ( $P = 0.78$ ) and averaged 85%. Amylase affected neither blood concentration of glucose ( $P = 0.80$ ) nor of insulin ( $P = 0.26$ ), but glucagon was lower without ( $P < 0.0001$ ) than with amylase. The infusion of starch increased fecal excretion of total VFA (acetate, propionate, and butyrate) by 53% ( $P < 0.05$ ), which indicates increased carbohydrate fermentation in the hindgut and incomplete digestion of starch in the small intestine. However, the excretion of total VFA was not affected by amylase ( $P = 0.66$ ). Lactate excretion was higher at the second day of starch infusion ( $P < 0.05$ ) without than with amylase, which suggests lower flow of starch from the small intestine to the hindgut due to a possible effect of amylase addition in animals not adapted to starch digestion. However, lactate excretion returned near to baseline values within 2 d, which was probably due to increase of lactate-utilizing bacteria and the

adaptation of the microbial population in the hindgut. Further studies with higher starch levels and addition of amylase are recommended.

**Key words:** amylase, cattle, hindgut fermentation, lactate, starch, volatile fatty acid

## 2.2 INTRODUCTION

Starch usually provides an important proportion of energy in diets for lactating cows and high producing beef cattle. However, on average only 11% to 90% of the postruminal starch flow disappear in the small intestine (Owens et al., 1986; Giuberti et al., 2014; Moharrery et al., 2014). Studies with diets containing high amount of grain (Karr et al., 1966) or with abomasal infusion of corn starch (Little et al., 1968; Kreikemeier et al., 1991; Matthé, 2001; Richards et al., 2002) have shown that digestion of starch in the small intestine is limited. Some aspects influencing the digestibility of starch in the small intestine are intrinsic feed factors like the structural formation (Giuberti et al., 2014) and the grain type. Feed processing methods (Owens et al., 1986), particle size, and interactions with other non-starch components affect the extent of starch digestion as well (Giuberti et al., 2014). Also reduced capacity of glucose absorption, the time for starch hydrolysis, the access of enzymes to starch granules or time and surface exposure (Owens et al., 1986), and limited supply and activity of pancreatic amylase (Huntington, 1997) have been stated as possible factors limiting starch digestion. Thus, supply of exogenous amylase may be an option to improve starch digestibility in the small intestine. In line with this, the effect of amylase addition on starch digestion in diets of dairy cows has been evaluated (Gencoglu et al., 2010; Weiss et al., 2011; McCarthy et al., 2013; Nozière et al., 2014; Vargas-Rodriguez et al., 2014). However, amylase was added to the concentrate making it difficult to differentiate the site and extent of the effect of amylase. Therefore, the present study aimed to evaluate the effect of an exogenous amylase on postruminal digestion of starch infused into the abomasum of cattle not adapted to starch digestion. We hypothesized that small intestinal digestion of starch in unadapted cattle is limited by amylase activity, which might be

improved by exogenous administration of amylase, and that this effect might be especially pronounced during adaptation to the starch digestion.

## 2.3 MATERIALS AND METHODS

The animal study reported herein was performed in accordance with the animal German Animal Welfare Act (Federal Republic of Germany, 2014) and approved by the Animal Welfare Commission of the Ministry of Energy, Agriculture, Environment and Rural Areas of the federal state of Schleswig-Holstein (V 244-7224.121-25).

### 2.3.1 *Animals and Diets*

Four adult heifers (2 German Black Pied and 2 Jersey × German Black Pied), with an average BW of  $565 \pm 29$  kg at the beginning of the experiment and fitted with rumen cannulas (#2C, 10 cm i.d.; Bar Diamond, Inc., Parma, ID) were used. The heifers were fed 5.5 kg/d of a diet targeted to contain no starch and consisted of 64.9% grass hay (primary growth of *Lolium perenne*-dominated sward), 33% dried beet pulp (J. August Plambeck GmbH, Brügge, Germany), 0.9% feed urea (Piarumin; Stickstoffwerke Piesteritz GmbH, Wittenberg, Germany) and 1.2% of a mineral and vitamin premix (Panto-Mineral; Hamburger Leistungsfutter GmbH, Hamburg, Germany) on DM basis in 2 equal meals at 0700 and 1500 h. The ingredients and chemical composition of the diet is shown in Table 2. The diet was formulated to meet requirements for maintenance according to the GfE (2001). Animals had free access to drinking water. The heifers were housed in a tie stall with individual cubicles and bedded with rubber mats.

### 2.3.2 *Experimental Design and Procedure*

The experiment consisted of 2 periods lasting 23 d each with a 10-d phase for adaptation to the diet containing no starch followed by a 13-d phase of abomasal infusion and sample collection. Between periods, a 7-wk period without treatment application was set to avoid carryover effects of adaptation to starch digestion. The animals were assigned randomly to a crossover design.

Firstly, from day 1 to 3 of each infusion period isotonic saline solution (0.9% NaCl wt/vol; B. Braun Melsungen AG, Melsungen, Germany) was infused into the abomasum to obtain baseline values of fecal and blood variables.

**Table 2.** Ingredients and chemical composition of the diet

Item	% of dietary DM
Ingredient	
Grass hay	64.9
Dried beet pulp	33.0
Feed urea	0.90
Mineral and vitamin mix <sup>1</sup>	1.20
Chemical composition <sup>2</sup>	
Crude ash	6.60
Crude protein	9.28
Ether extract	0.45
NDF	55.7
ADF	32.6
ADL	4.50

<sup>1</sup>Contained (per kilogram) 210 g calcium, 100 g sodium, 40 g magnesium, 30 g phosphorus, 1,000 mg copper, 8,000 mg zinc, 5,000 mg manganese, 60 mg iodine, 50 mg selenium, 40 mg cobalt, 5,000 mg vitamin E, 1,000,000 IU vitamin A and 100,000 IU vitamin D3.

<sup>2</sup>Calculated from chemical composition of individual ingredients.

Treatments were daily abomasal infusion of native corn starch containing 95.7% starch, 0.4% of CP, and 0.2% of crude ash (CA) on DM basis (Maisita 21.000; Agrana AG, Vienna, Austria) with or without an exogenous amylase. The starch product was mixed at a rate of 1 kg starch per 10 liters of water and maintained in suspension by continuous stirring. Abomasal infusion began half an hour after morning feeding and lasted for 10 h/d from day 4 to 13 of each infusion period. The average effective total dosage of starch was 880 g/d. A peristaltic pump (rotarus Flow 50; Hirschmann Laborgeräte GmbH & Co KG, Eberstadt, Germany) was used for infusion. The  $\alpha$ -amylase (EC.3.2.1.1; 13,000 U/mL, Distizym BA-N, 1,4- $\alpha$ -D-Glucan-Glucanohydrolase; Erbslöh Geisenheim AG, Geisenheim, Germany) solution (2% vol/vol in distilled water) was infused into the tube used for starch infusion at a rate of 50 mL/h using a syringe pump (540060-HP Single Syringe; TSE Systems GmbH, Bad Homburg, Germany; syringe used: Omnifyx Solo; B. Braun Melsungen AG, Melsungen, Germany). According to

manufacturer, the amylase was produced from a selected strain of *Bacillus subtilis*, with an optimum activity at pH 5.8 to 6.0 and at 70 to 80 °C. The amylase activity of Distizym BA-N was determined using the bicinchoninic assay where Distizym BA-N is added to a 1% soluble corn starch solution (wt/vol) at 37 °C for 10 min at pH 7.5. The reducing sugars were determined at a wavelength of 560 nm and expressed as units (U) using a maltose standard, where 1 U of  $\alpha$ -amylase activity is defined as 1  $\mu$ mol maltose formed per minute. According to the manufacturer, 1,560 U of Distizym BA-N/kg of grain starch is the recommended standard dosage in alcohol production for the liquefaction of starch-containing mashes. In our experiments we used a clearly higher dosage (147,727 U/kg starch) to compensate for any possible impairment of enzyme activity by low pH in the abomasum. The abomasal infusion was performed using a tool to insert lines into the abomasum described by Westreicher-Kristen and Susenbeth (2017). Succinctly, an insertion device with the infusion line was passed through the rumen cannula, then through the sulcus omasi, and finally through the omaso-abomasal orifice into the abomasum. The insertion device and the infusion lines remained in the abomasums for the entire phase of abomasal infusion of 13 d each and 24 h a day. Their position in the abomasums was controlled twice daily according to Westreicher-Kristen and Susenbeth (2017) to ensure that the suspension containing the starch and the amylase solution was always completely infused into the abomasum. To infuse the solutions into abomasum, a thin Tygon tubing (2.06 mm i.d.; Hirschmann, Eberstadt, Germany) was passed through the whole infusion line from the peristaltic pump into the abomasum with the insertion device. Total fecal excretion was determined using titanium dioxide ( $\text{TiO}_2$ ; Kronos 1171; Harold Scholz & Co. GmbH, Recklinghausen, Germany). For this, 5 g  $\text{TiO}_2$  were weighed into gelatine capsules (HKG size 30  $\times$  90 mm, 59 mL, limpid-transparent, type 36; Capsula GmbH, Ratingen, Germany) and ruminally administered to each heifer twice daily immediately after each feeding. The  $\text{TiO}_2$  administration was initiated 5 d before the phase of abomasal infusion to reach the equilibrium of  $\text{TiO}_2$  excretion after initial administration (Glindemann et al., 2009). For blood sampling,

heifers were surgically fitted with an indwelling long-term catheter (Long Term Catheter Guidewire Style A1410, 14 ga × 20 cm; MILA International Inc., Florence, KY) in 1 jugular vein at the second day of each phase of abomasal infusion. For this, heifers were sedated with 0.7 mL Rompun 2% (Xylazine; Bayer AG, Leverkusen, Germany) and surgical intervention was carried out under aseptic conditions and local anesthesia with 4 mL of Isocain 2% (Procainhydrochlorid; Selectavet Dr. Otto Fischer GmbH, Weyarn-Holzolling, Germany). The catheters were held in place by suturing them onto the skin. To protect the catheters from blockage with coagulated blood, they were permanently infused with isotonic saline solution (0.9% NaCl wt/vol; B. Braun Melsungen AG, Melsungen, Germany) at a rate of approximately 1 mL/min during the whole phases of blood sampling.

### 2.3.3 *Sampling and Sample Preparation*

During each sampling phase, 5 samples (~200 g fresh matter each) of the grass hay and dried beet pulp were collected and pooled by feed, divided into 2 subsamples and ground to pass a 2-mm and a 1-mm sieve (ZM1; Retsch GmbH, Haan, Germany) for chemical analysis. Fecal grab samples (~500 g fresh matter) were collected during the 13-d sampling phase from the rectum (exception day 4 because of transition time between the phases of NaCl and starch infusion) 4 times a day at 0900, 1200, 1500, and 1800 h.

The applied sampling pattern was chosen to compensate for possible diurnal variation in the concentration and excretions of TiO<sub>2</sub> via feces (Glindemann et al., 2009). After each collection time, fecal samples were cooled down for ~15 min at -20 °C to quickly minimize microbial activity. Afterwards, samples were placed in a cooling room at 4 °C and composited per animal at the end of each sampling day. From this, 6 subsamples (4 × 100 g and 2 × 250 g) were obtained and stored at -20 °C for analyses. Blood samples were collected at day 3 (baseline), 4, 6, and 8 of each sampling phase. The first daily blood sample was taken directly after beginning of the infusion at 0730 h (time point zero), followed by blood sampling after 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, and 10 h. For each sampling, the first 2 mL of blood were discarded.

Afterwards, blood samples (~9 mL) were drawn into collection tubes containing potassium EDTA as anticoagulant to obtain plasma (Monovette K3 EDTA, 92 × 16 mm; Sarstedt AG & Co., Nümbrecht, Germany) and placed on ice immediately for ~10 min. Additionally, blood samples (2 × ~9 mL) were drawn into collection tubes containing clotting activators to obtain serum (Serum Monovette, 92 × 16 mm; Sarstedt AG & Co., Nümbrecht, Germany) and maintained at room temperature for 60 min. Plasma and serum samples were obtained by centrifugation (1,041 × g, at 20 °C for 10 min) and samples were stored at –20 °C until analysis.

#### 2.3.4 Analytical Procedures

*Diets.* Feed samples (1-mm particle size) were analyzed in duplicate for concentrations of moisture, CA, CP, and ether extract according to the official analytical methods in Germany (VDLUFA, 2007; methods 3.1, 8.1, 4.1.1, and 5.1.1, respectively). Feed samples (2-mm particle size) were analyzed in triplicate for NDF, ADF, both expressed on ash-free basis, and ADL as described by Van Soest et al. (1991) using an ANKOM 200 Fiber Analyzer (ANKOM Technology, Macedon, NY). Heat-stable  $\alpha$ -amylase was added during NDF extraction. Native corn starch used for abomasal infusion was analyzed for starch concentration based on polarimetric procedure (method 7.2.1; VDLUFA, 2007).

*Feces.* At the end of the experiment, feces samples were defrosted at room temperature overnight and homogenized for the following described analyses. Samples were analyzed in duplicate for moisture, CA, and N according to VDLUFA (2007), and for concentration of TiO<sub>2</sub> as described by Brandt and Allam (1987). In brief, Kjeldahl digestion solution obtained by heating fecal sample for 6 h in 96% (vol/vol) sulfuric acid was filtered (MN 640 w, black ribbon No. 41, 150 mm i.d.; Macherey-Nagel GmbH & Co. KG, Düren, Germany) and added with 35% (vol/vol) hydrogen peroxide. The TiO<sub>2</sub> concentration was measured based on light absorbency in a spectrophotometer (Jenway 6300; Bibby Scientific Ltd, Stone, UK) at a wavelength of 405 nm. Concentration of starch was determined in lyophilized samples (0.2-mm particle size) based on glucose liberated after incubation with thermostable  $\alpha$ -amylase



(Thermamyl; Novozymes, Bagsvaerd, Denmark) at 95 °C for 45 min and amyloglycosidase (Roche Diagnostics GmbH, Mannheim, Germany) at 60 °C for 30 min as described by Brandt et al. (1987). Glucose concentration was measured based on light absorbency after incubation with glucose oxidase (GOD FS; DiaSys Diagnostic Systems GmbH, Holzheim, Germany) at 37 °C for 10 min in a spectrophotometer (Jenway 6300; Bibby Scientific Ltd, Stone, UK) at a wavelength of 500 nm. Concentration of VFA (acetate, propionate, and butyrate) was analyzed in a gas chromatograph (ATI Unicam 610; Unicam Chromatography GmbH & Co. KG, Kassel, Germany) equipped with a flame ionization detector. Separation of VFA was performed at 103°C for 25 min along a 2 mm column (GP 10%, SP-1000, 1% phosphoric acid, 100/120 Chromosorb WAW; Supelco, Inc., Bellefonte, PA). The concentration of lactate was determined using a Lactate-UV Test Kit (D-Lactic acid/L-Lactic acid-UV Test; Boehringer Ingelheim Pharma GmbH & Co. KG, Mannheim, Germany). The concentration of purine bases (PB) was determined in lyophilized samples in duplicate by HPLC (Merck-Hitachi HPLC; Merck KGaA, Darmstadt, Germany) using a reversed-phase C18 Hypersil Gold column (250 × 4 mm; Thermo Fisher Scientific GmbH, Dreieich, Germany) after sample hydrolysis in perchloric acid as described in detail by Dickhoefer et al. (2016).

*Blood.* Serum was analyzed for glucose concentration using the glucose hexokinase method (Glucose Hexokinase Fluid 5 + 1; mti-diagnostics GmbH, Idstein, Germany) and for insulin concentration by immunoradiometric assay (Insulin IRMA KIT; Immunotech, Inc., Beckman Coulter, CA). Glucagon was analyzed in plasma by radioimmunoassay (Glucagon RIA, MI13021; IBL International GmbH, Hamburg, Germany).

#### 2.3.5 Calculations and Statistical Analysis

Fecal DM excretion was calculated as TiO<sub>2</sub> administration (g/d) divided by the TiO<sub>2</sub> concentration (g/kg DM) in the feces, assuming a fecal recovery of TiO<sub>2</sub> of 100% (Glindemann et al., 2009). Fecal excretion of OM, total N, microbial N, starch, VFA, and lactate was

calculated based on their concentrations in feces (g/kg DM) and fecal DM excretion. The OM concentration in feces was calculated as  $1000 - CA$  (g/kg DM). The apparent disappearance of starch from the small intestine was calculated as the difference between amounts of infused starch and disappeared from the hindgut and excreted with the feces. The apparent disappearance of starch by fermentation from the hindgut was estimated based on the increment of microbial N excretion due to starch infusion compared to baseline values. The concentration of microbial N in feces was calculated based on PB excretion using a PB-N:microbial N ratio of 0.116 according to Chen (1989). The baseline values of microbial N excretion were obtained from the 3-d phase of NaCl infusion. In line with this, it was assumed that 1 g microbial N originates from 100 g of fermented starch according to Ørskov et al. (1970). They measured the microbial N synthesis when starch was infused through cannula into the caecum of sheep. The apparent disappearance of starch from total intestine tract was calculated as the difference between infusion and excretion amount of starch. For baseline, apparent total tract digestibility of DM ( $ATTD_{DM}$ ) and OM ( $ATTD_{OM}$ ) was calculated as the quotient of the total amount that apparently disappeared (intake – fecal excretion) and the amount of feed intake. For the starch infusion phase, the amount of starch infused was additionally considered as intake.

All data were statistically analyzed by the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). Differences between baseline and starch infusion in fecal excretion of DM, OM, total and microbial N, VFA, and lactate were calculated prior to statistical analysis by subtracting the baseline values (average of 3-d NaCl infusion) from values with starch infusion. The LSM and SEM of the differences between baseline and starch infusion were calculated and compared using the LSMEANS procedure with repeated measurements. Period, d, treatment, and d × treatment interactions were included as fixed effects and animal as a random factor. An autoregressive covariance structure for fecal variables was chosen because of equally spaced sampling times. Fecal excretion of total and microbial N, lactate, and propionate on a day-to-day basis were analyzed using the same model. Differences between baseline and starch

infusion in blood variables were calculated prior to statistical analysis by subtracting the baseline values (day 3 with NaCl infusion) from values with starch infusion for each time point. The LSM and SEM of hourly concentrations of blood variables and of the differences between baseline and starch infusion were calculated and compared using the LSMEANS procedure with repeated measurements. Period, d, treatment, time, and d × treatment and time × treatment interactions were included as fixed effects and animal as a random factor. An unstructured covariance structure for blood was chosen because of unequally spaced sampling times. Treatment effects were considered significantly different at  $P < 0.05$  and tendencies with  $P$ -values between 0.05 and 0.10.

## 2.4 RESULTS

### 2.4.1 Fecal Excretion and ATTD

The effects of starch infusion with or without amylase on fecal excretion of DM, OM, N, lactate, and VFA as well as ATTD are presented in Table 3. Baseline values of total and microbial N excretion averaged 34.6 and 8.8 g/d, respectively, and were increased with starch infusion ( $P < 0.05$ ). The increments of total and microbial N with starch infusion compared to baseline were not influenced by amylase administration ( $P = 0.46$  and  $0.81$ , respectively) and averaged 7.1 and 1.1 g/d, respectively. Changes of total and microbial N excretion on a day-to-day basis are shown in Fig. 1A and B, respectively, and were not affected by amylase administration ( $P = 0.53$  and  $0.49$ , respectively). Total VFA, acetate, and butyrate excretions increased with starch infusion ( $P < 0.05$ , for all; Table 3), whereas propionate excretion was unaffected ( $P = 0.65$ ). Molar proportion of butyrate increased and of acetate and propionate decreased through starch infusion ( $P < 0.05$ ), but they were unaffected by amylase treatment ( $P = 0.73$ ,  $0.86$ ,  $0.74$ , respectively). On a day-to-day basis, propionate excretion was higher at the second day of starch infusion with than without amylase ( $P < 0.05$ ; Fig. 2A). Afterwards, propionate excretion decreased to similar values of propionate excretion as without amylase. Baseline values of

lactate excretion averaged 0.02 g/d and increased numerically when starch was infused to 7.9 g/d (average) but without any influence without ( $P = 0.13$ ) or with amylase ( $P = 0.30$ ). Lactate excretion was not affected by amylase treatment ( $P = 0.72$ ; Table 3). On a day-to-day basis, lactate excretion was higher at the second day of starch infusion without than with amylase (72.8 vs. 43.3 g/d, respectively;  $P < 0.05$ ) (Fig. 2B). Furthermore, lactate excretion was drastically reduced without differences between treatments to 11.4 and 0.62 g/d in the third and fourth day of starch infusion, respectively.

**Table 3.** Fecal excretion of DM, N, lactate and VFA in heifers infused abomasally with NaCl (basal) or 880 g/d corn starch with or without amylase administration

	Basal <sup>1</sup>	Starch <sup>2</sup>	Basal <sup>1</sup>	Starch + Amylase <sup>2</sup>	SEM <sup>3</sup>	Effect of Amylase
		$\Delta$		$\Delta$		$P$
DM, kg/d	1.56	+ 0.16*	1.54	+ 0.20*	0.02	0.24
OM, kg/d	1.33	+ 0.15*	1.33	+ 0.18*	0.02	0.26
Total N, g/d	34.6	+ 7.38*	34.5	+ 6.80*	0.97	0.46
Microbial N <sup>4</sup> , g/d	8.81	+ 1.08*	8.91	+ 1.17*	0.43	0.81
Lactate, g/d	0.02	+ 9.39	0.02	+ 6.35	6.02	0.72
Total VFA <sup>5</sup> , g/d	17.9	+ 11.1*	20.0	+ 9.11*	3.22	0.66
Acetate, g/d	13.5	+ 6.69*	15.0	+ 6.37*	2.43	0.93
Propionate, g/d	2.81	- 0.25	3.09	- 0.63	0.53	0.60
Butyrate, g/d	1.56	+ 4.98*	1.82	+ 3.73*	1.28	0.37
VFA, % of total VFA						
Acetate	74.9	- 4.42*	75.3	- 4.20*	1.98	0.86
Propionate	16.1	- 7.38*	15.6	- 7.13*	0.96	0.74
Butyrate	8.99	+ 11.8*	9.08	+ 11.3*	2.27	0.73
ATTD <sub>DM</sub> , %	71.4	+ 1.36*	71.8	+ 0.79*	0.33	0.24
ATTD <sub>OM</sub> , %	73.8	+ 1.27*	73.8	+ 0.82*	0.43	0.25

\*Significant difference between baseline and starch infusion within each treatment ( $P < 0.05$ ).

<sup>1</sup>Data presented for baseline represent LSM of 3 d with NaCl infusion within each treatment ( $n = 4$ ).

<sup>2</sup>Values represent LSM of the difference between baseline and starch infusion without or with amylase administration ( $n = 4$ ).

<sup>3</sup>SEM of the LSM of  $\Delta$  (difference between baseline and starch infusion).

<sup>4</sup>Calculated as 0.116 g purine bases N/g microbial N according to Chen (1989).

<sup>5</sup>Sum of acetate, propionate and butyrate.

Lactate excretion further decreased numerically from 0.34 to 0.09 g/d from day 5 to 10 of starch infusion, however did not numerically return to the baseline values (0.02 g/d). The baseline values of ATTD<sub>DM</sub> and ATTD<sub>OM</sub> averaged 71.6% and 73.8%, respectively. These values

increased with starch infusion ( $P < 0.05$ ), but the increments were not affected by amylase supplementation ( $P = 0.24$  and  $0.25$ , respectively; Table 3).

#### 2.4.2 *Apparent Disappearance of Starch from Intestinal Tract*

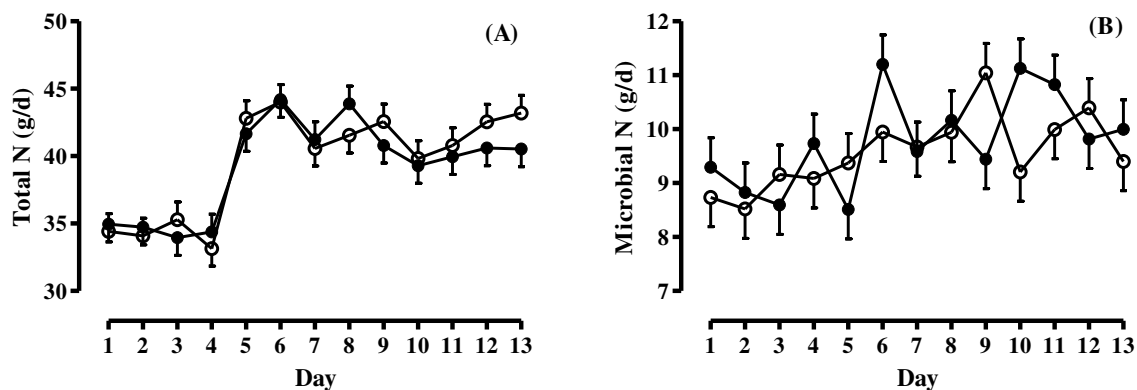
Excretion and apparent disappearance of infused starch with or without amylase are shown in Table 4. Fecal excretion of starch was very small and averaged 4.4 and 5.6 g/d with and without amylase, respectively; though the difference was not significant ( $P = 0.64$ ). Consequently, the apparent disappearance of starch from the total intestinal tract averaged 99%. The starch disappearance from the small intestine averaged 85% and was not improved by the addition of amylase ( $P = 0.78$ ). From the starch reaching the hindgut, 96% apparently disappeared.

#### 2.4.3 *Blood Glucose, Insulin and Glucagon Concentration*

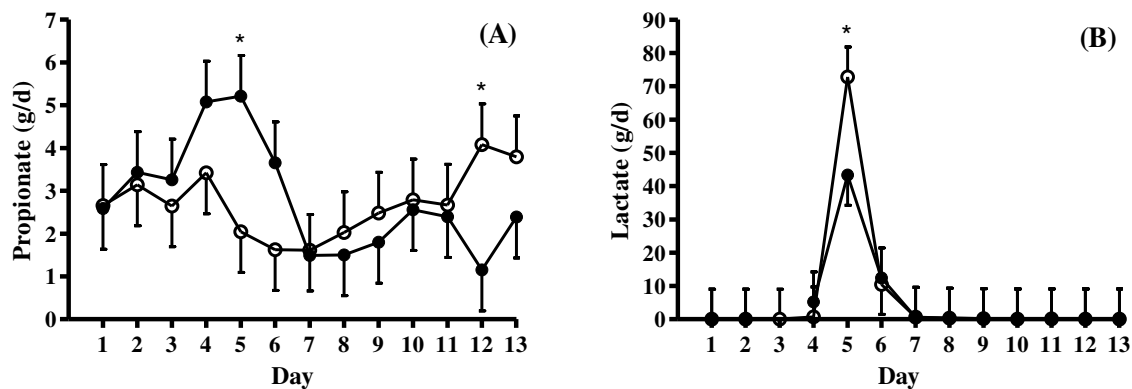
Blood glucose, insulin, and glucagon concentrations in heifers infused abomasally with starch without and with amylase treatment are shown in Table 5. Compared to baseline, glucose concentration was unaffected by starch infusion ( $P = 0.27$ ), whereas insulin concentration increased ( $P < 0.05$ ). Additional administration of amylase had no effect on glucose ( $P = 0.80$ ) and insulin ( $P = 0.26$ ) concentrations. Glucagon concentration did not differ from baseline values when starch plus amylase were infused ( $P = 0.45$ ), but decreased with starch infusion without amylase administration ( $P < 0.05$ ). Consequently, amylase administration affected blood glucagon concentration ( $P < 0.0001$ ). Diurnal variations of blood glucose, insulin, and glucagon concentrations during abomasal starch infusion are illustrated in Fig. 3. During the first phase of starch infusion (time points 0 to 1.5 h) concentrations of glucose and insulin increased. Thereafter (time points 1.5 to 6 h) they remained relatively constant (plateau phase). Concentrations of glucagon remained higher (time points 1.5 to 6 h) when starch was infused with than without amylase ( $P < 0.05$ ; Fig. 3C).

## 2.5 DISCUSSION

During the present trial, neither feed intake nor heifers' behavior was influenced by the presence of the infusion device in the abomasum. For the present study, we intended to infuse starch at a level that would not develop severe diarrhea based on results reported by Kreikemeier et al. (1991).



**Figure 1.** Daily fecal excretion of total N (A) and microbial N (B) of heifers abomasally infused with 0.9% NaCl (1-3 d) or 880 g/d corn starch (4-13 d) without (○) or with (●) amylase administration. Values are LSM  $\pm$  SEM ( $n = 4$ ). Graph A: day  $P < 0.0001$ , treatment  $P = 0.53$ , day  $\times$  treatment interactions  $P = 0.79$ . Graph B: day  $P = 0.01$ , treatment  $P = 0.49$ , day  $\times$  treatment interactions  $P = 0.01$ .



**Figure 2.** Daily fecal excretion of propionate (A) and lactate (B) of heifers abomasally infused with 0.9% NaCl (1-3 d) or 880 g/d corn starch (4-13 d) without (○) or with (●) amylase administration. Values are LSM  $\pm$  SEM ( $n = 4$ ). \*Indicate significant differences between treatments ( $P < 0.05$ ); otherwise  $P \geq 0.05$ . Graph A: day  $P = 0.27$ , treatment  $P = 0.87$ , day  $\times$  treatment interactions  $P = 0.31$ . Graph B: day  $P < 0.0001$ , treatment  $P = 0.64$ , day  $\times$  treatment interactions  $P = 0.93$ .

In our study, feces had a soft consistence only during the first 2 d after beginning of starch infusion. During the afternoon of the first day of starch infusion (~8 h after initiation of infusion), animals started to excrete feces with a softer consistence and lactic acid smell. Excreted feces during the second and third day of starch infusion were even softer and with a more intensive acidic smell. However, these effects were reduced during the following days. In general, cases of diarrhea were not observed and animals remained healthy during the infusion periods. It has to be noted that the results of the present study are valid only for the amount and rate of infusion of 880 g starch/d. However, similar infusion rates and even higher amounts of starch into the abomasum are reported in the literature, which indirectly supports the present approach (Oldick et al., 1997; Knowlton et al., 1998; Reynolds et al., 2001; Gressley and Armentano, 2007).

### *2.5.1 Excretion of Total and Microbial N*

It is well known that fecal N excretion is associated with DM intake (6 g/kg; Van Soest, 1994). Using this figure, total N would equal 32.7 g/d for baseline, which is quite similar to our measured value (34.5 g/d of total N). As expected, N excretion was increased with starch infusion when compared to baseline values. Simultaneously, excretion of microbial N was increased, which indicates an enhanced fermentation of carbohydrates and in turn a higher microbial mass in the hindgut. Fecal microbial N was estimated from PB excretion using a PB-N:microbial N ratio of 0.116. This ratio was determined by Chen (1989) in mixed (liquid- and solid-associated) ruminal microbes. This could be a matter of concern because this ratio could be different in ruminal and fecal microbes. Moreover, previous studies demonstrated that the PB-N:N ratio of ruminal microbes markedly vary depending on the microbial composition with values ranging from 0.03 in liquid-associated to 0.14 in solid-associated microbes (Dickhoefer et al., 2016).

**Table 4.** Fecal excretion and apparent disappearance of starch from different segments of the intestinal tract of heifers infused abomasally with 880 g/d corn starch without or with amylase treatment

	Treatment <sup>1</sup>		SEM	<i>P</i>
	Starch	Starch + Amylase		
Fecal excretion, g/d	5.63	4.38	1.86	0.64
Apparent disappearance, g/d				
Small intestine	752	742	35.6	0.78
Hindgut <sup>2</sup>	122	133	34.7	0.75
Total tract	874	875	1.86	0.65
Apparent disappearance, %				
Small intestine <sup>3</sup>	85.5	84.4	4.05	0.78
Hindgut <sup>3</sup>	97.2	95.4	1.38	0.33
Total tract	99.4	99.5	0.21	0.64

<sup>1</sup>Data presented are LSM of phase of starch infusion without or with amylase treatment ( $n = 4$ ).

Previous to starch and starch + amylase treatments, a 3-d phase of NaCl infusion was applied for baseline measurements.

<sup>2</sup>Calculated as 1 g microbial N/100 g starch fermented (Ørskov et al., 1970). Microbial N calculated based on purine bases excretion using a purine bases-N: microbial N ratio of 0.116 according to Chen (1989).

<sup>3</sup>Based on the amount reaching each respective segment of the intestine.

However, as far as we know, values of PB-N:N ratio for fecal microbial matter of ruminants are missing. It must be considered that the factor used may have led to an over- or underestimation of microbial N in baseline values, but the latter probably affected all estimations at the same degree, therefore not affecting differences between treatments. In the present study around 16% of the additional N excretion due to starch infusion was of microbial origin. The CP content of the infused starch was very low (~0.4% in DM) and its contribution to CP appearing in the hindgut can be neglected. Consequently, 84% of the increased N excretion by starch infusion can be considered as endogenous N. This represents around 6.8 g endogenous N/kg infused corn starch, which is far higher than values for fecal endogenous losses of 2.2 and 1.9 g N/kg DM intake given by GfE (2001) and NRC (2001). The main reason for this difference is that the latter values are related to intake and the greatest proportion of the feed does not reach the abomasum (e.g., fermentation and absorption in the rumen). In line with this and assuming a disappearance of feed DM by twothird in the rumen, both values are rather well in agreement.



### 2.5.2 Excretion of VFA and Lactate

The VFA, which are not absorbed and metabolized by colonic epithelial cells or transferred into the blood, are excreted via feces. Therefore, the increased fecal excretion of total VFA when starch was infused into the abomasum indicates enhanced carbohydrate fermentation in the hindgut. In this study, total VFA excretion increased by 53% with starch infusion. Similarly, Siciliano-Jones and Murphy (1989) reported an increase of 80% of total VFA concentrations in the cecum of steers when diets were changed from high forage to high grain proportions. Moreover, changes of the molar proportions of VFA in feces may provide information about which carbohydrate source was primarily fermented. The baseline values of the molar proportion of acetate, propionate, and butyrate averaged 75%, 16%, and 9%, respectively. With starch infusions, they were changed to 71%, 9%, and 20%, respectively. Similarly, Ørskov et al. (1970) found reduced proportion of acetate in both colon and cecum when starch was infused into the terminal ileum of sheep, and a 3-fold incremented proportion of butyrate in the colon, cecum, and feces. Consequently, the increased proportion of butyrate in feces indicates starch fermentation in the hindgut. The latter is supported by the observation in our study that lactate excretion increased drastically at the second day of starch infusion. According to Zhang and Cheryan (1991), starch is an ideal substrate for production of lactic acid by fermentation.

**Table 5.** Concentration of glucose and insulin in serum, and glucagon in plasma of heifers infused abomasally with 880 g/d corn starch without or with amylase treatment

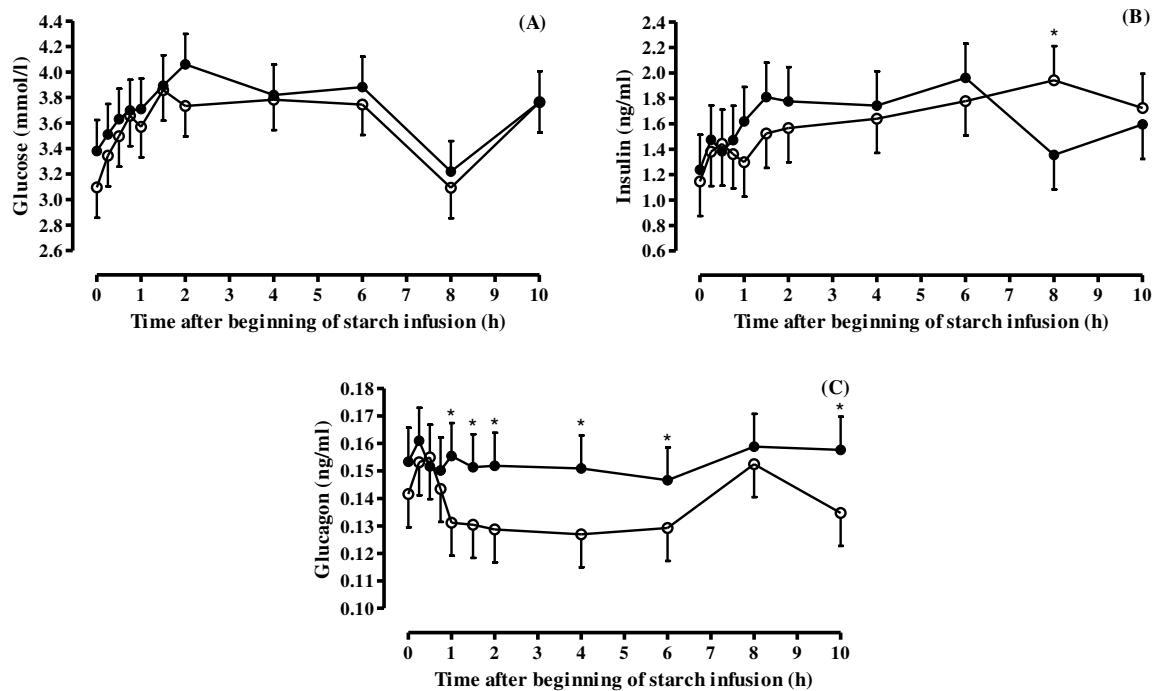
	Basal <sup>1</sup>	Starch <sup>2</sup>	Basal <sup>1</sup>	Starch + Amylase <sup>2</sup>	SEM <sup>3</sup>	Effect of Amylase <i>P</i>
		$\Delta$		$\Delta$		
Glucose, mmol/L	4.02	- 0.24	4.19	- 0.28	0.22	0.80
Insulin, ng/mL	1.16	+ 0.46*	1.22	+ 0.60*	0.14	0.26
Glucagon, ng/mL	152	- 19.0*	143	+ 6.94	9.10	<.0001

\*Significant difference between baseline and starch infusion within each treatment ( $P < 0.05$ ).

<sup>1</sup>Data presented for baseline represent LSM obtained at d 3 of NaCl infusion from 1.5 to 6 h after beginning of infusion within each treatment ( $n = 4$ ).

<sup>2</sup>Values represent LSM of the difference between baseline and starch infusion with or without amylase treatment from 1.5 to 6 h after beginning of infusion ( $n = 4$ ).

<sup>3</sup>SEM of the LSM of  $\Delta$  (difference between baseline and starch infusion).



**Figure 3.** Hourly concentrations of glucose (A) and insulin (B) in serum, and glucagon in plasma (C) of heifers ( $n = 4$ ) infused abomasally with 880 g/d corn starch without (○) or with (●) amylase treatment. Hourly concentrations represent LSM  $\pm$  SEM based on blood samples collected at d 4, 6 and 8 from starch infusion phase (without baseline phase with 0.9% NaCl infusion, d 1 to 3) \*Indicate significant differences between treatments ( $P < 0.05$ ); otherwise  $P \geq 0.05$ . Graph A: day  $P < 0.0001$ , time  $P < 0.0001$ , treatment  $P = 0.05$ , time  $\times$  treatment interactions  $P = 0.98$ , day  $\times$  treatment interactions  $P = 0.50$ . Graph B: day  $P < 0.0001$ , time  $P < 0.001$ , treatment  $P = 0.55$ , time  $\times$  treatment interactions  $P = 0.03$ , day  $\times$  treatment interactions  $P = 0.80$ . Graph C: day  $P = 0.57$ , time  $P < 0.001$ , treatment  $P < 0.0001$ , time  $\times$  treatment interactions  $P = 0.09$ , day  $\times$  treatment interactions  $P = 0.09$ .

Furthermore, the gradual reduction of lactate excretion suggests an adaptation of the microbiota in the hindgut. Earlier studies have shown that the main fermentation product of lactate-utilizing bacteria, isolated from human feces, is butyrate (Duncan et al., 2004). Accordingly, the reduction of lactate excretion in the present study was accompanied by an increase of butyrate excretion (data not shown). Moreover, it is important to remark that the increase of lactate excretion at the second day of starch infusion was lower with than without amylase, probably due to lower availability of substrate for starch-fermenting bacteria, which support the hypothesis that amylase improved the digestion of starch in the small intestine in the non-adapted stage. However, the fact that lactate excretion during the whole phase of starch infusion never reached numerically the baseline values suggests that starch was never completely

digested in the small intestine and that animal adaptation was not fully reached. It can be alternatively hypothesized that high starch flow to the hindgut occurred during many days, but the adaptation of microbiota in the hindgut – especially of lactate-utilizing bacteria – masked possible effects of amylase addition on starch digestion in the intestine by utilizing lactate in advanced stages of the starch infusion phase. Therefore, changes in composition of microbial community in the hindgut may have arisen without changes in total microbial N mass, and consequently, expected effects based on microbial N excretion could not have been observed. Another interesting observation is the results of DM content of feces. The baseline value of DM concentration of feces averaged 16.4%, remained unchanged with amylase administration with 16.3% at the second day of starch infusion, but tended to be lower without amylase administration (14.8%,  $P = 0.07$ ; data not shown). Watery feces might indicate hindgut acidosis (Gressley et al., 2011) without amylase administration. An increment of propionate is normally expected by fermentation of soluble carbohydrates (e.g., starch). Because reduction of lactate excretion indicated that starch digestion was improved with amylase, a reduction of propionate excretion with amylase administration could be expected as well. Nevertheless, propionate excretion was higher with than without amylase administration during the second day of starch infusion. The latter suggests that glucose from hydrolyzed disaccharides and trisaccharides in the small intestine was probably not absorbed due to limitation of glucose absorption. Consequently, these highly soluble carbohydrates flew to the hindgut and were fermented to propionate. Finally, it is important to remark that results of propionate and lactate excretion and DM content of feces at the second day of starch infusion match with each other, which suggests that amylase addition improves the digestion of starch in the small intestine of not adapted animals.

### 2.5.3 *Apparent Disappearance of Starch*

In the present study around 85% of infused starch apparently disappeared from the small intestine, which is within the range of 11% to 90% for cattle (Owens et al., 1986; Giuberti et

al., 2014; Moharrery et al., 2014). From total starch that flows into the hindgut, 8 to 95% can be fermented by microbes (Moharrery et al., 2014). In the present study, the apparent disappearance of starch from the hindgut was high (average 96%). This was probably due to starch source used and the slow passage rate through the hindgut, which is characteristic in animals with low feed intake or at maintenance level as in our study. Moreover, the amount of starch that reached the hindgut was low (average 128 g/d), which can be related to the starch source used in the trial. Ørskov et al. (1970) reported in a study with sheep that starch infused into the cecum only in amounts higher than 138 g/d largely appeared in the feces. Thus, they showed that the hindgut has a substantial capacity for starch fermentation. In accordance, the apparent disappearance of starch from the total tract in our trial was nearly complete (>99%) and agrees with results with abomasal infusion of purified corn starch (Reynolds et al., 2001) and partially hydrolyzed starch (Knowlton et al., 1998). In the present study, the increase of microbial N excretion compared to the baseline was used as a proxy to calculate the amount of starch entering the hindgut. However, since microbial N was estimated from PB excretion, the calculated quantity of starch depends on the accuracy of the assumed values for the microbial yield per g starch fermented and the proportion of PB-N to total microbial N. As mentioned before, excretions of the different VFA indicate that besides starch probably mainly soluble sugars were fermented in the hindgut. The Cornell Net Carbohydrate and Protein System assumes that microbes that ferment soluble sugars produce around 18% more microbial CP than starch-fermenting microbes (Khezri et al., 2009). In line with this, an assumption of higher efficiency of microbial CP synthesis in the hindgut would result in a smaller amount of carbohydrates reaching the hindgut.

#### *2.5.4 Blood Glucose, Insulin, and Glucagon Concentration*

In the present study peripheral blood glucose concentration was not affected by starch infusion or amylase administration, which is in agreement with results of other findings (Larsen et al., 1956; Huber et al., 1961; Little et al., 1968; Huntington and Reynolds, 1986; Knowlton et al.,

1998). This might be explained by an enhanced uptake of glucose in peripheral tissues or inhibition of liver gluconeogenesis due to increasing pancreatic insulin secretion. The observed increase in serum insulin due to starch infusion is in agreement with observations from Knowlton et al. (1998). In vitro studies with hepatocytes from cows indicated that hepatic gluconeogenesis from propionate is insulin-independent (Donkin and Armentano, 1995). Another explanation for the missing effect of infused starch on blood glucose concentration could be an enhanced utilization of glucose by gut tissues. Wahle et al. (1971) demonstrated that appreciable amounts of glucose are consumed by small intestinal tissue of sheep resulting in lactate as one of the end products of this glycolytic process. Kreikemeier et al. (1991) demonstrated that only 38% of starch disappearing from the small intestine could be accounted as net glucose absorption when starch was infused into the abomasum of Holstein steers. Similar observations were made by Huntington and Reynolds (1986). However, changes in insulin and glucagon concentrations with starch infusion indicated that glucose was absorbed in rates sufficient to affect the secretion of these hormones. On the other hand, concentrations of insulin and glucagon might have changed independently of blood glucose concentration. For example, an elevated insulin secretion was observed in earlier experiments with intravenous infusions of butyrate and propionate in sheep (Manns and Boda, 1967; Horino et al., 1968). Therefore, insulin secretion might have increased due to higher concentrations of these substances, which result from starch fermentation in the hindgut as discussed earlier. However, Huntington and Reynolds (1986) did not detect an effect of abomasal starch infusion on plasma concentration of propionate and butyrate. This might be due to the fact that the liver removes propionate and butyrate at rates nearly equal to their net absorption so that blood concentrations are normally quite low (Reynolds, 1995). In our study, blood glucose concentration seemed to be mainly influenced by feeding and not by starch infusion as glucose concentration decreased within the last 2 h before the second feeding and increased afterwards although infusion was constant. The low concentration of glucose 8 h after beginning of infusion might be due to a

reduced availability of propionate due to fasting, which would lead to a reduced gluconeogenesis. Finally, although the differences in glucagon concentrations found in the present study are difficult to explain, they might indicate some unknown effects of amylase administration.

#### *2.5.5 Limiting Factors of Starch Disappearance from Small Intestine*

A review of possible limitations of starch disappearance from small intestine in ruminants has been published by Owens et al. (1986): the time for starch hydrolysis, the access of enzymes to starch granules, the capacity for glucose absorption, and the activity of hydrolyzing enzymes involved. In the small intestine of ruminants, pancreatic amylase hydrolyzes amylose and amylopectin resulting in limit dextrins and oligosaccharides of 2 or 3 glucose units (Huntington, 1997). Disaccharidases located at the brush border membrane (e.g., maltase and isomaltase) are required for complete hydrolysis to glucose (Harmon, 1992). Karr et al. (1966) suggested the pancreatic amylase secretion to be the limiting factor of starch digestion in the small intestine, whereas Kreikemeier and Harmon (1995) stated a limitation due to the glucosidase activity at the brush border membrane. Huntington (1997) suggested that starch disappearance in the small intestine of not adapted ruminants is probably limited by the capacity for absorption of end products from starch digestion rather than by starch hydrolysis itself, whereas starch hydrolysis in adapted ruminants seems to be limited by amylase activity. However, our results (i.e., higher lactate excretion without amylase at the second day of starch infusion) demonstrated that the activity of amylase is also a limiting factor in cattle not adapted to starch digestion. Contrary, Remillard et al. (1990) found no effects of abomasal-infused amylase on intestinal digestibility of starch and suggested that amylase was not the first limiting factor in digestion of starch. However, animals were adapted to starch digestion through a preceding period of feeding. Missing effects of amylase administration in later days of starch infusion might be explained by reduced secretion of endogenous amylase due to amylase infusion. Swanson et al. (2002) observed a decreased secretion of pancreatic amylase when starch hydrolysate was infused into

the abomasum of beef steers at rates of 20 to 40 g/h. Therefore, an indirect effect of amylase infusion on amylase secretion due to increased amounts of hydrolyzed starch in the lumen of the small intestine might exist. On the other hand, missing effects of amylase administration in later days of starch infusion might be explained by an adaptation due to an increased secretion of endogenous amylase. An adaptation of amylase activity in the small intestine was demonstrated in earlier studies as dietary components changed from forage to grain (Russell et al., 1981; Kreikemeier et al., 1990). In line with this, adaptation is also based on glucose uptake by induction of the sodium-dependent glucose cotransporter (SGLT1) in the small intestine as reviewed by Shirazi-Beechey (1995). In contrast, adaptation due to an increased secretion of brush border glucosidases seems to be limited (Harmon, 1992). Therefore, the missing effect of exogenous amylase in the last days of starch infusion in the present study might also be explained by an insufficient activity of these enzymes.

In conclusion, increased fecal excretion of microbial N and VFA indicates increased carbohydrate fermentation in the hindgut, and therefore limited digestion of starch in the small intestine. Lower lactate excretion during the beginning of starch infusion with amylase administration indicates an effect of amylase in not adapted animals, and suggests that addition of exogenous amylase might have the potential to improve intestinal digestibility of starch in not adapted animals. Missing effects of amylase administration after adaptation might be due to a sufficient activity of endogenous amylase at the infused level of starch of this trial or due to a rapid adaptation of hindgut microbes. Therefore, further studies are required to clarify whether the activity of endogenous amylase might be a limiting factor at higher levels of starch infusion. Intestinal digestibility of starch reported in this study is within the range of those values reported in the literature, which indirectly indicates a relative reliability of the present approach of calculating starch digestibility.

## 2.6 ACKNOWLEDGMENTS

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## Manuscript II

### Effects of abomasally infused amylase and increasing amounts of corn starch on fecal excretion of starch, total and microbial nitrogen, and volatile fatty acids in heifers<sup>1</sup>

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### 3.1 ABSTRACT

The aim of the present study was to study the effect of exogenous amylase on postruminal disappearance of increasing amounts of corn starch being infused into the abomasum of heifers, and to detect a possible limitation of starch digestion in the small intestine. Four rumen-fistulated heifers (two German Black Pied and two Jersey × German Black Pied) with an initial BW of  $565 \pm 6$  kg were fed 5.6 kg DM/d of a diet *targeted* to contain only a negligible amount of starch. Animals were assigned randomly to a cross-over trial with two experimental periods lasting 35 d each with 10 d of diet adaption followed by 25 d of sample collection. During the sampling period, each animal was abomasally infused with native corn starch at five levels (953, 1213, 1425, 1733 and 1993 g DM/d) each for a 5-d period with and without exogenous amylase, respectively. At day 6 to 10 the heifers received an abomasal infusion of starch in amounts of 724 g/d. Feces were sampled four times a day during the collection periods. Titanium dioxide was ruminally administered (10 g/d) to estimate fecal excretion. Purine bases in feces were determined and used as a marker for microbial N excretion. Fecal excretion of microbial N increased linearly with increasing level of starch infusion ( $P < 0.001$ ), indicating a constant proportion of the infused starch being fermented in the hindgut. In contrast, the apparent digestibility of starch from the total postruminal tract decreased linearly from 90 to 80% ( $P < 0.001$ ) when the intestinal starch supply increased from 1 to 2 kg/d. There is strong evidence based on the increasing starch excretion with feces and the indication of a constant proportion of infused starch being fermented in the hindgut for a decreasing efficiency of starch digestion in the small intestine with increasing intestinal supply. Amylase administration increased fecal excretion of butyrate ( $P = 0.04$ ) and tended to increase isovalerate excretion ( $P = 0.06$ ). However, amylase did not affect fecal excretion of microbial N or starch, suggesting that pancreatic amylase activity may not be the primarily limiting factor of postruminal starch digestion in heifers when corn starch is abomasally infused in amounts up to 2 kg/d.



**Key words:** amylase, cattle, fecal composition, hindgut fermentation, small intestinal digestion, starch

### 3.2 INTRODUCTION

Previous studies with diets containing high proportion of grain (Karr et al., 1966) or with corn starch infused into the abomasum (Little et al., 1968; Kreikemeier et al., 1991; Richards et al., 2002) or duodenum (Matthé, 2001; Brake et al., 2014) have shown a limited digestion of starch in the small intestine of cattle. Possible limiting factors are well reviewed by Owens et al. (1986) and Huntington (1997) and include the capacity for glucose absorption, the time for starch hydrolysis, the activity of hydrolyzing enzymes, adequate working conditions and the absence of enzyme inhibitors as well as the access of enzymes to starch granules. Thus, the supply of exogenous amylase could be seen as a strategy to improve starch digestibility in the small intestine. It was shown that up to 880 g/d corn starch can be digested in the small intestine of dry cows (Matthé, 2001) and 830 g/d in beef steers (Branco et al., 1999). However, intestinal digestibility of 880 g/d of corn starch infused into the abomasum of heifers was not improved when exogenous amylase was administered (Westreicher-Kristen et al., 2018). Missing effect of amylase administration was suggested to be due to a sufficient activity of endogenous amylase at this low level of starch infusion. Thus, further studies are required to clarify whether the activity of endogenous amylase might be a limiting factor at higher levels of abomasal starch infusion. The herein study aimed to investigate the postruminal digestion of different levels of starch infused into the abomasum of heifers without and with exogenous administration of amylase. We hypothesized that intestinal digestibility of starch might be limited by endogenous amylase activity with amounts of infused starch higher than 1 kg/d and that the disappearance of starch in the small intestine of adapted animals can be improved by exogenous amylase.

### 3.3 MATERIALS AND METHODS

The animal study reported herein was performed in accordance with the German Animal Welfare Act (Federal Republic of Germany, 2014) and approved by the Animal Welfare Commission of the Ministry of Energy, Agriculture, Environment and Rural Areas of the Federal State of Schleswig-Holstein, Germany (V 244-7224.121-25).

#### *3.3.1 Animals and Diets*

Four fully-grown heifers (two German Black Pied and two Jersey × German Black Pied) with an initial BW of  $565 \pm 6$  kg and fitted with rumen cannulas (#2C, 10 cm i.d.; Bar Diamond, Inc., Parma, ID) were used. The heifers were fed 5.6 kg/d of a diet targeted to contain only a negligible amount of starch and consisting of 65.4% grass hay (primary growth of *Lolium perenne*-dominated sward), 32.5% dried beet pulp (J. August Plambeck GmbH, Brügge, Germany), 0.9% feed urea (Piarumin; Stickstoffwerke Piesteritz GmbH, Wittenberg, Germany) and 1.2% of a mineral and vitamin premix (Panto-Mineral; Hamburger Leistungsfutter GmbH, Hamburg, Germany) on DM basis in two equal meals at 0700 and 1500 h. The ingredients and chemical composition of the experimental diet is shown in Table 6. The diet was formulated to meet maintenance requirements according to the German feeding standards (GfE, 2001).

#### *3.3.2 Experimental Design and Procedure*

The experiment consisted of two periods lasting 35 d each with 10-d for adaptation to the diet followed by 25-d for treatments application and sample collection. Between periods, a 5 wk period without treatment application was set to avoid carryover effects of adaptation to starch digestion. During the 25-d sampling periods each animal received an abomasal infusion of corn starch at five infusion levels increasing each 5 d from a targeted dosage of 1000 to 1250, 1500, 1750 and 2000 g/d. The exact amounts of starch infused were determined by recording the amount of residual infusate solution. The effective dosages were 953, 1213, 1425, 1733 and 1993 g/d.

**Table 6.** Ingredients and chemical composition of the diet.

Item	% of dietary DM
Ingredient	
Grass hay	65.4
Dried beet pulp	32.5
Feed urea	0.90
Mineral and vitamin mix <sup>1</sup>	1.20
Nutrient <sup>2</sup>	
Crude ash	6.51
Crude protein	8.48
Ether extract	0.62
NDF <sub>org</sub>	58.5
ADF <sub>org</sub>	33.0
ADL	3.37

<sup>1</sup>The mineral and vitamin mixture contained 210 g calcium, 100 g sodium, 40 g magnesium, 30 g phosphorus, 1,000 mg copper, 8,000 mg zinc, 5,000 mg manganese, 60 mg iodine, 50 mg selenium, 40 mg cobalt, 5,000 mg vitamin E, 1,000,000 IU vitamin A and 100,000 IU vitamin D3 (as fed).

<sup>2</sup>Calculated from the analyzed nutrient composition of individual ingredients.

Prior to these experimental periods animals were adapted to starch infusion and digestion at an effective dosage of 724 g/d at d 6 to 10, to have similar increases of starch supply ( $\approx$  250 g/d) for all experimental starch levels including the lowest. The native corn starch contained 95.7% starch, 0.4% CP and 0.2% crude ash on DM basis (Maisita 21.000; AGRANA AG, Vienna, Austria). The starch product was mixed in 10 L water and kept suspended by continuous stirring. The abomasal infusion was performed using a tool to insert lines into the abomasum described by Westreicher-Kristen and Susenbeth (2017). The infusion began half an hour after morning feeding and lasted 10 h/d. A peristaltic pump (rotarus Flow 50; Hirschmann Laborgeräte GmbH & co KG, Eberstadt, Germany) was used for infusion.

The animals were assigned randomly to a crossover design. In each experimental period two animals received an additional infusion of an exogenous  $\alpha$  amylase (EC.3.2.1.1; 13,000 U/mL, Distizym BA-N, 1,4- $\alpha$ -D-Glucan-Glucanohydrolase; Erbslöh Geisenheim AG, Geisenheim, Germany). The  $\alpha$  amylase was mixed with distilled water and continuously infused into the line for starch infusion throughout each 10-h infusion phase at a rate of 50 mL/h using a syringe pump (540060-HP Single Syringe; TSE Systems GmbH, Bad Homburg, Germany). The

amylase activity of Distizym BA-N was determined in a bicinchoninic assay as described by Westreicher-Kristen et al. (2018). A dosage of Distizym BA-N of 260,000 U/kg starch was administered.

Total fecal excretion was determined using titanium dioxide (TiO<sub>2</sub>; Kronos 1171; Harold Scholz & Co. GmbH, Recklinghausen, Germany). For this purpose, 5 g TiO<sub>2</sub> were ruminally administered via gelatin capsules (HKG size 30 × 90 mm, 59 mL, lipid-transparent, type 36; Capsula GmbH, Ratingen, Germany) to each heifer twice daily immediately after each feeding. The TiO<sub>2</sub> administration was initiated 5 d before the phase of sample collection to reach the equilibrium of TiO<sub>2</sub> excretion after initial administration (Glindemann et al., 2009).

### *3.3.3 Sampling, Sample Preparation and Analytical Procedures*

During each experimental period, 6 samples (~200 g fresh matter each) of the grass hay and dried beet pulp were collected and pooled by feed for chemical analyses. The feed samples (1-mm particle size) were analyzed in duplicate for concentrations of moisture, crude ash (CA), crude protein (CP) and ether extract according to the official analytical methods in Germany (VDLUFA, 2007). Feed samples (2-mm particle size) were analyzed in triplicate for NDF, ADF, both expressed on ash-free basis, and ADL as described by Van Soest et al. (1991) using an ANKOM 200 Fiber Analyzer (ANKOM Technology, Macedon, NY). Heat-stable  $\alpha$ -amylase was added during NDF extraction. Native corn starch used for abomasal infusion was analyzed for starch concentration based on polarimetric procedure (method 7.2.1; VDLUFA, 2007). Fecal grab samples (~500 g fresh matter) were collected from the rectum during the 25-d sampling phase 4 times a day at 0900, 1200, 1500 and 1800 h. Immediately after collection samples were kept at -20 °C for ~15 min to quickly minimize microbial activity. Afterwards, samples were placed in a cooling room at 4 °C and merged per animal at the end of each sampling day. Six subsamples per day (minimum 100 g per sample) were taken and stored at -20 °C for analyses. Subsamples were thawed and pooled by animal and level of starch infusion

at the end of each experimental period. Samples of the first day of each infusion level were excluded because they were assumed to be possibly affected by the previous level of starch infusion due to delayed excretion. The pooled fecal samples were analyzed in duplicate for moisture, CA, N and NDF according to VDLUFA (2007). Fecal concentration of TiO<sub>2</sub> was determined according to Brandt and Allam (1987) as described by Westreicher-Kristen et al. (2018). Concentration of starch was determined as total  $\alpha$ -linked glucose polymers in lyophilized samples (0.2-mm particle size) by two-step enzymatic hydrolysis according to Brandt et al. (1987), briefly described by Westreicher-Kristen et al. (2018). Concentration of VFA in feces (acetate, butyrate, propionate, valerate, isovalerate and isobutyrate) was determined using a gas chromatograph, and concentration of L-lactate using a Lactate-UV Test Kit as described by Westreicher-Kristen et al. (2018). The concentration of purine bases (PB) in feces was determined by HPLC according to Dickhoefer et al. (2016).

#### 3.3.4 Calculations and Statistical Analysis

Fecal excretion of DM was calculated as TiO<sub>2</sub> administration (g/d) divided by the TiO<sub>2</sub> concentration (g/kg DM) in feces, assuming a fecal recovery of 100%. Fecal excretion of OM, total N, microbial N, starch, VFA and lactate was calculated based on their concentrations in feces (g/kg DM) and fecal DM excretion. The OM concentration in feces was calculated as 1000 – CA (g/kg DM). The concentration of total lactate was calculated assuming a ratio of L- and D-lactate of 50:50. The apparent total tract disappearances of OM (ATTD<sub>OM</sub>) and NDF (ATTD<sub>NDF</sub>) were calculated as the quotient of the total amount that apparently disappeared (intake – fecal excretion) and the amount of intake including the amount of OM infused as starch. The apparent disappearance of starch from total postruminal tract (ATTD<sub>Starch</sub>) was calculated as the quotient of the total amount that apparently disappeared (infusion – fecal excretion) and the amount of starch infused. The concentration of microbial N in feces was calculated based on PB excretion using a PB-N: microbial N ratio of 0.116 according to Chen (1989).

All data were statistically analyzed by the MIXED procedure of SAS (SAS Institute, Inc., Cary, NC). Period, infusion level, treatment and infusion level  $\times$  treatment interactions were included as fixed effects and animal as a random factor. The LSM and SEM of each parameter were calculated and compared using the LSMEANS statement. Linear and quadratic orthogonal contrasts were tested for the effects of starch infusion level using the CONTRAST statement. Unequal spacing between starch infusion levels was considered by generating the coefficients for linear and quadratic contrasts using the IML procedure of SAS. Treatment effects were considered significantly different at  $P < 0.05$  and tendencies with P-values between 0.05 and 0.10. Simple linear regression analyses were performed (PROCREQ) to determine the fecal excretion of microbial N and starch as well as starch disappearance from the small intestine as a function of the amount of starch infused.

### 3.4 RESULTS

Fecal excretion of DM, OM, N, microbial N, starch, lactate and VFA as well as  $ATTD_{OM}$ ,  $ATTD_{NDF}$  and  $ATTD_{Starch}$  at increasing levels of starch infusion with or without exogenous amylase are presented in Table 7. Fecal excretion of DM and OM increased linearly with increasing level of starch infusion ( $P < 0.001$  for both). The  $ATTD_{OM}$  decreased linearly with infusion level ( $P = 0.02$ ) with values ranging between 72.9 and 70.1%. The administration of amylase did neither affect fecal excretion of DM ( $P = 0.98$ ) and OM ( $P = 0.75$ ) nor  $ATTD_{OM}$  ( $P = 0.69$ ). The  $ATTD_{NDF}$  was neither affected by level of starch infusion (linear:  $P = 0.80$ ; quadratic:  $P = 0.39$ ) nor by amylase administration ( $P = 0.95$ ) and averaged 69.2% for both treatments.

The fecal excretion of total N and microbial N increased linearly with increasing level of starch infusion ( $P = 0.02$  and  $P < 0.01$ , respectively) from 46.3 to 47.6 g/d and 9.3 to 10.7 g/d, respectively. On average, microbial N excretion increased by 0.12 g/100 g of additionally infused starch (Figure 4). Neither total N ( $P = 0.41$ ) nor microbial N excretion ( $P = 0.46$ ) were

affected by amylase. The proportion of microbial N in feces (as % of total N) was unaffected by amylase ( $P = 0.21$ ), but increased linearly with starch infusion ( $P = 0.01$ ) from 20.2 to 22.7%. Fecal excretion of VFA increased linearly with increasing level of starch infusion ( $P < 0.001$ ), but was not affected by amylase ( $P = 0.18$ ). Acetate and butyrate excretion increased with level of starch infusion ( $P < 0.001$  for both), while propionate excretion was not affected by infusion level (linear:  $P = 0.12$ ; quadratic:  $P = 0.43$ ). Acetate excretion and propionate excretion were not affected by amylase ( $P = 0.34$  and  $P = 0.90$ , respectively), but butyrate excretion was higher with amylase than without ( $P = 0.04$ ). Molar proportion of acetate and propionate decreased ( $P < 0.001$ ) and of butyrate increased ( $P < 0.001$ ) linearly with increasing level of starch infusion. Additionally, molar proportion of butyrate increased ( $P = 0.02$ ) and of acetate decreased due to amylase ( $P = 0.05$ ), while molar proportion of propionate was unaffected by amylase ( $P = 0.57$ ). Valerate excretion decreased linearly with starch infusion ( $P = 0.02$ ), but was unaffected by amylase administration ( $P = 0.53$ ). Excretions of isobutyrate and isovalerate responded quadratically ( $P = 0.02$  and  $P = 0.05$ , respectively) with increments until starch infusion levels of 1425 and 1213 g/d, respectively, and decrements with further increasing levels of infusion. The administration of exogenous amylase tended to increase the excretion of isovalerate ( $P = 0.06$ ), while isobutyrate was not affected by amylase ( $P = 0.34$ ). Lactate excretion increased linearly with increasing level of starch infusion ( $P < 0.001$ ), but was not affected by amylase ( $P = 0.50$ ) with values ranging between 0.6 and 4.0 g/d. Starch excretion increased linearly ( $P < 0.001$ ) on average with 29.2 g/100 g of additionally infused starch (Figure 5). However, the  $ATTD_{\text{Starch}}$  decreased linearly ( $P < 0.001$ ) from 89.9 to 80.3% when averaged for both treatments. Amylase affected neither starch excretion ( $P = 0.54$ ) nor  $ATTD_{\text{Starch}}$  ( $P = 0.39$ ).

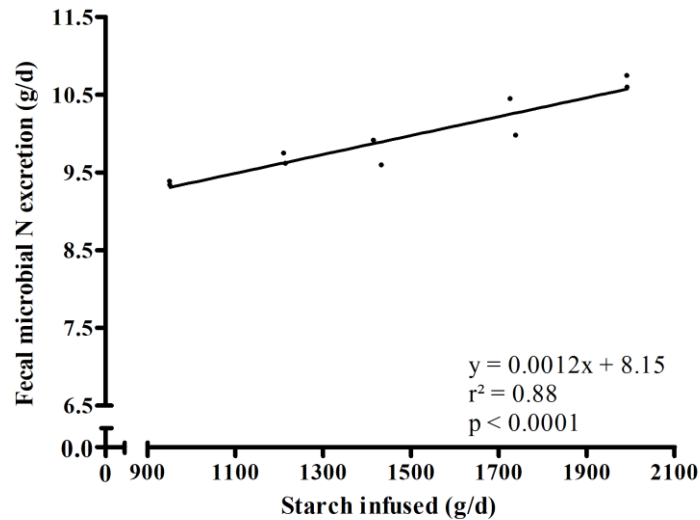
**Table 7.** Fecal excretion of DM, OM, N, VFA, starch and lactate in heifers infused abomasally with increasing amounts of starch with or without exogenous amylase administration

Item	Treatment <sup>1</sup>												SEM		P-value <sup>2</sup>				
	Starch				Starch + amylase				Starch + amylase										
	Amount of starch infused, g/d				Amount of starch infused, g/d				Amount of starch infused, g/d				L	Q	α				
DM, kg/d	1.97	1.96	2.06	2.17	2.34	1.95	1.97	2.05	2.22	2.32	1.95	1.97	2.05	2.22	2.32	0.77	<.001	0.04	0.98
OM, kg/d <sup>3</sup>	1.70	1.69	1.80	1.92	2.09	1.70	1.72	1.79	1.98	2.07	1.70	1.72	1.79	1.98	2.07	0.76	<.001	0.04	0.75
Starch, kg/d	0.09	0.13	0.20	0.28	0.39	0.10	0.16	0.18	0.34	0.39	0.10	0.16	0.18	0.34	0.39	0.07	<.001	0.24	0.54
Total N, g/d	46.2	45.5	46.9	47.1	47.3	46.4	44.8	46.0	46.0	47.9	46.4	44.8	46.0	46.0	47.9	1.10	0.02	0.10	0.41
Microbial N, g/d	9.38	9.61	9.59	9.97	10.6	9.33	9.74	9.91	10.4	10.7	9.33	9.74	9.91	10.4	10.7	0.53	<.001	0.58	0.46
Microbial N, % of N	20.3	21.1	20.5	21.1	22.4	20.2	21.7	21.7	22.7	22.4	20.2	21.7	21.7	22.7	22.4	1.00	0.01	0.92	0.21
Lactate, g/d <sup>4</sup>	0.59	1.26	1.67	2.68	3.96	0.64	1.44	1.57	2.20	3.11	0.64	1.44	1.57	2.20	3.11	0.78	<.001	0.40	0.50
VFA, g/d	34.0	43.2	49.0	50.9	53.0	39.1	45.0	49.3	56.0	57.2	39.1	45.0	49.3	56.0	57.2	4.52	<.001	0.22	0.18
Acetate, g/d	22.5	26.8	29.9	32.2	32.2	25.3	26.9	30.0	33.7	34.3	25.3	26.9	30.0	33.7	34.3	2.68	<.001	0.37	0.34
Propionate, g/d	2.36	2.98	2.81	2.15	2.15	2.89	2.49	2.70	2.15	2.05	2.89	2.49	2.70	2.15	2.05	0.62	0.12	0.43	0.90
Butyrate, g/d	8.11	12.3	15.3	15.7	17.9	10.0	14.5	15.5	19.2	19.9	10.0	14.5	15.5	19.2	19.9	1.88	<.001	0.13	0.04
Valerate, g/d	0.31	0.31	0.18	0.16	0.17	0.28	0.18	0.26	0.14	0.16	0.28	0.18	0.26	0.14	0.16	0.07	0.02	0.57	0.53
Isobutyrate, g/d	0.29	0.31	0.35	0.29	0.24	0.29	0.36	0.35	0.32	0.27	0.29	0.36	0.35	0.32	0.27	0.06	0.22	0.02	0.34
Isovalerate, g/d	0.39	0.45	0.45	0.33	0.32	0.39	0.49	0.47	0.49	0.41	0.39	0.49	0.47	0.49	0.41	0.08	0.33	0.05	0.06
Acetate, % <sup>5</sup>	68.5	63.8	62.1	64.2	61.8	66.3	61.3	62.3	61.2	61.2	66.3	61.3	62.3	61.2	61.2	1.22	<.001	0.02	0.05
Propionate, % <sup>5</sup>	7.48	6.99	6.01	4.27	4.28	8.10	5.68	5.65	4.03	3.66	8.10	5.68	5.65	4.03	3.66	1.50	<.001	0.62	0.57
Butyrate, % <sup>5</sup>	24.0	29.2	31.9	31.5	33.9	25.6	33.0	32.1	34.8	35.1	25.6	33.0	32.1	34.8	35.1	1.60	<.001	0.01	0.02
ATTD <sub>OM</sub> , % <sup>6</sup>	71.7	72.9	72.1	71.5	70.1	71.7	72.4	72.2	70.6	70.3	71.7	72.4	72.2	70.6	70.3	1.12	0.02	0.05	0.69
ATTD <sub>NDF</sub> , %	67.2	70.1	70.8	70.1	68.2	69.1	69.2	70.4	66.9	70.4	69.1	69.2	70.4	66.9	70.4	2.79	0.80	0.39	0.95
ATTD <sub>Starch</sub> , %	90.5	89.3	85.9	84.1	80.3	89.3	87.1	87.0	80.4	80.3	89.3	87.1	87.0	80.4	80.3	4.32	<.001	0.61	0.39

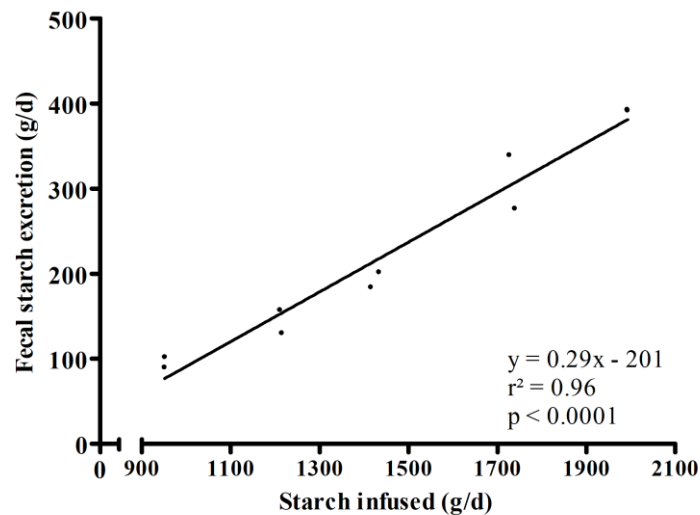
SEM = standard error of mean; DM = dry matter; OM = organic matter; N = nitrogen; VFA = volatile fatty acids; ATTD = apparent total tract disappearance.

<sup>1</sup>Values represent LSM of the last 4 d of each infusion level within each treatment ( $n = 4$ ).<sup>2</sup>Linear (L) and quadratic (Q) effects of starch infusion and effect of amylase administration ( $\alpha$ ).<sup>3</sup>OM was calculated as  $1000 - \text{CA}$  (g/kg DM). VFA are partly included as they were captured in DM analysis.<sup>4</sup>Lactate excretion including D- and L-isomers.<sup>5</sup>Proportion of the sum of acetate, propionate and butyrate.<sup>6</sup>OM infused as starch was considered as intake.





**Figure 4.** Fecal excretion of microbial N as function of the amount of starch infused.



**Figure 5.** Fecal excretion of starch as function of the amount of starch infused.

### 3.5 DISCUSSION

#### 3.5.1 Methodological Aspects

The lowest level of starch infusion was targeted to be 1 kg/d, because this was known to be an acceptable amount from this preceding experiment, where diarrhea was not observed. The rate of starch infusion was increased moderately to determine the capacity of starch digestion in animals adapted to starch digestion. Feces started to have a softer consistence a few hours after beginning of abomasal starch infusion and became softer and stickier with increasing level of starch infusion. However, cases of diarrhea were not observed throughout the whole infusion

periods. Additionally, feed intake was not influenced by the presence of the infusion device in the abomasum during the present trial.

Administration of each infusion level of starch during 5 consecutive days was assumed to be an acceptable sampling protocol as fecal excretion of measured parameters reached an equilibrium within 4 d of starch infusion in a pre-trial under similar experimental conditions (Westreicher-Kristen et al., 2018). Nevertheless, it must be noted that fecal samples were obtained during adaptation phase to increasing amounts of starch infused and not at steady state conditions after an adaptation period. However, the animals were expected to be adapted to starch digestion by the preceding period where only moderately lower amounts of starch (250 g/d) were infused. Another critical point might be the fact, that starch was infused only during 10 out of 24 h a day causing diurnal variations. However, we assumed that the diurnal flow of starch entering the large intestine does not affect the extent of starch fermentation. Fecal samples were taken frequently and can be seen as representative for the total amount excreted. The exogenous amylase used in the herein experiment was from bacterial origin; to compensate possible reduced enzyme activity by low pH, a clearly higher enzyme concentration than the dosage recommended by the manufacturer (1,560 U/kg of grain starch) was applied.

### *3.5.2 Postruminal Disappearance of Starch*

The ATTD<sub>OM</sub> decreased with increasing level of starch infusion reflecting an increased excretion of MCP, VFA, and lactate due to hindgut fermentation as well as an increased excretion of starch due to incomplete digestion in the total postruminal tract. It must be noted, that the ATTD<sub>OM</sub> might be overestimated based on the fact that VFA were included in OM excretion only in the extent of which they were captured in DM analysis. The ATTD<sub>Starch</sub> decreased with increasing amounts of starch infused, from 90 to 80% with intestinal supplies of 1 and 2 kg/d, respectively. This is in agreement with results of previous experiments, where a decreasing postruminal disappearance with increasing starch flow to the small intestine was

observed (Little et al., 1968; Knowlton et al., 1998; Matthé, 2001). For example, Matthé (2001) reported a decreasing disappearance of starch from the total postruminal tract from 95% to 79% with intestinal supplies of 876 and 1753 g/d in dry cows, which is comparable to the herein observed values. However, when comparing the results from different experiments the large number of influencing factors, such as the animal, the level of feed intake, the dietary composition, the nature of the administered starch as well as the experimental design (e.g. time for adaption to starch digestion) must be considered.

In the herein experiment, fecal excretion of microbial N and VFA was taken as indicators for starch fermentation in the hindgut as discussed previously by Westreicher-Kristen et al. (2018). The missing increase of the molar proportion of propionate with increasing starch fermentation, which is observed in the rumen, might be explained by its fast absorption from the hindgut (Bergman, 1990). The quadratic response of isovalerate and isobutyrate excretion with increments until starch infusion levels of 1213 and 1425 g/d, respectively, and decrements with further increasing levels might either reflect changes in the rate of absorption of these VFA or changes in the provision of branched-chain amino acids as precursor substances (Smith and Macfarlane, 1997).

Based on the linear increase of microbial N excretion, a rather constant proportion of infused starch was assumed to be fermented in the hindgut. As the  $ATTD_{NDF}$  was not affected by level of starch infusion, an unaffected fiber fermentation in the hindgut can be assumed. Therefore, microbial N excretion increased by starch fermentation only, accounting on average by 0.12 g/100 g of additionally infused starch. Assuming an efficiency of CP synthesis in the hindgut of 1 g microbial N/100 g of starch fermented as demonstrated by starch infusion into the caecum of sheep (Ørskov et al., 1970), on average 12% of the infused starch was fermented in the hindgut. This is in close agreement with the results of our previous experiment, where 1.1 g/d microbial N was additionally excreted with 880 g/d starch infusion under similar

experimental conditions, indicating that on average 12.5% of infused starch were fermented in the hindgut (Westreicher-Kristen et al., 2018). Furthermore, these values are similar to that of 14% reported by Branco et al. (1999), who infused starch hydrolysate into the abomasum of steers, which were fed fescue hay at maintenance level feeding (1.8% of BW). However, microbial N excretion was estimated based on fecal excretion of PB in the herein experiment and inaccuracies due to this method were already discussed by Westreicher-Kristen et al. (2018). Microbial N excretion averaged 9.9 g/d comprising ~21% of total fecal N output, which indeed appears to be rather low. For example, Knowlton et al. (1998) observed proportions of microbial N in feces between 45% and 57% when corn-based diets were fed to lactating dairy cows. Furthermore, according to Hungate (1966) the efficiency of hindgut CP synthesis might range between 0.9 and 1.7 g microbial N/100 g carbohydrates fermented. If the level of starch infusion somehow altered the efficiency of starch fermentation in the hindgut (i.e. by altering the rate of protein degradation in the hindgut), this might have masked differences in the extent of starch fermentation between infusion levels. Consequently, values of starch disappearance from the hindgut based on microbial N excretion as herein presented must be interpreted with care.

The linear increase of starch excretion at intestinal supplies greater than 953 g/d indicates a rather constant proportion of 29% of the additionally infused starch being excreted with feces. However, considering the decreasing  $ATTD_{\text{Starch}}$  from 90% to 80% and assuming a constant proportion of 12% of additional infused starch being fermented in the hindgut, starch disappearance from the small intestine decreased from 78% to 68% at intestinal supplies of 953 and 1993 g/d, respectively (Figure 3). This is in line with the results from previous experiments reporting a decreasing efficiency of starch digestion with increasing intestinal supplies in cattle (Karr et al., 1966; Kreikemeier et al., 1991; Matthé, 2001).

### 3.5.3 *Effects of Abomasally Infused Amylase on Postruminal Starch Digestion*

Missing effects of exogenous amylase on fecal excretion of starch, MCP and total VFA imply an equal extent of starch disappearance from the total postruminal tract as well as an equal extent of hindgut fermentation. Therefore, a similar disappearance of starch or end-products of starch hydrolysis in the small intestine can be assumed for both treatments as well. Consequently, pancreatic amylase activity does not seem to be the primarily limiting factor of starch digestion in the small intestine, when starch escapes ruminal fermentation in amounts up to 2 kg/d under conditions of the present experiment. Nevertheless, whether starch or end-products of starch hydrolysis were fermented in the hindgut and excreted via feces could not be differentiated from the herein experiment. Therefore, it cannot be excluded that starch hydrolysis in the small intestine was improved by amylase administration, but this effect could have been masked by an insufficient activity of other hydrolyzing enzymes or limited capacity for glucose absorption. It is unlikely that protein supply to the animals' metabolism limited the secretion of enzymes at higher levels of starch infusion, since microbial protein production at energy maintenance feeding level exceeds considerably the protein requirement of the animal. However, it cannot be excluded that limited time for starch hydrolysis at higher infusion levels due to a rapid passage of digesta through the small intestine as discussed by Owens et al. (1986) was partly responsible for the incomplete digestion. Furthermore, reduced endogenous secretion of pancreatic amylase due to amylase infusion might have masked possible effects, however this is not supported by observations of Remillard et al. (1990), whereat Swanson et al. (2002) observed a reduced secretion of pancreatic amylase due to an infusion of starch hydrolysate at rates of 20-40 g/h. Therefore, an indirect effect of amylase infusion on amylase secretion due to an increased amount of hydrolyzed starch in the lumen of the small intestine might exist. Additionally, as the activity of the exogenous amylase in the duodenal chyme was not determined in this experiment, some degradation of exogenous amylase in the abomasum

can be expected but might not have limited possible enzyme effects, since the high dosage of amylase have compensated possible activity losses.

Higher proportion of butyrate and lower proportion of acetate with than without amylase administration might indicate differences in the proportion of  $\alpha$ -linked glucose polymers (starch vs. end-products of starch hydrolysis) reaching the hindgut. Additionally, isovalerate excretion was higher with amylase administration, which is suggested to be caused by hindgut fermentation of nitrogenous compounds (Smith and Macfarlane, 1997). These might originate from amylase infusion or an enhanced flow of endogenous N to the hindgut as already suggested from the results of the previous experiment (Westreicher-Kristen et al., 2018). However, total and microbial N excretion as well as the proportion of microbial N in feces were not affected by amylase administration. Hence, an effect of exogenous amylase on endogenous N excretion seems unlikely. Assuming an unaffected excretion of dietary N, the additional amount of endogenous N excreted can be calculated as the difference of additional total N and microbial N excretion, which is equal to 0.03 g/100 g of additionally infused starch. In contrast, endogenous N excretion increased on average by 0.68 g/100g starch infused in the preceding experiment with animals not completely adapted to starch digestion (Westreicher-Kristen et al., 2018). Therefore, it can be speculated that the greater endogenous N excretion in the preceding experiment was primarily based on adaptive mechanisms, in contrast to the better adapted animals in the present experiment.

#### *3.5.4 Comparison of the Energetic Efficiency of Ruminal and Postruminal Starch Digestion*

Calculations and observations of Harmon and McLeod (2001) indicate that the partial energetic efficiency of ruminal starch fermentation, defined as starch energy retained in body tissue divided by starch ME intake, is 70% to 75% of that of starch digested and absorbed from the small intestine. Latter reflects the sum of energy losses attributed to digestion and absorption (e.g. methane energy, urine energy, fecal energy due to microbial residues from ruminal

fermentation and endogenous secretion) as well as energy losses due to heat production by fermentation, digestion and utilization of digestion end-products for maintenance and production. However, results from the present experiment demonstrate that the digestion of starch in the small intestine in amounts exceeding 1.0 kg/d is inevitable accompanied by an enhanced fermentation of starch in the hindgut. Based on our calculations only 68% to 78% of starch entering the small intestine disappeared from the small intestine, while 12% were fermented in the hindgut and up to 20% excreted with feces. Although less methane is produced by caecal compared to ruminal fermentation, the energetic efficiency is clearly lower, since the energy of the microbial matter is completely excreted with feces (McLeod et al., 2006). When the values of the energetic efficiency of starch digestion given by Harmon and McLeod (2001) are corrected by assuming a digestibility of starch in the small intestine of 68% instead of 78%, the increase in the partial energetic efficiency by shifting starch digestion from the rumen to the small intestine equals 32% to 44%, which is in line with the value of 42% reported by Owens et al. (1986).

However, previous experiments reported that only 35% to 38% of starch disappearing from the small intestine could be accounted for as net glucose absorption in steers due to an enhanced glucose utilization by the portal-drained visceral tissues (Huntington and Reynolds, 1986; Kreikemeier et al., 1991). Furthermore, Gilbert et al. (2015) reported that a noteworthy amount of starch disappearing from the small intestine underwent microbial fermentation in the distal part of the ileum in milk-fed calves, which might as well occur in adult cattle, and would explain the rather low net absorption of glucose in the previous mentioned experiments. Beside the limited net absorption of glucose from starch digestion in the small intestine, an increased excretion of endogenous N during phase of adaption (Westreicher-Kristen et al., 2018) as well as a reduced energy supply for ruminal microbes and therefore a reduced MCP supply to the host animal must be considered when shifting starch digestion from the rumen to the small intestine.

In conclusion, the results of the present experiment indicate a decreasing efficiency of starch digestion in the small intestine with increasing intestinal supplies as well as a considerably amount of MCP and starch excreted with feces. It can be speculated that these effects and the expected reduction of MCP supply by ruminal fermentation might override potential energetic advantages of postruminal starch digestion at amounts greater than 1 kg/d. Furthermore, the results did not indicate a limitation of starch digestion in the small intestine by pancreatic amylase under conditions of the present experiment. However, starch hydrolysis might have been enhanced by exogenous amylase but not detected by fecal parameters due to limited activity of disaccharidases or a restricted capacity for glucose absorption.

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## 4 General Discussion

Shifting starch digestion from the rumen to the small intestine is assumed to increase the energy supply to high producing beef cattle and lactating dairy cows due to a greater energetic efficiency of starch digestion in the small intestine compared to ruminal fermentation (Owens et al., 1986; Huntington et al., 2006; McLeod et al., 2006). This advantage led to a growing interest to investigate the capacity for starch digestion in the small intestine of ruminants. Several experiments with diets containing high proportion of ruminally undegradable starch (Karr et al., 1966; Russell et al., 1981a; Remillard et al., 1990; Zinn, 1990) or infusions of native starch into the abomasum (Kreikemeier et al., 1991; Knowlton et al., 1998a; Branco et al., 1999) or duodenum (Matthé, 2001; Richards et al., 2002; Brake et al., 2014) of beef steers and dairy cows were conducted in the past to investigate the capacity for starch digestion and absorption in the small intestine of cattle. A declining percentage of starch disappearing from the small intestine indicated a decreasing efficiency of starch digestion in the small intestine with increasing intestinal supply (Karr et al., 1966; Kreikemeier et al., 1991; Matthé, 2001). Accordingly, the main focuses of the present thesis were to assess the capacity for starch digestion in the small intestine by infusion of corn starch in amounts up to 2.0 kg/d into the abomasum of heifers and to investigate a possible limitation of intestinal starch digestion by insufficient activity of pancreatic amylase.

### 4.1 METHODOLOGICAL ASPECTS

#### 4.1.1 *Experimental Design and Procedure*

Animals were fed at maintenance level (1% of BW on DM basis) to minimize variations in the rate of passage (Mertens, 1987) and therefore, variations of starch digestibility. Nevertheless, starch was infused only 10 h out of 24 h a day, so starch flow through the digestive tract and the fecal excretion of starch and other affected parameters like microbial crude protein (MCP), volatile fatty acids (VFA) and lactate might have been subjected to diurnal variations.

The daily fecal excretion was estimated based on the recovery of ruminally administered titanium dioxide (TiO<sub>2</sub>) in fecal samples as this procedure was demonstrated to have a high reliability (De Souza et al., 2015). The administration in two dosages per day in combination with four equally spaced sampling times a day was chosen to further improve the prediction accuracy. A recovery rate of 100% of the administered amount in fecal samples was assumed as demonstrated by Glindemann et al. (2009) for the applied sampling pattern in combination with an initiation of the administration 5 d before the beginning of the sampling period.

#### *4.1.2 Estimation of Starch Disappearance from Small Intestine*

Starch disappearance from the small intestine within the experiments of this thesis was estimated based on the fecal starch excretion and the extent of starch fermentation in the hindgut, which was quantified from excreted microbial N. Advantageously, the animals had not be fitted with ileal cannulas which is critical due to the microbial colonization and mobility of this intestinal segment as described by Harmon and Richards (1997). On the other hand, several factors might have led to an over- or underestimation of starch disappearance from the small intestine.

Fecal excretion of microbial N was estimated from purine bases (PB), which were determined in fecal samples by high-pressure liquid chromatography (HPLC) as described by Dickhoefer et al. (2016). A ratio of PB-N:microbial N of 0.116 was assumed for calculation. This ratio was determined by Chen (1989) in mixed ruminal microbes which is a matter of concern as this ratio might differ between ruminal and fecal microbes. Furthermore, the ratio of PB-N:microbial N was demonstrated to vary significantly depending on the microbial composition with values ranging between 0.04 to 0.14 in liquid-associated microbes and 0.03 to 0.12 in particle-associated microbes as reviewed by Dickhoefer et al. (2016). Therefore, microbial N excretion might have been to some extent over- or to a greater extent underestimated in the herein presented experiments. The average proportion of microbial N from total N in feces was

estimated to be 24% in the first experiment (Chapter 2.5.1) and 21% in the second experiment (Chapter 3.4). These values appear comparatively low, as for example Knowlton et al. (1998b) reported proportions between 45 and 57% when differently processed corn grains were fed to lactating dairy cows. The mean of published PB-N:microbial N ratios in ruminal microbes from cattle as compiled by Dickhoefer et al. (2016) is  $0.09 \pm 0.03$  ( $n = 7$ ), which is quite similar to the applied value in the herein reported experiments. Assuming the ratio of 0.09, microbial N excretion would increase by 5.0 g/d. However, differences between treatments (with or without amylase) as well as differences between the infusion levels might hardly be affected by variations in the PB-N:microbial N ratio.

When estimating microbial N from PB excretion also non-microbial PB from dietary or endogenous origin must be considered. According to Vicente et al. (2004) this fraction accounts for 30% to 40% of duodenal PB on average. As reported by McAllan and Smith (1972) dietary PB are rapidly degraded in the rumen and might therefore represent only negligible amounts of duodenal PB. Moreover, adenine and guanine disappeared completely during passage through the small intestine after infusion into the proximal duodenum of steers as reported by McAllan (1980). Therefore, an extensive degradation of dietary PB in the herein reported experiments can be assumed. Nevertheless, endogenous PB (i.e. PB of sloughed epithelial cells) are excreted in feces and might have led to an overestimation of microbial N excretion. References on the concentration of PB in bovine epithelial cells are lacking, but according to Vicente et al. (2004) total nucleic acids account for 7% of tissue N whereby a ratio of 7.33 mmol PB per g nucleic acid N can be assumed. Alternative methods for the quantification of microbial N in feces as other internal markers (i.e. diaminopimelic acid) or external isotopic markers (i.e.  $^{15}\text{N}$  and  $^{35}\text{S}$ ) also imply several limitations as summarized by Broderick and Merchen (1992). The latter recommended that PB or  $^{15}\text{N}$  should be used for estimating ruminal CP synthesis.

Besides the assumption of a constant PB-N:microbial N ratio, the efficiency of CP synthesis in the hindgut needs to be known in order to estimate the quantity of starch fermented in the hindgut. In the herein reported experiments the value of 1 g microbial N/100 g was used as determined by Ørskov et al. (1970) in studies where corn starch was infused into the caecum of sheep. However, Hungate (1966) reported in a literature review that the efficiency of hindgut CP synthesis ranges between 0.9 and 1.7 g microbial N/100 g carbohydrate fermented. Considering this variation and the additional microbial N excretion of 0.12 g/100 g of starch infused, the extent of starch fermentation in the hindgut might have ranged between 7.1% and 13.3% of infused starch in the herein presented experiments. Consequently, starch disappearance from the small intestine might have differed by up to 5%.

#### *4.1.3 Basal Flow of $\alpha$ -linked and free Glucose*

In the second experiment starch was analyzed as the total amount of  $\alpha$ -linked glucose polymers. In contrast, in the first experiment fecal excretion of other  $\alpha$ -glycosides than starch was neglected by calculating the difference of free glucose in fecal samples before and after incubation with thermostable amylase. To enable a comparison of both studies, the fecal excretion as well as the apparent total tract disappearance of total  $\alpha$ -linked glucose from different segments of the gastrointestinal tract was recalculated for the first experiment in Table 8. Fecal excretion of total  $\alpha$ -linked glucose observed with starch infusion was corrected for the amount of  $\alpha$ -linked glucose occurring with infusion of isotonic saline solution providing a starch free diet. This basal flow of  $\alpha$ -linked glucose can be assumed to be based on fecal excretion of  $\alpha$ -glycosides of endogenous or bacterial origin (Branco et al., 1999). When starch excretion is determined as the total amount of  $\alpha$ -linked and free glucose and corrected for basal excretion of 24.6 g/d, the apparent total tract disappearance resulted in 95% instead of 99% (Chapter 2.4.2). Furthermore, the disappearance of  $\alpha$ -linked and free glucose from the small intestine then amounts for 81% instead of 85% (Chapter 2.4.2).



In the second experiment no control period without starch infusion was performed. Yet, a similar basal flow of  $\alpha$ -linked and free glucose as in the first experiment can be assumed due to similar experimental conditions (same animals and similar diets). Therefore, starch disappearance from the total tract might have been 1% to 2% higher than the reported values of 68% to 78% (Chapter 3.5.2).

**Table 8.** Fecal excretion and apparent disappearance of total  $\alpha$ -linked and free glucose from different segments of the intestinal tract of heifers infused abomasally with 880 g/d corn starch without or with amylase treatment (recalculated results of manuscript I)

	Treatment		SEM	<i>P</i>
	Starch	Starch + Amylase		
Fecal excretion, g/d <sup>1</sup>	61.0	61.1	9.05	0.99
Basal excretion, g/d <sup>2</sup>	24.1	25.1	1.20	0.50
$\Delta$ Excretion, g/d <sup>3</sup>	36.9	36.0	9.54	0.92
Apparent disappearance, g/d				
Small intestine	717	707	20.5	0.67
Hindgut <sup>4</sup>	122	133	17.9	0.61
Total tract	812	814	6.42	0.89
Apparent disappearance, %				
Small intestine <sup>5</sup>	81.5	80.3	2.33	0.67
Hindgut <sup>5</sup>	74.4	75.6	3.82	0.79
Total tract	95.3	95.4	0.73	0.92

<sup>1</sup>LSM and SEM with starch infusion without or with amylase treatment ( $n = 4$ ).

<sup>2</sup>LSM and SEM with NaCl infusion prior to starch infusion ( $n = 4$ ).

<sup>3</sup>Calculated as fecal excretion – basal excretion.

<sup>4</sup>Calculated from additional excreted microbial N assuming an efficiency of CP synthesis of 1 g microbial N/100 g starch fermented (Ørskov et al., 1970).

<sup>5</sup>Based on the amount reaching each respective segment of the intestine.

Nevertheless,  $\alpha$ -linked glucose from bacterial origin might have been excreted in a greater extent with than without starch infusion in the first experiment or with increasing level of starch infusion in the second. Assuming a N content of microbial DM of 4.9 – 7.9 g N/100 g (Dickhoefer et al., 2016) and the observed increase of microbial N excretion of 0.12 g/100 g additionally infused, on average 0.6 – 0.9 g microbial DM/100 g of additionally infused starch were excreted. Considering that polysaccharides account for up to 20% of microbial DM (Storm and Ørskov, 1983), microbial polysaccharides might have contributed to fecal excretion of  $\alpha$ -linked glucose in amounts up to 0.2 g/100 g starch infused. Furthermore, the increased

excretion of endogenous N with starch infusion observed in the first experiment, might be based on an enhanced secretion of mucin glycoproteins. As up to 70% of the molecular weight of mucin glycoproteins are comprised by polysaccharides (Forstner, 1978), mucopolysaccharides might also have contributed to fecal excretion of  $\alpha$ -linked glucose, leading to an underestimation of starch disappearance from the total postruminal tract.

#### 4.2 STARCH DISAPPEARANCE FROM THE INTESTINAL TRACT

The results of experiments with diets containing high proportion of ruminally undegraded starch demonstrated that ruminants are able to digest large quantities of dietary starch postruminally, including starch digestion in the small intestine and fermentation in the hindgut. For example, a remarkable capacity for postruminal starch digestion up to 5.1 kg/d was observed in lactating dairy cows by McCarthy et al. (1989). In line with this, corn starch infused into the abomasum of heifers in amounts of 0.9 kg/d disappeared nearly completely (> 99%) from the total postruminal tract of heifers in our first experiment (Chapter 2.5.3). Considering the total amount of  $\alpha$ -linked glucose excreted with feces as well as the basal excretion of  $\alpha$ -linked glucose with infusion of isotonic saline solution, total tract disappearance averaged 95%. In our second experiment, starch disappearance from the total tract decreased from 90% to 80% with increasing amounts of starch infused from 1.0 to 2.0 kg/d (Chapter 3.4). Considering a basal excretion of  $\alpha$ -linked glucose in amounts of 24.6 g/d as observed in the first experiment under similar experimental conditions (e.g. animals, level of feed intake and dietary composition), starch disappearance from the total postruminal tract is considerably higher and decreased from 93% to 82%. Similar observations were made by Matthé (2001), who reported a reduction of postruminal starch disappearance from 95% to 79% with duodenal infusions of 0.9 and 1.8 kg/d, respectively.

#### 4.2.1 *Starch Disappearance from the Small Intestine*

For the evaluation of nutrient and energy supply by postruminal starch digestion, the extent of praecaecal starch digestion and absorption needs to be quantified and distinguished from hindgut fermentation. This quantification is difficult due to the large number of influencing factors, such as animal, dietary composition, level of feed intake, type of administered starch, analytical procedures as well as the experimental design (e.g. time for adaption to starch digestion). According to Owens and Soderlund (2006) the average disappearance of starch from the small intestine (as percentage of the intestinal flow) is greater in beef steers compared to lactating dairy cows, which was suggested to be caused by a lower DM intake (DMI) and the resulting lower rate of passage. It has to be noted, that this conclusion is based on a meta-analysis of different productions trials but not on a direct comparison within trials. However, the main factor influencing starch digestion in the small intestine might be the nature of the administered starch, which is known to vary not only between starch sources, but also between differently processed grain starch from the same grain type. For example, Zinn (1990) reported an apparent disappearance of steam-flaked corn starch in the small intestine of 94%, in contrast to 54% of dry-rolled corn starch. Furthermore, Rémond et al. (2004) reported a decreasing disappearance of starch from the small intestine from 67% to 47% with increasing particle size of ground corn from 0.7 to 3.7 mm. Experiments with abomasal and duodenal infusions of native starches circumvent influences of corn processing to directly address the effect of an increasing intestinal supply on starch disappearance from the small intestine. However, Matthé (2001) demonstrated that also differences between the postruminal disappearance of native starches from different grain types (corn vs. wheat) in cattle exist.

In the first experiment, the apparent disappearance of native corn starch from the small intestine of heifers was estimated to be 85% with abomasal infusion of 0.9 kg/d (Chapter 2.5.3). Considering the total amount of  $\alpha$ -linked glucose in feces as well as the basal excretion, the disappearance of  $\alpha$ -linked glucose from the small intestine in the first experiment was 81%.

This is within the range of published values of experiments with abomasal or duodenal infusions of corn starch in beef steers and dry cows (Table 9).

**Table 9.** Disappearance of abomasally or duodenally infused corn starch from the small intestine and the total postruminal tract of steers, dry cows and heifers

Reference	Animal	Type of starch	Starch flow to small intestine (kg/d)	Rate of starch infusion (g/h)	Starch disappearance			
					Small intestine		Postruminal tract	
					% <sup>1</sup>	kg/d	% <sup>1</sup>	kg/d
a)	Steers	NCS	0.20	8	59	0.12	94	0.19
			0.40	17	36	0.12	83	0.33
			0.60	25	46	0.26	67	0.18
b)	Steers	NCS	0.20	20	88	0.18	nd	nd
			0.40	40	66	0.26	nd	nd
			0.60	60	55	0.33	nd	nd
c)	Steers	CSH	0.32	10	97	0.15	99	0.27
			0.50	20	100	0.43	100	0.55
			0.92	40	88	0.83	100	1.09
d)	Dry cows	NCS	0.88	73	70	0.61	95	0.83
			1.75	146	50	0.88	79	1.38
e)	Steers	NCS	1.30	43	56	0.73	94	0.97
f)	Heifers	NCS	0.88	88	85	0.75	99	0.87

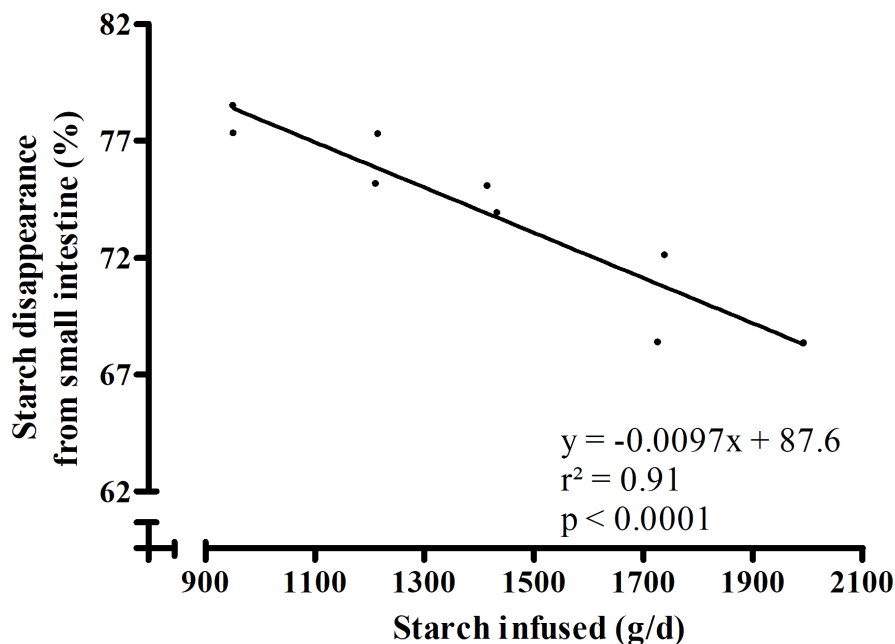
a: Little et al. (1968); b: Kreikemeier et al. (1991); c: Branco et al. (1999); d: Matthé (2001); e: Richards et al. (2002); f: results of our first experiment.

NCS = native corn starch; CSH = corn starch hydrolysate (digested by amylase prior to infusion); nd = not determined.

<sup>1</sup>Expressed as percentage of starch flow to the small intestine.

When all available values for the disappearance of corn starch from the small intestine of beef steers and heifers resulting from infusion trials as presented in Table 9 as well as results from feeding trials with ground or cracked corn starch (Table 1) are pooled and subjected to a linear regression analysis, starch disappearance from the small intestine averages  $69 \pm 8.7\%$  at an intestinal starch flow up to 1.2 kg/d (Figure 6). Values of the disappearance of corn starch from the small intestine of beef steers or heifers at intestinal starch flows exceeding 1.2 kg/d were lacking so far. However, starch disappearance from the small intestine was demonstrated to decrease from 78% to 68% when the intestinal supply was increased from 1.0 to 2.0 kg/d in our second experiment. This decrease is in line with the findings of Matthé (2001), who reported a

reduction of starch disappearance from the small intestine of dry cows from 70% to 50% when the intestinal supply increased from 0.9 to 1.8 kg/d. Hence, a decreasing efficiency of starch digestion in the small intestine of cattle with intestinal supplies exceeding 1.0 kg/d has to be expected.



**Figure 6.** The amount of starch disappearing from the small intestine of beef steers and heifers as a function of the intestinal starch flow. Data from our first experiment, Karr et al. (1966), Little et al. (1968), Russell et al. (1981b), Remillard et al. (1990), Zinn (1990), Kreikemeier et al. (1991), Branco et al. (1999) and Richards et al. (2002).

#### 4.2.2 Possible Factors Limiting Starch Disappearance from the Small Intestine

Possible factors limiting starch digestion in the ruminants' small intestine are well reviewed by Owens et al. (1986) and Huntington (1997). They include the capacity for glucose absorption, the time for starch hydrolysis, the access of enzymes to starch granules as well as the activity of hydrolyzing enzymes as dependent on a sufficient secretion, adequate working conditions and the absence of enzyme inhibitors. Results of previous experiments investigating the primarily limiting factor of postruminal starch digestion are contradicting. Mayes and Ørskov (1974) postulated that postruminal digestion of corn starch is limited by the capacity for glucose absorption when corn starch was infused into the abomasum of lambs. However, it has been

shown that glucose uptake by the Na<sup>+</sup>-dependent glucose transporter (SGLT1) is rapidly extended by increasing dietary glucose supply (Shirazi-Beechey, 1995). In steers, Bauer et al. (1995) observed a threefold increase in glucose uptake within 4 d when infused abomasally with starch hydrolysate. Correspondingly, the greater portal glucose flux with glucose infusion compared to starch infusion in the experiment of Kreikemeier and Harmon (1995) was taken as evidence that other factors than glucose absorption are limiting starch digestion in the small intestine. Huntington (1997) concluded from theoretical calculations and previous observations in literature that starch digestion in the small intestine is rather limited by the activity of hydrolyzing enzymes at least with intestinal supplies up to 1.5 kg/d in steers and up to 3.0 kg/d in lactating dairy cows.

The experiments reported herein aimed to demonstrate a limitation of starch digestion in the ruminants' small intestine due to an insufficient activity of pancreatic amylase. Higher excretion of lactate without amylase administration as observed in the first experiment indicated an effect of exogenous amylase on postruminal digestion of starch in animals not adapted to starch digestion (Chapter 2.5.2). It can be speculated that the infused amylase enhanced starch hydrolysis in the small intestine, and, thereby, affected the proportions of  $\alpha$ -glycosides (e.g. maltose, isomaltose and starch) and free glucose reaching the large intestine. However, the equal extent of MCP and VFA excretion in both treatments did not support the assumption of a limitation of postruminal starch digestion by insufficient activity of amylase in animals not adapted to starch digestion at an intestinal starch flow of 0.9 kg/d. Hence, the quantity of  $\alpha$ -glycosides and free glucose flowing to the hindgut potentially did not differ between treatments. Furthermore, the higher lactate excretion without amylase administration was only observed at the second day of starch infusion, indicating a fast adaption to postruminal starch digestion. This could either be explained by changes of the microbial population in the hindgut (e.g. increase of lactate-utilizing bacteria) or by an enhanced secretion of pancreatic amylase resulting in similar proportions of  $\alpha$ -linked and free glucose in ileal digesta for both treatments.

This is in line with the observations in the second experiment, where lactate excretion was unaffected by amylase administration when animals were adapted to starch digestion (Chapter 3.4). Furthermore, the results of the second experiment indicated that even at intestinal starch flows up to 2.0 kg/d starch disappearance from the small intestine is not limited by pancreatic amylase when animals were adapted to starch digestion within infusion periods of 30 d (Chapter 3.5.3). In a similar manner, Remillard et al. (1990) infused an exogenous amylase into the abomasum of steers and did not observe an effect on intestinal starch disappearance when animals were adapted to starch digestion within a 14-d preliminary period.

Mechanisms for adaptation to starch digestion by cattle's small intestine include an enhanced uptake of glucose (Bauer et al., 1995; Shirazi-Beechey, 1995) as well as an enhanced secretion of pancreatic amylase (Harmon, 1992). The latter was observed in earlier experiments when forage was replaced by grain in the diet (Harmon, 1992; Kreikemeier et al., 1990; Russell et al., 1981a). It was concluded that this effect depends mainly on the increased energy intake rather than the increased intake of dietary starch itself. On the other hand, Swanson et al. (2002) demonstrated that the presence of glucose or starch hydrolysate in the small intestine decreases the secretion of pancreatic amylase in ruminants. Similar observations were made by Walker and Harmon (1995). Therefore, missing effects of exogenous amylase in animals adapted to starch digestion might either be explained by reduced secretion of pancreatic amylase due to an increased luminal concentration of hydrolyzed starch or an enhanced endogenous secretion in animals not infused with amylase.

The possibility of inadequate working conditions must also be considered when interpreting missing effects of an exogenous amylase. The activity of hydrolyzing enzymes depends mainly on the pH with an optimum of 6.9 for amylase (Russell et al., 1981a). It can be suggested, that starch infusion decreases digesta pH below this optimum as an increasing proportion of concentrates in the diet is known to reduce pH (Russell et al., 1981a). However, Zinn and Owens (1980) reported a compensation for low intestinal pH by pancreatic bicarbonate

secretion. In line with this, exogenous supply of bicarbonate by jejunal infusion did not increase postruminal disappearance of abomasally infused corn starch (Remillard et al., 1990), therefore, lower digesta pH seems not to be the primary factor limiting postruminal starch digestion due to insufficient enzyme activity. Furthermore, the high dosage of amylase applied in the herein reported experiments should have compensated for any decrease in enzyme activity by suboptimal pH in the duodenum or the presence of enzyme inhibitors.

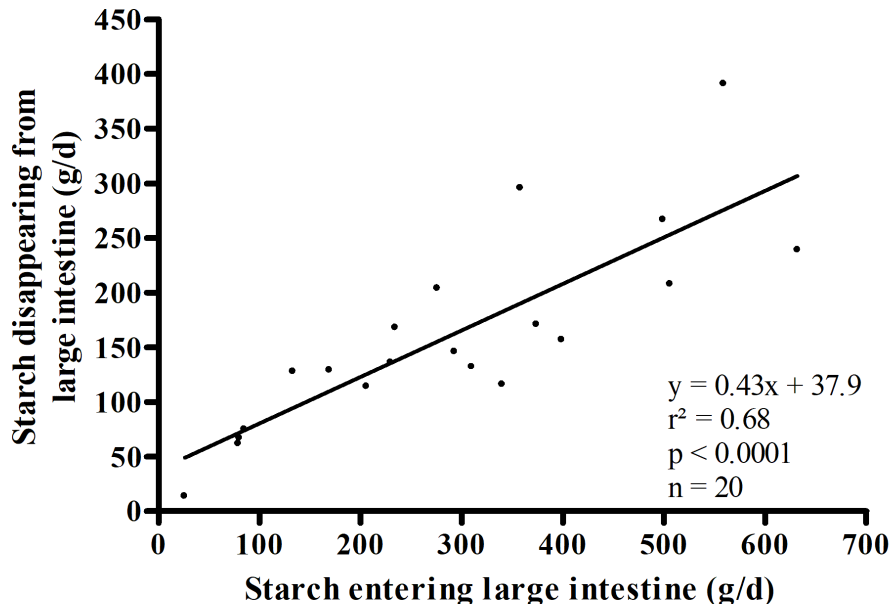
Assuming a sufficient activity of amylase at intestinal supplies up to 2.0 kg/d, a limitation due to an insufficient activity of other hydrolyzing enzymes must be considered. In line with this, Kreikemeier and Harmon (1995) suggested an insufficient activity of mucosal disaccharidases as large amounts of short-chain  $\alpha$ -glucosides and only little free glucose were flowing past the ileum after abomasal infusion of corn starch in beef cattle. Likewise, Gilbert et al. (2015b) suggested a limitation of intestinal starch digestion by maltase activity when varying sources of carbohydrates (gelatinized corn starch, maltodextrin and maltose) were fed to milk-fed calves. Therefore, experiments with subsequent infusions of starch and disaccharidases would be interesting to investigate the possibility of improving postruminal starch by exogenous supply of these hydrolyzing enzymes.

On the other hand, a limited access of hydrolyzing enzymes to starch granules cannot be excluded as possible limiting factor of intestinal starch digestion in the herein presented experiments. Larger starch granules are known to be less rapidly hydrolyzed in the small intestine compared to smaller ones because of their smaller available surface area in relation to their volume (Aggarwal and Dollimore, 1998). Therefore, if large starch granules were present in the infused native corn starch, they could have escaped enzymatic hydrolysis in the small intestine and reached the hindgut. This would also explain the rather low fermentability of the infused starch in the hindgut (Chapter 3.5.2) as larger starch granules are known to be less rapidly fermented (Owens et al., 1986).



#### 4.2.3 Starch Disappearance from the Large Intestine

Starch or end-products of starch hydrolysis, if praecaecally not absorbed, are fermented in the hindgut comparable to ruminal fermentation. In the second experiment a constant proportion of 12% of abomasally infused corn starch was fermented in the hindgut although starch flow to this segment increased disproportionately with increasing level of starch infusion due to the decreasing efficiency of starch digestion in the small intestine (Chapter 3.5.2). Hence, the hindgut seems to be incapable of compensating incomplete starch digestion within the small intestine. This leads to an enhanced excretion of starch with feces. When the results from the herein presented experiments and other trials with feeding or postruminal infusions of corn starch in beef steers are pooled and subjected to a linear regression analysis, on average 43% of starch entering the large intestine are fermented in this segment (Figure 7). This value is in line with the results from Harmon et al. (2004) and Owens and Soderlund (2006).



**Figure 7.** The amount of starch disappearing from the large intestine of beef steers and heifers as a function of the amount flowing to this segment. Data from both herein presented experiments, Karr et al. (1966), Little et al. (1968), Russell et al. (1981b) and Zinn (1990).

### 4.3 EVALUATION OF POSTRUMINAL STARCH DIGESTION

#### 4.3.1 *Net Glucose Absorption from Intestinal Starch Digestion*

With regard to the purpose of an increased glucose and energy supply to the animal's metabolism not only the disappearance of starch in the small intestine but net absorption of glucose and the transport of glucose to peripheral tissues must be considered. In line with this, the abomasal infusion of corn starch at a rate of 88 g/h was unable to increase glucose concentration in peripheral blood, although starch disappearance from the small intestine was estimated to exceed 80% (Chapter 2.5.4). Similarly, the concentration of glucose was not increased in jugular blood after abomasal infusion of corn starch in lactating dairy cows (Knowlton et al., 1998a) or beef steers (Kreikemeier et al., 1991) at rates of 68 and 60 g/h, respectively. In addition, Huntington and Reynolds (1986) did not observe an increased glucose concentration in blood samples drawn from the iliac artery after infusion of corn starch at a rate of 22.5 g/h into the abomasum of heifers, although the concentration of glucose in blood samples drawn from the portal vein slightly increased. The missing effect of starch infusion on glucose concentration in peripheral blood might be explained by endocrine regulation via an increased insulin:glucagon ratio in peripheral blood as observed in the first experiment (Chapter 2.4.3). Similar observations were made by Knowlton et al. (1998a). Insulin and glucagon are known to be the key regulators of blood glucose concentration whereby these hormones have countervailing effects. Insulin decreases blood glucose concentration by increasing liver glycogen synthesis, glucose uptake into peripheral tissues and inhibiting liver gluconeogenesis (Saltiel and Kahn, 2001). Glucagon raises blood glucose by increasing hepatic gluconeogenesis and glycogenolysis and decreasing glycolysis and glycogen synthesis (Jiang and Zhang, 2003). Therefore, the missing effect of starch infusion and increased glucose absorption on blood glucose concentration might be explained by increased liver glycogen synthesis, an increased glucose uptake into peripheral tissues or a decreased rate of gluconeogenesis. Yet, results from in vitro data with hepatocytes from cows indicated that hepatic gluconeogenesis from

propionate is insulin-independent (Donkin and Armentano, 1995) and propionate is the major precursor substance for gluconeogenesis in ruminants. Thus, increasing liver glycogen synthesis or glucose uptake into peripheral tissues might be the predominant modes of action for insulin.

However, the missing effects of postruminal starch infusions might as well be based on glucose utilization by gut tissues, as indicated by negative values for net glucose absorption with water infusion in experiments of Kreikemeier et al. (1991) and Huntington and Reynolds (1986). Wahle et al. (1971) suggested that appreciable amounts of luminal glucose are directly metabolized to lactate within the enterocytes during absorption. In contrast, Reynolds (2005) suggested an increased utilization of arterial glucose by the portal-drained viscera (PDV) after abomasal infusion of corn starch, rather than a glucose metabolism during absorption. The PDV tissues, including the digestive tract, pancreas, spleen and the associated adipose tissues, show a high rate of tissue turnover and secretory processes (Piccioli Cappelli et al., 1997). The greater glucose use by PDV might not only be based on enhanced secretory processes as well as gut cell metabolism and maintenance but also on fat retention in visceral fatty tissues. Correspondingly, McLeod et al. (2007) demonstrated that an increased lipogenesis in gut tissues occurred when glucose was infused into the abomasum of steers. Similar observations were made by Rust (1983), when glucose was infused into the abomasum of lambs. 87% of the additionally provided energy was assumed to be used for lipogenesis at gut tissues. Findings of Kreikemeier et al. (1991) indicated that only 38% of starch disappearing from the small intestine of steers could be accounted for as net glucose absorption when corn starch was infused into the abomasum of steers. Huntington and Reynolds (1986) reported a similar value of 35% when corn starch was infused into the abomasum of heifers, which was considered to be caused by an enhanced glucose utilization by gut tissues.

Previous experiments demonstrated that microbial fermentation not only occur in the hindgut but also in the distal part of the ileum (Mayes and Ørskov, 1974; Gilbert et al., 2015a). Consequently, the low value of net glucose absorption reported in the experiments of Kreikemeier et al. (1991) and Huntington and Reynolds (1986) might not only be based on an enhanced glucose use by gut tissues but also on ileal fermentation and absorption of VFA. In the experiment of Gilbert et al. (2015a), only 34% of praecaecally disappearing starch were attributed to enzymatic hydrolysis whereas 66% were fermented in the ileum. However, in the experiment of Kreikemeier and Harmon (1995), the concentration of VFA in ileal digesta was not increased after abomasal infusion of corn starch when compared to water infusion. In contrast, Huntington and Reynolds (1986) observed an increased net absorption of lactate with abomasal infusion of starch in heifers, which could be based either on ileal fermentation or on gut metabolism as described before. Nevertheless, noteworthy amounts of infused starch were fermented in ileum and caecum in the herein presented experiments as demonstrated by increased VFA and microbial N excretion. In line with this, an enhanced absorption of propionate and butyrate would also explain the increased insulin secretion observed in the first experiment as these substances are also known to stimulate insulin secretion if absorbed amounts exceed the hepatic utilization capacity (Horino et al., 1968; Nocek and Tamminga, 1991). In conclusion, even if starch entering the small intestine would be completely hydrolyzed, the advantage of shifting starch digestion from the rumen to the small intestine would probably be diminished by two reasons: the limited transport of glucose to peripheral tissues due to its metabolization in the enterocytes and the increased utilization of arterial glucose by the PDV tissues.

#### *4.3.2 Effects of Postruminal Starch Digestion on Nitrogen Utilization*

One major disadvantage of shifting starch digestion from the rumen to the small intestine is probably the reduced energy supply for ruminal microbes and, therefore, the reduced supply of MCP from ruminal fermentation to the host animal. According to Czerkawski (1978) an average

efficiency of ruminal MCP synthesis of  $19.3 \pm 2.0$  g microbial N/kg of truly fermented organic matter can be assumed. In line with this, data of previous studies demonstrated smaller quantities of microbial N flowing to the duodenum of lactating dairy cows (237 vs. 286 g/d) when corn-based diets instead of barley-based diets were fed with ruminal starch degradabilities of 35% and 69%, respectively (Yang et al., 1997). Similar observations were made by Poore et al. (1993) when steam-flaked sorghum grain with ruminal degradability of 74% was replaced by dry-rolled sorghum grain with ruminal degradability of 48% and microbial N flow decreased from 448 to 349 g/d. However, the efficiency of ruminal MCP synthesis does not only rely on the extent and rate of carbohydrate degradation, but also on the rate of protein degradation (Russell et al., 1992) and other factors, like ruminal pH, ruminal dilution rate and the feeding frequency on high concentrate diets (Broderick and Merchen, 1992). For example, a ruminal pH < 6 is known to reduce the efficiency of ruminal MCP synthesis (Russell et al., 1992). Thus, shifting starch digestion from the rumen to the small intestine might be beneficial for the efficiency of MCP synthesis if it prevents a reduction of ruminal pH by decelerating ruminal fermentation. Furthermore, ruminants fulfill their demand for digestible protein not only by ruminal MCP synthesis, but also by intestinal digestion of ruminally undegraded protein. Hence, reduced MCP supply from ruminal fermentation with greater postruminal starch digestion might be compensated by increasing the proportion of ruminally undegraded protein in the diet. However, this might be questionable from the economic perspective as eligible feedstuffs rich in ruminally undegraded protein tend to be rather expensive.

Another concern about postruminal starch digestion regarding N metabolism is an incomplete digestion and absorption of starch in the small intestine, which causes an enhanced fermentation of starch in the hindgut and therefore, an increased excretion of microbial N with feces. According to Armstrong and Smithard (1979) there is no evidence that amino acids in microbial matter resulting from hindgut fermentation are released and absorbed from the large intestine. In the herein presented experiments fecal excretion of microbial N increased on average by

0.12 g/100 g of starch infused. In line with this, the apparent total tract disappearance of N (ATTD<sub>N</sub>) decreased from 57.2% with infusion of isotonic saline solution to 48.9% with abomasal starch infusion in amounts of 880 g/d as observed in the first experiment. In the second experiment, the ATTD<sub>N</sub> further decreased from 39.7% to 38.5% with infusion amounts of 0.9 and 2.0 kg/d, respectively (Table 10). On the other hand, urinary N excretion might have decreased with starch infusion as observed by Reynolds et al. (2001) due to an enhanced transfer of N to the hindgut. However, this parameter was not determined in the herein presented experiments.

**Table 10.** Intake, fecal excretion and apparent total tract disappearance (ATTD<sub>N</sub>) of nitrogen at increasing amounts of corn starch being infused into the abomasum of heifers

Experiment	Amount of starch infused, g/d						
	0	880	953	1213	1425	1733	1993
	1	1	2	2	2	2	2
Intake, g/d	80.9	81.4	76.8	77.0	77.1	77.2	77.4
Dietary N	80.9	80.9	76.3	76.3	76.3	76.3	76.3
Infused N <sup>1</sup>	0.0	0.5	0.5	0.7	0.8	0.9	1.1
Fecal excretion, g/d	34.6	41.6	46.3	45.1	46.4	46.6	47.6
Microbial N	8.9	10.0	9.4	9.7	9.8	10.2	10.7
Dietary and endogenous N	25.7	31.6	36.9	35.4	36.6	36.4	35.9
ATTD <sub>N</sub> , %	57.2	48.9	39.7	41.4	39.7	39.6	38.5

<sup>1</sup>Analyzed N content of the infused starch product is 0.05%.

Beside the increased excretion of microbial N, the results of the first experiment indicated an increased excretion of endogenous N when corn starch was infused into the abomasum of heifer not adapted to starch digestion. An amount of 0.68 g endogenous N/100 g starch infused was additionally excreted when compared to infusion of isotonic saline solution. However, the possible underestimation of microbial N cannot be excluded (Chapter 4.1.2), so endogenous N excretion might be overestimated. Nevertheless, the increased excretion of endogenous N might be based on mechanisms of adaption to starch digestion in the intestinal tract like an enhanced secretion of pancreatic juice (Swanson et al., 2002) or an enhanced mucin secretion. This is supported by the fact that the incremental increase of endogenous N excretion, calculated as the difference of total N and microbial N additionally excreted, showed a lower value of

0.02 g/100 g of infused starch when animals were adapted to starch infusion in the second experiment (Chapter 3.4).

#### *4.3.3 Efficiency of Energy Utilization from Starch Digestion*

Both, ruminal fermentation and intestinal digestion of starch are inevitable accompanied by energetic losses and costs. In ruminal fermentation, energy losses are mainly related to methane production and heat of fermentation. The latter is assumed to be rather constant and to amount for up to 6.4% of the gross energy (GE) of the carbohydrate fermented (Hungate, 1966). Methane production depends on several factors, including the level of feed intake, the dietary composition and the digestibility of dietary components (Danielsson, 2016). According to Blaxter and Clapperton (1965) the percentage of GE converted into methane ranges between 6.2% and 10.8% at maintenance level feeding, whereby the higher value was observed for pelleted meadow-fescue grass while the lower value was observed for sugar-beet pulp given as sole feed with apparent ruminal DM degradabilities of 55% and 87%, respectively. McLeod et al. (2006) stated that methane losses from starch fermentation account for 6.8% to 9.6% of the GE, so that direct costs of ruminal fermentation sum up to 16% of starch GE on average.

Indirect energetic costs of ruminal starch fermentation must also be considered. Energy requirements for absorption of VFA from the rumen by passive diffusion are negligible (McLeod et al., 2006), but energy in form of adenosine triphosphate (ATP) is expended during glucose generation from propionate by hepatic gluconeogenesis. According to Krebs et al. (1967) 6 mol ATP are required for the generation of 1 mol glucose from pyruvate. Assuming a minimum heat loss associated with the formation and use of 1 mol ATP of 75 kJ approximately 16% of the potential energy of glucose (2.8 MJ/mol) is expended (Baldwin, 1968). On the other hand, a portion of the fermented energy is captured in the resulting microbial matter and can be released by enzymatic hydrolysis in the small intestine (McLeod et al., 2006). Thereby a digestibility of ruminal microbes in the small intestine of 90% can be assumed (Black, 1971).

Energetic costs of starch hydrolysis and glucose absorption in the small intestine must be considered when starch is digested postruminally. According to Harmon and McLeod (2001), 0.6% of the digestible energy (DE) of starch are expended due to the release of heat as the result of glycosidic bond cleavage during starch hydrolysis. Additionally, the absorption of glucose via SGLT1 utilizes ATP in an indirect manner as it depends on the action of the Na<sup>+</sup>/K<sup>+</sup>-ATPase (Shirazi-Beechey, 1995). According to Baldwin (1968), energy losses attributed to absorption account for 1 mol ATP per mol glucose, which equals 2.6% of the potential energetic value of glucose. A portion of glucose is absorbed by passive diffusion, but this mechanism is assumed to be of little importance in cattle contributing less than 8% to total transport (Krehbiel et al., 1996). According to Harmon and McLeod (2001) total energy losses attributed to starch hydrolysis and glucose absorption in the small intestine of 3.2% of starch DE can be assumed (Harmon and McLeod, 2001). However, additional energetic losses, like costs for the incorporation of glucose transporters into the brush border membrane as well as an enhanced enzyme secretion must be considered. Yet, these processes can hardly be quantified.

To predict production responses of growing or lactating cattle to changes in the site of starch digestion, not only the energetic losses attributed to digestion and absorption, but also the energetic efficiency of end-product utilization for maintenance and production must be considered. Owens et al. (1986) demonstrated by multiple linear regression of gain-to-feed ratios and site of starch digestion in beef steers, that the total energetic efficiency of ruminal starch fermentation, defined as energy retained in body tissues divided by metabolizable energy (ME) from starch intake, is 70% of the energetic efficiency of starch digested and absorbed from the small intestine. This is quite similar to the observation of Harmon and McLeod (2001), that ruminal starch fermentation is only 73% as efficient as starch digestion in the small intestine. Those calculations reflect not only the sum of energy losses attributed to digestion and absorption (e.g. methane energy, urine energy, fecal energy due to microbial residues from ruminal fermentation and endogenous secretion), but also the energy losses due to heat



production by fermentation, digestion and utilization of digestion end-products for maintenance and production.

However, results from the herein presented experiments demonstrate that postruminal starch digestion, especially when originating from corn, is inevitable accompanied by starch fermentation in the hindgut due to an incomplete digestion and absorption within the small intestine. Although less methane is produced by caecal fermentation, hindgut fermentation is assumed to be less efficient than ruminal fermentation, because the resulting microbial matter and the therein incorporated energy (15% to 30% of DE) is excreted with feces (McLeod et al., 2006). According to Harmon and McLeod (2001) starch fermentation in the hindgut can be assumed to be only 54% as efficient than starch digestion in the small intestine. Hence, an increased fermentation of starch in the hindgut can be assumed to override any energetic benefits of starch digestion in the small intestine.

In Table 11 a model calculation of the energy yield of 1 kg starch being either fermented in the rumen to an extent of 75% and further digested postruminally or digested in the small intestine to an extent of 75% to 80% is presented. Starch disappearance from the total postruminal tract was assumed to be 95% as observed in our first experiment at an intestinal starch flow of 0.9 kg/d. It was assumed that starch disappearance from the total tract of ruminal escape starch is 99% and starch disappearance from the small intestine is 93% at this low level of intestinal starch flow as observed by Karr et al. (1966). These calculations indicate, that the energy provided by 1 kg starch being digested in the small intestine is greater compared to ruminal fermentation, if starch disappearance from the small intestine exceeds 77%. It must be noted that the energetic costs for gluconeogenesis from propionate were not considered as costs of ruminal fermentation. Likewise, indirect costs of starch digestion in the small intestine, like energetic costs for the incorporation of glucose transporters into the brush border membrane

and enzyme secretion are not included into calculations. Furthermore, the energy incorporated in microbial matter resulting from ruminal fermentation must additionally be considered.

**Table 11.** Model calculation of the energy yield of 1 kg starch being either fermented to an extent of 75 % in the rumen or being digested in the small intestine assuming starch disappearances between 75 and 80%

Ruminal starch flow, kg	1.00			
Ruminal disappearance, %	75			
Intestinal starch flow, kg	0.25	1.00	1.00	1.00
Disappearance from small intestine, %	93	80	77	75
Total tract disappearance, % <sup>1</sup>	99	95	95	95
Ruminally fermented energy, MJ <sup>2</sup>	13.1			
Energy losses by fermentation <sup>3</sup>	2.1			
Energy yield as VFA	11.0			
Small intestine digested energy, MJ <sup>2</sup>	4.1	14.0	13.5	13.1
Energy costs for hydrolysis and absorption <sup>4</sup>	0.1	0.4	0.4	0.4
Energy yield as glucose	3.9	13.6	13.0	12.7
Hindgut fermented energy, MJ <sup>2</sup>	0.1	2.6	3.2	3.5
Energy losses by fermentation <sup>5</sup>	0.0	0.9	1.1	1.2
Energy yield as VFA	0.1	1.7	2.1	2.3
Energy yield, MJ	15.1	15.3	15.1	15.0

<sup>1</sup>Based on the results of our first experiment at an intestinal starch flow of 0.9 kg/d and results from Karr et al. (1966) for ruminal escape starch.

<sup>2</sup>Calculated as kg starch fermented or digested in respective segment  $\times$  17.5 kJ/g (gross energy of starch)

<sup>3</sup>Calculated as ruminally fermented energy  $\times$  0.16.

<sup>4</sup>Calculated as small intestine digested energy  $\times$  0.032.

<sup>5</sup>Assuming energy losses of 40% of hindgut fermented energy, including hexose energy incorporated in excreted microbial matter.

Results from our second experiment as well as results from previous experiments with abomasal or duodenal infusions of native starches indicated a decreasing efficiency of starch digestion in the small intestine and the total postruminal tract (e.g. increased excretion of starch with feces) with increasing intestinal starch supply (Chapter 4.2.1). Therefore, the energy of 5 kg of starch provided by digestion at decreasing ruminal degradabilities from 80% to 60% resulting in increasing starch flows to the small intestine from 1.0 to 2.0 kg/d is presented in Table 12. Values of starch disappearance from the total postruminal tract and the small intestine at increasing intestinal starch supplies are based on the results from the second experiment. Energy losses attributed to hindgut fermentation due to methane formation, thermal heat and the incorporated energy in the resulting microbial matter, which is excreted with feces, were

assumed to sum up to 40% of the energy from starch fermentation in the hindgut. It must be noted, that presumed values of starch disappearance from the small intestine are not based on ruminally undegraded starch as under practical feeding conditions, but an infusion of native corn starch. Furthermore, indirect costs of starch digestion in the small intestine, like energetic costs for the incorporation of glucose transporters into the brush border membrane and enzyme secretion are not included into calculations. Likewise, energetic costs for gluconeogenesis from propionate were not considered as costs of ruminal fermentation. Nevertheless, these calculations indicate that reducing starch fermentation in the rumen in order to increase postruminal digestion in amounts exceeding 1.0 kg/d does not improve the energy supply for maintenance and productive purpose. Therefore, at intestinal starch flows this high, the decreasing efficiency of starch digestion in the small intestine with increasing intestinal supplies can be assumed to override any gains from greater energetic efficiency of intestinal starch digestion.

**Table 12.** An estimate of energy yield of 5 kg ingested corn starch being fermented in variable proportions in the rumen

Ruminal starch flow, kg/d	5.00	5.00	5.00	5.00	5.00
Ruminal disappearance of starch, %	80	75	70	65	60
Intestinal starch flow, kg/d	1.00	1.25	1.50	1.75	2.00
Disappearance from small intestine, % <sup>1</sup>	78	75	73	71	68
Total postruminal disappearance, % <sup>1</sup>	90	88	86	82	80
Ruminally fermented energy, MJ <sup>2</sup>	70.0	65.6	61.3	56.9	52.5
Energy losses by fermentation <sup>3</sup>	11.1	10.4	9.7	9.0	8.3
Energy yield as VFA	58.9	55.3	51.6	47.9	44.2
Small intestine digested energy, MJ <sup>2</sup>	13.7	16.4	19.2	21.7	23.8
Energy costs for hydrolysis and absorption <sup>4</sup>	0.4	0.5	0.6	0.7	0.8
Energy yield as glucose	13.2	15.9	18.5	21.0	23.0
Hindgut fermented energy, MJ <sup>2</sup>	2.1	2.8	3.4	3.4	4.2
Energy losses by fermentation <sup>5</sup>	0.8	0.9	1.1	1.1	1.4
Energy yield as VFA	1.3	1.9	2.3	2.2	2.8
Energy yield, MJ	73.4	73.0	72.4	71.2	70.0

<sup>1</sup>Based on the results of the second experiment and related to the respective intestinal starch flow.

<sup>2</sup>Calculated as kg starch digested in respective segment × 17.5 kJ/g (gross energy of starch)

<sup>3</sup>Calculated as ruminally fermented energy × 0.16.

<sup>4</sup>Calculated as small intestine digested energy × 0.032.

<sup>5</sup>Assuming energy losses of 40% of hindgut fermented energy, including hexose energy incorporated in excreted microbial matter.

#### *4.3.4 Production Response of Lactating Dairy Cows and Beef Cattle*

In line with the previous calculations, Nocek and Tamminga (1991) concluded in a literature review that production studies with feeding of differently processed grain starch did not show a clear evidence for benefits of enhancing postruminal starch digestion in lactating dairy cows. Furthermore, Firkins et al. (2001) summarized from the results of several production trials, that milk yield decreased when steam-flaked corn with a ruminal starch degradability of 52% was replaced by steam-rolled corn with lower ruminal starch degradability of 35%. In contrast, McCarthy et al. (1989) observed an increased milk yield when barley-based diets were replaced by corn-based diets with lower ruminal starch degradability (76% vs. 52%, respectively). In a similar manner, Huck et al. (1998) reported an increased weight gain and feed efficiency of yearling steers when steam-flaked sorghum was partially replaced by dry-rolled corn with lower ruminal starch degradability. However, these benefits must not necessarily be based on an improved energetic efficiency of glucose supply from postruminal starch digestion, as they might also result from slower ruminal starch digestion and therefore reduced incidences of ruminal acidosis (Owens et al., 1998). Additionally, an improved efficiency of ruminal protein synthesis due to a decelerated ruminal fermentation can be suggested (Chapter 4.3.3). Furthermore, processing of cereal grains usually not only alters the site of starch digestion, but also the digestibility of starch in the total tract as well as DMI. For example, DMI was higher with corn-based diets than with barley-based diets (23.3 vs. 20.5 kg/d, respectively) in the experiment of McCarthy et al. (1989). In contrast, DMI was unaffected in the review of Firkins et al. (2001), but the digestibility of starch in the total tract was clearly higher for steam-flaked corn than for steam-rolled corn (96% vs. 87%, respectively). Hence, greater glucose availability for lactose synthesis from glucose or VFA absorption can be assumed to be the main reason for higher milk yields rather than a greater energetic efficiency of starch utilization. In conclusion, increased milk yield of dairy cows and weight gains of beef cattle might be attained under

practical feeding conditions when postruminal starch flow is increased without decreasing the total tract disappearance of starch or if attributed to greater DMI.

#### 4.4 CONCLUSIONS

The results from this thesis demonstrate that postruminal digestion of corn starch in cattle is inevitable accompanied by an increased fermentation of starch in the hindgut, reflecting an incomplete digestion in the small intestine. Starch disappearance from the small intestine decreased with increasing amounts being infused into the abomasum of heifers, indicating a decreasing efficiency of starch digestion in the small intestine. However, the administration of exogenous amylase did not improve starch disappearance from the small intestine, so other factors than pancreatic amylase activity seem to be primarily limiting postruminal starch digestion in cattle. Yet, even if postruminally supplied starch is completely hydrolyzed within the small intestine, greater glucose availability from starch digestion is assumed to be overridden by an enhanced utilization of glucose by gut tissues. In line with this, glucose concentration in peripheral blood was unaffected by abomasal infusion of 0.9 kg/d of corn starch, although 85% of the infused starch disappeared from the small intestine. It is suggested that the absorbed glucose rather increases fat deposition in intestinal tissues than glucose availability for growth or lactation performance in cattle (McLeod et al., 2007). Therefore, postruminal digestion of starch must not necessarily improve the production efficiency of cattle, although it prevents methane and heat energy losses attributed to ruminal fermentation. Considering only the energetic aspects, starch can efficiently be digested in the small intestine in amounts up to 1.0 kg/d. At greater intestinal flows, an enhanced fermentation of starch in the hindgut and a decreasing total tract disappearance of starch eliminate energetic advantages of starch digestion in the small intestine.

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## 5 Summary

Diets containing high proportion of cereal grain are typically fed to lactating dairy cows and high-producing beef cattle in order to meet their enhanced energy requirements. Hence, these animals consume large amounts of dietary starch, contributing up to 80% of cereal grains' dry matter. Compared to ruminal fermentation of starch, which is attributed to noteworthy energy losses in form of methane and thermal heat, starch digestion in the small intestine is assumed to be energetically more efficient. Therefore, shifting starch digestion from the rumen to the small intestine is assumed to increase the total energetic efficiency of starch utilization in cattle. For this purpose, the capacity for starch digestion in the small intestine of cattle and possible limiting factors need to be determined. Previous experiments demonstrated a decreasing efficiency of starch digestion in the small intestine with increasing intestinal supply. Possible factors limiting starch digestion in the small intestine are well discussed in literature, including an insufficient activity or secretion of hydrolyzing enzymes, limited time for starch hydrolysis, a restricted capacity for glucose absorption, as well as limited access of enzymes to starch granules.

Aim of the present thesis was to investigate the capacity for starch digestion in the small intestine of cattle and demonstrate a limitation due to an insufficient activity of pancreatic amylase. For this purpose, corn starch was infused into the abomasum of four rumen-cannulated heifers with or without exogenous amylase. In the first experiment, corn starch was infused in moderate amounts of 0.9 kg/d, aiming to prevent cases of diarrhea due to excessive fermentation of starch in the hindgut. Baseline values for fecal and blood parameters were determined by infusion of isotonic saline solution prior to starch infusion. Higher fecal excretion of microbial crude protein (MCP) and volatile fatty acids (VFA) with than without starch infusion indicated an enhanced fermentation of starch in the hindgut, which indicates an incomplete digestion of the infused starch in the small intestine. However, the efficiency of starch digestion in the small intestine was quite high as 85% of the infused starch were estimated

to disappear from the small intestine. In line with this, exogenous amylase did not improve starch digestion, which was suggested to be based on a sufficient secretion of pancreatic amylase at this level of starch infusion. Glucose concentration in peripheral blood drawn from the jugular vein was unaffected by starch infusion, but the insulin:glucagon ratio increased. The latter might reflect the endocrine regulation of blood glucose due to an enhanced glucose absorption, but could also result from increased VFA absorption from hindgut fermentation.

In the second experiment, corn starch was abomasally infused at 5 infusion levels, which stepwise increased from 1.0 to 2.0 kg/d, with or without exogenous amylase. Results demonstrated a decreasing efficiency of starch digestion in the small intestine of cattle as observed in previous experiments. Starch disappearance decreased from 78 to 68% with intestinal starch supplies of 1.0 and 2.0 kg/d, respectively. Furthermore, the results demonstrated a limited ability of the hindgut to compensate the decreasing efficiency of starch digestion in the small intestine, leading to an increasing excretion of starch with feces with increasing intestinal flow of starch. Amylase administration did not improve starch digestion in the small intestine, indicating other factors rather than pancreatic amylase activity being the primarily factor limiting starch digestion in the small intestine of cattle.

In conclusion, the decreasing efficiency of starch digestion with increasing intestinal supply in combination with the increasing excretion of starch and MCP with feces, can be assumed to override any gains in the energetic efficiency of starch digestion in the small intestine compared to ruminal fermentation.



## 6 Zusammenfassung

Getreidereiche Rationen werden häufig eingesetzt, um den erhöhten Energiebedarf von laktierenden Milchkühen und Mastrindern mit hoher Leistung zu decken. Im Zuge dessen nehmen die Tiere große Mengen an Stärke über das Futter auf, welche bis zu 80% der Trockenmasse von Getreidekörnern ausmacht. Im Vergleich zur Fermentation von Stärke im Pansen, welche mit nennenswerten Energieverlusten in Form von Methan und Fermentationswärme einhergeht, wird der Stärkeverdauung im Dünndarm eine höhere energetische Effizienz zugesprochen. Somit könnte die gezielte Verlagerung der Stärkeverdauung vom Pansen in den Dünndarm eine erhöhte energetische Effizienz der Stärkenutzung beim Rind bewirken. Für diesen Zweck wäre es wichtig, die Kapazität und mögliche begrenzende Faktoren der Stärkeverdauung im Dünndarm von Rindern zu bestimmen. Bisherige Studien zeigten, dass die Effizienz der Stärkeverdauung im Dünndarm mit zunehmender Anflutung von Stärke abnimmt. Als mögliche begrenzende Faktoren werden eine ungenügende Aktivität oder Sekretion von hydrolysierenden Enzymen, eine begrenzte Zeit für die Stärkehydrolyse, eine unzureichende Kapazität der Glukoseabsorption, sowie ein begrenzter Zugang für hydrolysierende Enzyme zu den Stärkegranula diskutiert.

Ziel der vorliegenden Arbeit war es, die Kapazität der Stärkeverdauung im Dünndarm beim Rind zu bestimmen und eine Limitierung der selbigen durch die Aktivität der Pankreas-Amylase zu zeigen. Zu diesem Zweck wurde Maisstärke mit oder ohne gleichzeitige Infusion einer exogenen Amylase in den Labmagen vierer pansenfistulierter Färsen infundiert. In einem ersten Versuch wurde eine moderate Menge an Maisstärke von 0.9 kg pro Tier und Tag infundiert, um Durchfälle aufgrund einer exzessiven Fermentation von Stärke im Dickdarm zu vermeiden. Zur Bestimmung der Basalwerte wurde vorab eine Infusion einer isotonischen Kochsalzlösung vorgenommen. Die mit Stärkeinfusion erhöhte Ausscheidung an mikrobiellem Rohprotein (MXP) und flüchtigen Fettsäuren (FFS) über den Kot deutete auf eine Fermentation von Stärke im Dickdarm und somit eine unvollständige Verdauung und Absorption der

infundierten Stärke im Dünndarm hin. Dennoch kann von einer recht hohen Effizienz der Stärkeverdauung ausgegangen werden, da schätzungsweise 85% der infundierten Stärke im Dünndarm verschwanden. In Übereinstimmung hiermit bewirkte die exogene Amylase keine Verbesserung der Stärkeverdaulichkeit im Dünndarm, was vermutlich in einer ausreichenden endogenen Sekretion der Pankreas-Amylase begründet war. Die Glukose-Konzentration im peripheren Blut, welches aus der Jugularvene entnommen wurde, wurde nicht durch die Stärkeinfusion beeinflusst, jedoch wurde ein weiteres Verhältnis von Insulin:Glukagon festgestellt. Letzteres könnte sowohl die endokrine Regulation der Blutglukose, als auch eine erhöhte Absorption von FFS im Zuge der Dickdarmfermentation widerspiegeln.

In einem zweiten Versuch wurden Stärkemengen in 5 Stufen ansteigend von 1 auf 2 kg pro Tier und Tag mit oder ohne zusätzliche Infusion einer exogenen Amylase infundiert. Die Ergebnisse zeigten eine abnehmende Effizienz der Stärkeverdauung im Dünndarm mit zunehmender Stärkeanflutung. Die Verdaulichkeit der Stärke sank von 78% bei einer Stärkeanflutung von 1 kg pro Tag auf 68% bei 2 kg pro Tag. Darüber hinaus zeigten die Ergebnisse ein begrenztes Potenzial des Dickdarms, die abnehmende Effizienz der Stärkeverdauung im Dünndarm zu kompensieren, sodass ein zunehmender Anteil der infundierten Stärke über den Kot ausgeschieden wurde. Die Zulage der exogenen Amylase bewirkte keine Verbesserung der Stärkeverdauung im Dünndarm, sodass andere Faktoren als die Aktivität der Pankreas-Amylase die Stärkeverdauung im Dünndarm von Rindern zu begrenzen scheinen.

Zusammenfassend lässt sich sagen, dass davon ausgegangen werden kann, dass die abnehmende Effizienz der Stärkeverdauung im Dünndarm sowie die zunehmende Ausscheidung an Stärke und MXP über den Kot mit zunehmender Stärkeanflutung am Dünndarm, mögliche energetische Vorteile der Stärkeverdauung im Dünndarm gegenüber der Pansenfermentation aufheben.

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