

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

**Response of urinary purine derivatives excretion to nutritional
factors affecting ruminal microbial protein synthesis in cattle**

Dissertation

Zur Erlangung des Doktorgrades der
Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

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M.Sc. Veronika Sandra Ahnert

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1 General introduction

The ruminant digestive system maintains a symbiotic relationship with ruminal microorganisms. This symbiosis enables the host animal to utilize fibrous plant biomass and non-protein nitrogen (N) as food resources that would not be utilizable otherwise and make ruminants independent of external sources of B-vitamins and amino acids (Van Soest, 1982). The ruminal fermentation is a result of physical and microbiological activities converting complex lignocellulosic plant material into microbial biomass and valuable fermentation end products (i.e. short-chain fatty acids) that can be used by the host animal. Microbial fermentation occurs under continuous-culture conditions, whereby the frequent intake of feed, removal of end products, and outflow of microbial biomass enables a continual growth of ruminal microbes (Edwards et al., 2008). The microbial biomass which is not recycled within the rumen and leaves the rumen accounts for more than 50 % of protein available for absorption at the small intestine (Clark et al., 1992) and greatly contributes the animals' supply of essential amino acids. The microbial crude protein (MCP) is characterized by a high and relatively constant quality because it is rich in most of the essential amino acids (Clark et al., 1992). The rumen microbial ecosystem is complex and highly diverse, consisting of bacteria, archaea, protozoa, and fungi. Growth of rumen microbes mainly depends on the availability of N sources (i.e. true protein, non-protein N) and of energy for protein synthesis coming from the fermentation of structural and non-structural carbohydrates which provides carbon skeletons (Clark et al., 1992; Stern et al., 1994; Bach et al., 2005). If dietary rumen degradable protein is in excess relative to the amount required by rumen microbes, ammonia-N is absorbed, metabolized to urea in the liver, and excreted via urine and milk. Therefore, the synchrony of the availability of ruminal degradable protein and carbohydrate may influence microbial growth and efficiency of MCP synthesis (Kim et al., 1999; Dewhurst et al., 2000; Bach et al., 2005), although reports in the literature are inconsistent (Reynolds and Kristensen, 2008; Hall and Huntington, 2008). The yield of microbial biomass is not solely determined by the availability of nutrients for microbial growth, but also by the energy used by rumen microbes for maintenance which in turn depends on the maintenance requirements and growth rate of the microbial species (Dewhurst et al., 2000). Therefore, the efficiency of MCP synthesis (i.e. g MCP/ kg organic matter fermented) is not constant. Besides these nutritional factors mentioned, other non-nutritional factors such as ruminal pH value (Strobel and Russell, 1986), rate of digestion and passage (Firkins et al., 1998), and defaunation (Jouany et

al., 1988; Broudiscou and Jouany, 1995) influence microbial growth and efficiency of MCP synthesis.

Despite the advantages of ruminal fermentation, the conversion of dietary protein into the products (e.g. meat, milk) is less efficient compared to non-ruminants (Tamminga, 1992; Dewhurst et al., 2000) because only 15 to 40 % of ingested dietary crude protein (CP) is retained in products (Calsamiglia et al., 2010). This lower efficiency of N utilization and the emission of N is one of the predominant environmental concerns (Dijkstra et al., 2013). Major losses of N in cattle production systems come from fertilizers and manure (urine and feces) causing volatilization of ammonia and nitrous oxide, and nitrate contamination in soil and ground water (Tamminga, 1996). As urinary N is more susceptible to leaching and volatile N losses compared to fecal N, reducing the urinary N excretion will contribute to reduce the environmental impact (Dijkstra et al., 2013). Strategies to improve the efficiency of N assimilation by ruminal microbes include feeding dietary degradable CP concentrations that meet microbial requirements and reducing excess of rumen degradable N (Tamminga, 1992; Reynolds and Kristensen, 2008). Therefore, ruminant nutritionists are challenged to optimize ruminal processes in terms of maximizing yield and efficiency of nutrient utilization as well as reducing the environmental impact of livestock production. The accurate measurement of MCP synthesis is essential in order to investigate the factors affecting microbial yield and efficiency of MCP synthesis (Reynal et al., 2005), to improve the accuracy of protein feeding to ruminants, and to reduce environmental burdens from ruminant production (Dijkstra et al., 2013).

1.1 Protein evaluation systems

A profound understanding and manipulation of MCP synthesis are essential for optimizing the production performance of ruminant livestock (Stern et al., 1994; Dijkstra et al., 1998). The current German protein evaluation system for dairy cows and heifers of utilizable CP at the duodenum (uCP; GfE, 2001) is based on empirical equations which are a direct description of observed data developed under experimental conditions. The synthesis of MCP is predicted assuming a fixed yield of MCP that is generated per unit of metabolizable energy intake or organic matter fermented, assuming that N is not limiting. Within such a system, effects such as the substrate type and dietary concentration, microbial interactions, and other dietary factors influencing MCP synthesis are not considered (Dijkstra et al., 1998). Other protein evaluation systems currently in use such as the Cornell Net Carbohydrate and Protein System

in the USA (CNCPS; Fox et al., 2004), the Feed into Milk system in the UK (FiM; Thomas, 2004), and the DVE/OEB₂₀₁₀ system in the Netherlands (VanDuinkerken et al., 2011) are mathematical models which include empirical and mechanistic approaches. As example, these systems predict the energy yield and supply to rumen microbes depending on the substrate fermented and thus, might be able to predict MCP synthesis more precisely (Dijkstra et al., 1998). Furthermore, the DVE/OEB₂₀₁₀ considers variation in the efficiency of MCP synthesis due to differences in fractional outflow rates of particle- and liquid-associated microbes which determines different requirements of energy for their maintenance. Irrespective of the systems, data observed are mainly derived from studies with surgically modified animals kept under experimental conditions. However, these conditions may not be representative to practical feeding conditions and may be insufficient to accurately predict MCP supply to the animal (Titgemeyer, 1997). Hence, on-farm methods based on indirect indicators are required that are able to monitor rumen function and MCP supply in intact animals in order to support farmers and advisers in feeding decisions and to improve the efficiency of dietary N utilization (Dewhurst et al., 1996).

1.2 Estimation of duodenal microbial crude protein flow

The quantification of MCP synthesis *in vivo* is difficult due to the complexity of the rumen system, and more specifically the great diversity of microbial species and their interactions, and different nutrient requirements and metabolism (Dewhurst et al., 2000; Bach et al., 2005). Several methods have been developed for estimation of duodenal MCP flow, however, all have limitations that affect the accuracy of prediction (Dijkstra et al., 1998; Dehority, 2003). *In vivo* methods for measuring duodenal MCP flow and for validation of prediction equations rely on the surgical cannulation of animals and the use of appropriate markers to label the flow of digesta and the microbial biomass. Such invasive method leads to animal care concerns, is expensive, time-consuming, affect the animals' dry matter (DM) intake, and yields variable results and therefore reproducibility and representativeness of measurements are poor (Titgemeyer, 1997). To measure MCP flow *in vivo* the proportion of MCP of total duodenal CP flow must be determined. For this, MCP must be distinguished from dietary rumen un-degraded CP and endogenous CP (Dewhurst et al., 2000). Measuring duodenal MCP flow depends on the use of suitable microbial markers in which a particular microbial constituent is related to cell number, weight, or N content (Broderick and Merchen, 1992). Moreover, knowledge of the marker to N ratios in different species of ruminal bacteria and protozoa is required (Broderick and Merchen, 1992). Microbial markers are classified into

internal and external markers. Internal markers are natural components that are unique in microorganisms, such as 2,6-diaminopimelic acid (Hutton et al., 1971), D-Alanine (Garrett et al., 1982), nucleic acids (Smith and McAllan, 1970), and total purine bases (Zinn and Owens, 1986). External markers are stable or radioactive isotopes, including ^{15}N (Brandt et al., 1980) and $^{35}\text{SO}_4$ (Beever et al., 1974) that will get incorporated into MCP. *In vivo* and *in vitro* studies have shown pronounced differences in MCP estimates obtained with different markers (Siddons et al., 1982; Carro and Miller, 2002; Reynal et al., 2005). Moreover, most markers only capture bacterial biomass and are not suitable to estimate the contribution of protozoa to total microbial biomass (Dewhurst et al., 2000). Thus, none of the microbial markers known can be regarded as ideal and suitable (Obispo and Dehority, 1999). As summarized by Dehority (2003), "... no marker technique can be any better than our ability to isolate a specific microbial fraction from the rumen to serve as a reference standard. This fraction obviously needs to be representative of the microbial population which we wish to estimate."

1.2.1 Purine metabolism and urinary purine derivatives excretion

To determine MCP flow *in vivo*, simpler and more accurate methods are needed. Unicellular organisms have been recognized as markers for ruminal MCP synthesis due to their high concentrations of nucleic acids compared to the concentration in plant and mammalian cells (Broderick and Merchen, 1992). Although there is large variation, overall mean purine and protein content of mixed bacterial isolates is 1.4 % DM (or 1.9×10^{-8} $\mu\text{g}/\text{cell}$) and 44.0 % DM (or 59.8×10^{-8} $\mu\text{g}/\text{cell}$), respectively (Obispo and Dehority, 1999). The close relationship between the excretion of purine derivatives (PD) in urine and the amount of MCP synthesized in the rumen was first published in 1965 by Topps and Elliot. The authors were the first who showed the correlation between nucleic acid concentration in the rumen and excretion of PD in urine of sheep and proposed its use as an index of the efficiency of protein utilization in the ruminant. In the next decades, much research has been done investigating the excretion of PD, for instance, in urine of sheep (Puchala and Kulasek, 1992; Balcells et al., 1993; Chamberlain et al., 1993), cattle (Verbic et al., 1990; Chen et al., 1992, 1995b; Orellana Boero et al., 2001), and goats (Belenguer et al., 2002), as well as in milk of dairy cows (Giesecke et al., 1994; Vagnoni and Broderick, 1997) with the objective to use this as a parameter for estimating MCP supply to the ruminant and to aid understanding of the effects of dietary factors on MCP synthesis.

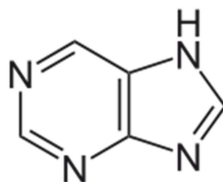


Figure 1 Purine ring

The purine bases adenine and guanine are major components of nucleic acids (deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)). Furthermore, purine nucleotides are part of many coenzymes (e.g. A (CoA), nicotinamide adenine dinucleotide (NAD)) as well as of adenosine triphosphate (ATP) and guanosine triphosphate (GTP). A purine is a heterocyclic aromatic organic compound, consisting of a pyrimidine ring fused to an imidazole ring (Figure 1). Purines are either synthesized *de novo* or reused from salvage pathway. In ruminants, the majority of purine bases reaching the duodenum are considered to originate from rumen microbes because ruminant feedstuffs have generally lower purine contents than rumen microbes and dietary nucleic acids were reported to be rapidly degraded in the rumen (McAllan and Smith, 1973; McAllan, 1982). In the duodenum, the high secretion of pancreatic nucleases hydrolyzes nucleic acids into nucleotides (base + pentose + phosphate), nucleosides (base + pentose), and free bases (McAllan, 1982). Free purine bases and nucleosides are readily absorbed via sodium dependent transporter into the mucosal epithelium. Since there is a high xanthine oxidase activity in the intestinal mucosa and plasma of cattle (Al-Khalidi and Chaglassian, 1965), most of the absorbed purines are rapidly oxidized to uric acid which cannot be incorporated into tissue nucleic acids. In sheep, xanthine oxidase activity is very low (Al-Khalidi and Chaglassian, 1965) which explains the high appearance of the non-oxidized purines hypoxanthine and xanthine in the portal and peripheral blood (Balcells et al., 1992). Hypoxanthine can be salvaged for incorporation into tissues nucleic acids, however, after oxidation of hypoxanthine by xanthine oxidase to uric acid a reutilization is not possible (Chen et al., 1990c; Balcells et al., 1992). All purines that are not salvaged or decomposed from tissues nucleic acids are catabolized to their derivatives, namely allantoin, uric acid, xanthine, and hypoxanthine. The concentration of plasma PD is maintained constant and the disposal of PD is mainly via the urine and other non-renal routes such as saliva (Chen et al., 1990a), gut (Berlin and Hawkins, 1968), and milk (Giesecke et al., 1994). Therefore, estimation of MCP supply from urinary PD excretion must account for the proportion of plasma PD excreted via non-renal routes. The end product of purine catabolism is species-specific (Stangassinger et al., 1995). In bovine plasma and urine, allantoin is quantitatively the main PD next to uric acid, whereas hypoxanthine and xanthine are almost

absent (Verbic et al., 1990). However, sheep excrete all four compounds (allantoin, uric acid, hypoxanthine and xanthine) in considerable amounts (Stangassinger et al., 1995). Hence, models to estimating MCP supply must account for differences between ruminant species.

1.2.2 Endogenous purine derivatives

In animal tissues there is a continuous process of purine nucleotides degradation and re-synthesis of purines either from *de novo* or from preformed purines by the salvage pathway (Chen and Ørskov, 2003). A small fraction of the purine nucleotides from tissues are excreted via the urine after degradation to PD which are the so called “endogenous PD”. For modelling MCP supply from urinary PD excretion, there is a necessity to quantify the contribution of endogenous PD to total PD excretion. In cattle, the endogenous PD excretion was measured by Verbic et al., (1990) and Chen et al. (1990c) using the “intra gastric nutrition technique” (Ørskov et al., 1979), by Giesecke et al. (1993) using the technique of “gastric emptying” (Balcells et al., 1991), and by Orellana-Boero et al. (2001) and González-Ronquillo et al. (2003) using the “stable isotope technique” (Pérez et al., 1998). Variation of the measured endogenous PD excretion was smaller when expressed in terms of metabolic body weight ($BW^{0.75}$; Orellana-Boero et al., 2001). Values of measured endogenous PD excretion in European cattle ranged between 0.428 (Verbic et al., 1990) and 0.514 mmol/kg $BW^{0.75}$ (Chen et al., 1990c) whereby the physiological state of the animal (e.g. dry or lactating cows) seems to have only a minor effect (Tas and Susenbeth, 2007).

1.2.3 Estimating microbial protein supply from urinary excretion of purine derivatives

The underlying principle of the PD approach is simple as illustrated in Figure 2. The duodenal flow of purines originates mainly from rumen microbial nucleic acids (McAllan and Smith, 1973; McAllan, 1982). As an exception, feeding diets containing fishmeal showed that a substantial proportion of purine bases escaped the rumen because fishmeal naturally has high amounts of nucleic acids (Djouvinov et al., 1998). Therefore, feeding fishmeal will potentially lead to inaccuracy when using urinary PD excretion to estimate MCP flow. The digestibility of purine bases in the small intestine is assumed to be similar to the mean digestibility of nucleic acids of 0.83 derived from literature values (Chen and Ørskov, 2003). The knowledge of the quantitative relationship between excretion of PD in urine and duodenal purine bases flow as well as the contribution of endogenous PD to total PD excretion in urine is prerequisite for using urinary PD excretion to predict duodenal MCP flow. Since the recovery of absorbed purine bases and the endogenous PD losses in urine differs among ruminant

species, prediction equations for duodenal MCP flow from urinary PD excretion were proposed for sheep (Chen et al., 1990b, 1992; Balcells et al., 1991), cattle (Verbic et al., 1990; Vagnoni et al., 1997), zebu cattle (Pimpa et al., 2001), buffalo (Pimpa et al., 2003), and goats (Belenguer et al., 2002). Duodenal MCP flow can be calculated from the predicted flow of purine bases, the purine-N to total-N ratio of microbial biomass, the N content of microbial purines, and the duodenal digestibility of microbial purines.

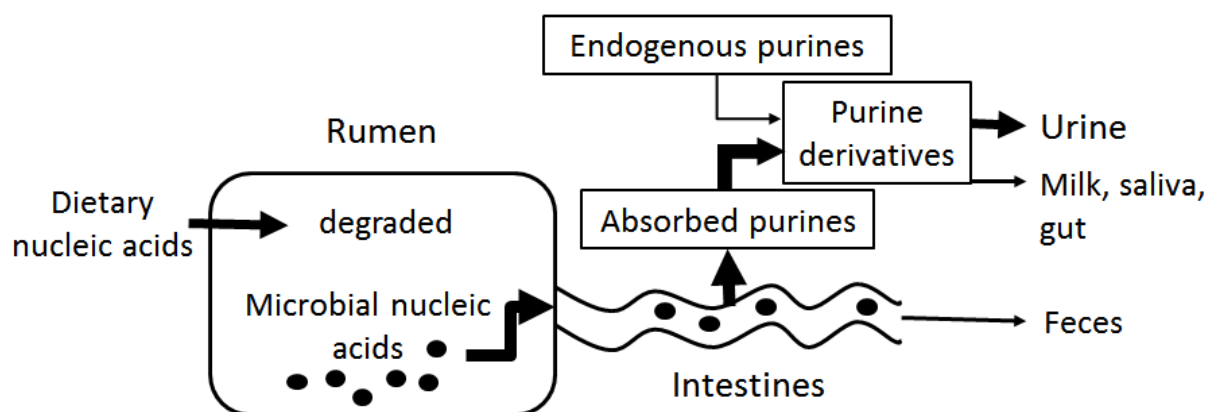


Figure 2 Principle of the method for estimation of microbial protein supply to the ruminant from urinary excretion of purine derivatives (modified after Chen and Gomes, 1992).

The model to estimate duodenal MCP flow in European cattle after Chen and Ørskov (2003) that was used in this thesis is based on the linear regression equation established by Verbic et al. (1990; Equation (1)) to predict the absorption of purine bases (x , mmol/day) from the total urinary PD excretion (y , mmol/day), the recovery rate of absorbed PD (slope), and the endogenous PD contribution (intercept) in terms of $BW^{0.75}$ (in kg).

$$y = 0.85x + 0.385 * BW^{0.75} \quad (1)$$

$$\text{Thus, } x = (y - 0.385 * BW^{0.75}) \div 0.85$$

The urinary PD method is based on the following assumptions:

- i) The ratio of purine-N to total-N of the ruminal microbial matter is considered to be constant.
- ii) The true intestinal digestibility of purine nucleosides and free bases is constant.
- iii) The species-specific excretion of endogenous PD is considered as constant in relation to the $BW^{0.75}$ of the animals.
- iv) The recovery rate of absorbed exogenous purines in urine is constant in cattle.

The advantage of the PD method is that it is non-invasive and that the analysis of PD in urine is easy, cheap, and rapid. The total collection of daily urine volume is required to calculate the total urinary PD excretion. However, spot urine samples could serve as an alternative to total urine collection which would be easy and feasible under practical feeding conditions (Chen et al., 1992). For this, daily urine volume can be estimated from the molar ratio of urinary PD to creatinine concentration in urine spot samples (Chen et al., 1995a). Besides, the mammary excretion of PD would be very attractive to avoid total collection of urine and sampling would be much easier, however, several studies have shown a high correlation with milk yield making it unsuitable as an indicator for MCP supply (Susmel et al., 1995; Shingfield and Offer, 1998). Urinary PD excretion has the potential to overcome the disadvantages of invasive methods and to be used in field and on-farm studies (Chen and Ørskov, 2003; Moorby et al., 2006).

1.3 Objectives and hypotheses of the thesis

Providing high-producing ruminants with adequate amounts of protein is of great importance in order to improve production performance and efficiency of N utilization and to prevent an oversupply with protein which in turn may cause metabolic stress, fertility disorders, and increase the emission of environmental pollutants. Protein synthesized by rumen microbes accounts for a major proportion of ruminants protein supply and thus, the quantification of duodenal MCP flow is a key issue in ruminant nutrition. The overall aim of this thesis was to investigate the response of urinary PD excretion to nutritional factors affecting ruminal MCP synthesis in two animal studies in order to evaluate the accuracy and validity of this indicator to reflect changes in MCP supply. The influence of an intraruminal infused rapidly fermentable carbohydrate to enhance and a condensed tannin source to reduce duodenal MCP flow were tested with regard to changes in urinary PD excretion, N excretion via feces and urine, and apparent total tract nutrient digestibilities. Also, the influence of these nutritional factors was discussed in view of modification of rumen metabolism to improve MCP synthesis and efficiency of N utilization (General discussion; Chapter 4).

Within the first study (Chapter 2), a trial was conducted to examine urinary PD excretion in response to ruminal carbohydrate supplementation by testing four dosages of glucose that were infused intraruminally in incremental amounts. It was hypothesized that the increase in duodenal MCP flow in response to glucose infusion will linearly increase urinary PD excretion. Furthermore, it was expected that even minor amounts of glucose affect urinary PD excretion.

The second study (Chapter 3) aimed to investigate the effects of intraruminal infusion of increasing dosages of Quebracho tannin extract, a commercial condensed tannin extract, on duodenal MCP flow using urinary PD excretion, apparent nutrient digestibility, and N turnover and partitioning of N excretion. While low to moderate condensed tannins concentrations may be beneficial by binding feed protein in the rumen and thus protecting them from immediate degradation and improve flow of essential amino acids to the small intestine (Jones and Mangan, 1977; Barry and Manley, 1984), higher concentrations of condensed tannins may exert their anti-microbial and anti-nutritional properties and reduce MCP synthesis and nutrient digestibility (Barry and McNabb, 1999). It was hypothesized that low Quebracho tannin extract dosages will reduce ruminal protein degradation without reducing MCP synthesis and fermentation of carbohydrates. Moderate to high dosages of Quebracho tannin extract are expected to reduce ruminal carbohydrate fermentation and the MCP synthesis. Moreover, the response of urinary PD excretion was tested in view of detecting potential increases in duodenal MCP flow and efficiency of MCP synthesis as well as identifying the dosage at which Quebracho tannin extract will decrease both, duodenal MCP flow and efficiency of MCP synthesis.

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2 Response of urinary purine derivatives excretion to different levels of ruminal glucose infusion in heifers.

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2.1 Abstract

The study investigated the response of urinary purine derivatives (PD) excretion to increasing levels of intraruminal glucose infusion to evaluate how well this indicator reflects induced changes in microbial crude protein flow. Four rumen-cannulated heifers (482 ± 25 kg body weight) were fed at maintenance energy level a basal diet (on fresh matter basis) of 4 kg/d hay, 1.5 kg/d concentrate and 60 g/d minerals in two equal meals. The trial comprised a control period (Control I) without glucose infusion followed by four consecutive periods in which all animals received 125 g, 250 g, 500 g, and 1000 g/d of glucose, respectively. For this, daily dosages of glucose and urea (90 g/d during all periods) were divided into three portions that were dissolved in water and directly administered into the rumen during morning and afternoon feedings and once during noon. After the highest glucose dosage, a second control period was carried out (Control II). Urinary PD excretion increased with glucose infusion of 125 g/d (71.4 mmol/d) and 1000 g/d (74.2 mmol/d) over the level at Control I (53.9 mmol/d (standard error of the mean (SEM) 3.4; $P \leq 0.012$). After withdrawing glucose infusion, PD excretion (79.0 mmol/d) did not return to Control I level ($P=0.001$). In contrast, fecal nitrogen (N) excretions linearly increased with incremental glucose infusion ($P < 0.001$) from 33.9 g/d at Control I to 39.7 g/d (SEM 0.5) at 1000 g/d glucose and were similar in Control I and II ($P=0.086$). The contradicting responses in the excretions of fecal N and urinary PD to increasing glucose infusions highlight the limited accuracy of the PD excretion as a non-invasive indicator when incremental dosages of rapidly fermentable carbohydrates are supplied.

2.2 Introduction

The microbial crude protein (MCP) synthesised in the forestomach system of ruminants accounts for an average 59 % of the non-ammonia nitrogen (N) entering the small intestine (reviewed by Clark et al., 1992), thus contributing to more than half of the essential amino acids supply to the host animal. The quantification of duodenal MCP flow is thus of great importance, for example, in determining the requirements of high producing ruminants for rumen undegradable feed protein. Moreover, profound understanding of the factors influencing ruminal MCP synthesis (i.e. availability of N and energy, rumen pH, rumen outflow rate, anti-nutritive factors, etc.) is prerequisite. To measure MCP flow under experimental conditions, *in vivo* studies rely on the use of double-cannulated animals and inert marker techniques that are difficult to apply, costly, time-consuming, and yield variable

results (Dewhurst et al., 2000). Non-invasive indicators in excreta and/or products, however, may allow for an estimation of duodenal MCP flow at the level of individual animals even under on-farm conditions. For decades, the excretion of purine derivatives (PD) via the urine of ruminants has been used as a microbial marker for post-ruminal MCP flow (Topps and Elliott, 1965; Verbic et al., 1990; Chen and Ørskov, 2003). The underlying assumptions are that (i) the majority of nucleic acids reaching the duodenum are of microbial origin because dietary nucleic acids are extensively degraded in the rumen (McAllan, 1982), (ii) a great proportion of the purine bases in microbial nucleic acids are absorbed and (iii) their derivatives are mainly excreted via the urine of the host animal (Verbic et al., 1990). Several studies have evaluated and emphasized the validity of this method, and numerous studies have used this marker to investigate the effects of diets that differ considerably in their nutritional value due to the use of various concentrate feeds (Chen et al., 1992; Chamberlain et al., 1993), feed intake levels (González-Ronquillo et al., 2004), and/or forage to concentrate ratios (Moorby et al., 2006). However, to our knowledge, few studies so far exist that evaluate the sensitivity of this indicator to reflect the effects of small increments in readily fermentable carbohydrates on urinary PD excretion.

Hence, the present study analyzes the response of urinary PD excretion to incremental amounts of intraruminally infused glucose to evaluate whether and how this non-invasive indicator will reflect the stimulated ruminal MCP synthesis. Glucose was chosen as a readily available energy source because it is readily and directly available for microbes and likely to be completely fermented in the rumen. Therefore, it was hypothesized (i) that urinary PD excretion linearly increases with increasing glucose dosages due to an increase in MCP flow at the duodenum and (ii) that even minor changes in glucose infusion are detectable with this microbial marker.

2.3 Material and Methods

2.3.1 Animals and feeding

A pre-trial and a main trial were conducted using three and four rumen-cannulated heifers (Jersey x German Black Pied Lowland), respectively. The pre-trial was performed to determine the time required to achieve a constant level of PD excretion after the onset of glucose and urea infusion to identify the required duration of the adaptation period to glucose dosing during the main trial. The animals were fully grown, non-pregnant, and were kept in a tie stall with free access to fresh drinking water. In both, the pre-trial and the main trial, the

basal diet consisted of 4 kg/d of grass hay, 1.5 kg/d of concentrate feed, and 60 g/d of a mineral mixture (on fresh matter (FM) basis). It was offered in two equal meals at 07:30 h and 16:30 h and covered the animals' metabolizable energy (ME) and utilizable crude protein (uCP) requirements for maintenance (GfE, 2001). The composition of the concentrate was as follows (on FM basis): rye grain (23.2 %), rapeseed meal (21.0 %), corn gluten (18.5 %), barley middlings (15.0 %), rye bran (12.0 %), beet pulp (4.5 %), corn grain (4.0 %), and calcium carbonate (1.1 %). Prior to each trial, the grass hay was chopped (5–10 cm length) and homogenized. Dry matter (DM) contents of the grass hay and concentrate feed were determined to calculate DM intake. Subsequently, rations for every meal and animal were weighed and stored until feeding. Chemical compositions of the hay and concentrate feeds are given in Table 1. During both trials, all animals completely consumed their diets, so that no feed refusals occurred.

Table 1 Chemical composition of the grass hay and the concentrate feed used in the basal diets of the pre-trial (n=3) and main trial (n=6; arithmetic means \pm standard deviations).

		Pre-trial		Main trial	
		Hay	Concentrate	Hay	Concentrate
DM	[% FM]	92.8 \pm 0.4	89.6 \pm 0.5	90.5 \pm 0.5	87.7 \pm 0.5
OM	[% DM]	95.0 \pm 0.3	93.2 \pm 0.3	94.6 \pm 0.3	93.2 \pm 0.1
CP		7.4 \pm 0.1	17.7 \pm 0.1	6.1 \pm 0.8	18.5 \pm 0.1
CL		1.2 \pm 0.1	3.2 \pm 0.2	0.8 \pm 0.2	3.2 \pm 0.1
CF		34.3 \pm 0.6	8.2 \pm 0.1	37.1 \pm 0.7	7.5 \pm 0.1
NDF		70.7 \pm 0.8	27.9 \pm 0.7	73.5 \pm 1.0	25.9 \pm 0.8
ADF		39.7 \pm 0.7	11.5 \pm 0.4	46.2 \pm 1.4	10.6 \pm 0.9
ADL		6.1 \pm 0.3	3.7 \pm 0.2	6.6 \pm 0.2	2.7 \pm 0.1
Starch		n.d.	26.9 \pm 0.9	n.d.	31.7 \pm 1.3
ME	[MJ/kg DM]	8.0 \pm 0.1	12.5 \pm 0.2	7.3 \pm 0.2	12.3 \pm 0.2
IvdOM	[%]	57.8 \pm 0.4	83.8 \pm 1.0	54.1 \pm 1.6	82.8 \pm 1.2

DM, dry matter; FM, fresh matter; OM, organic matter; CP, crude protein; CL, crude lipid; CF, crude fiber; NDF, neutral detergent fiber (inclusive residual ash); ADF, acid detergent fiber (inclusive residual ash); ADL, acid detergent lignin; n.d., not determined; ME, metabolizable energy; IvdOM, *in vitro*-digestibility of organic matter.

2.3.2 Experimental designs and procedures

The pre-trial lasted for 37 d and comprised a 10 d-adaptation period to the basal diet followed by 27 d of complete urine and feces collection. The latter period consisted of two phases.

Initially, the animals were offered the basal diet during days 11–20 to determine urinary PD excretion without glucose infusion. During days 21–37, they additionally received 1000 g/d glucose and 90 g/d urea. Urea was administered to assure a positive ruminal N balance (GfE, 2001) when glucose were administered at 1000 g/d. Daily supply of N from the basal diet and basal diet plus urea was 80 and 122 g N, respectively. Glucose and urea dosages were divided into two equal portions. Each portion was diluted in 500 ml lukewarm water and administered directly into the rumen via the cannulae during morning and evening feeding, respectively. The animals were weighed on days 9, 25, and 37. Their mean body weight (BW) was 520 kg (standard deviation 15 kg; n=3).

The main trial consisted of six periods starting with a control period (Control I) without glucose infusion followed by four consecutive periods during which all animals simultaneously received 125 g (G_{125}), 250 g (G_{250}), 500 g (G_{500}), and 1000 g/d (G_{1000}) of glucose and a second control period (Control II). Each period (except Control II) lasted for 11 d including 4 d of adaptation and 7 d of total urine and feces collection. The adaptation to glucose infusion was restricted to 4 d because urinary PD excretion reached a new level after 4 d of glucose dosing in the pre-trial (see results section). Glucose was administered by dividing the daily dose into three portions (25, 50, and 25 % of the total amount), each of which was dissolved in 500 ml of lukewarm water and administered directly into the rumen via the cannulae at 07:30 h (morning feeding), 12:30 h, and 16:30 h (afternoon feeding), respectively. Subsequent to G_{1000} , glucose dosing was stopped and after 4 d of adaptation, total urine and feces were collected again for 5 d (Control II) to test whether and how fast urinary PD excretion returned to the same level as that in Control I. During the whole trial, the animals received 90 g/d of urea in addition to the basal diet to assure a positive ruminal N balance (GfE, 2001) at all glucose dosages and to investigate the sole effects of glucose on N partitioning at constant N intake. Urea was diluted and administered in three portions (30 g each) together with the glucose during G_{125} to G_{1000} , and diluted in 100 ml of water during the two control periods. Hence, daily intake of N (including urea-N) was constant across all experimental periods at 114 g. The animals were weighed every two weeks. Their mean BW was 482 (standard deviation 25 kg; n=4) and was constant for all animals throughout the trial. Individual water intake was recorded twice a day using water meters attached to the drinking troughs.

2.3.3 Sample collection and preparation

One sample each of the grass hay (~ 2 kg FM) and the concentrate feed (~ 750 g FM) was collected on three occasions during the pre-trial and once during each period in the main trial and ground to pass through a 1 mm-screen (ZM1, Retsch mill, Germany). Subsequently, three subsamples (~ 50 g each) of each sample were taken for chemical analysis. Feces excreted by each heifer were manually collected from rubber mats placed behind the animals and weighed at every feeding. For urine collection, harnesses were attached to the animals' vulva and furnished with a hose to drain the urine directly into canisters. The latter were filled with about 150 ml of 20 % (v/v) sulphuric acid to reduce pH to < 3. Urine weight and volume were determined at every feeding. In both trials, total urine collected from each animal between two subsequent morning feedings were pooled and mixed.

In the pre-trial, three subsamples (500 ml each) of the pooled urine and feces (500 g each) of each animal were taken every day. For analyses of allantoin and uric acid, 500 ml of the pooled urine were filtered (Macherey-Nagel, average pore size 4–12 µm). Of this filtrate, 10 ml were diluted with distilled water (1:5, v/v) and six aliquots (5 ml each) of the diluted filtrate were taken. Three aliquots each were taken for measurement of concentrations of allantoin and uric acid in single determination (see next section).

In the main trial, again 500 ml of the pooled urine excreted between two subsequent morning feedings were filtered, 50 ml of the filtrate diluted with distilled water (1:5, v/v), and two aliquots of 15 ml each were obtained for PD analysis every day. The aliquots collected on days 1–2, 3–5, and 6–7, respectively (except for Control II), were combined to three pooled samples (30 ml each) prior to analysis, in which the amount of urine taken from each aliquot corresponded to the urine volume of the respective day. The remaining pooled urine and feces collected every day were stored frozen until the end of every sampling period (except for Control II). Then, urine and feces were thawed, homogenized, and three subsamples each of urine (500 ml each) and feces (500 g each) were taken. During Control II, pooled urine and feces were homogenized and subsamples taken and analyzed every day according to the procedure in the pre-trial. Samples from animals on days when considerable urine losses occurred were excluded from pooling and the data analysis (27 of a total of 164 cases (= 6 periods * 7 d (5 d in Control II) * 4 animals)). All samples and aliquots collected during the pre-trial and main trial were stored at -20°C until analysis.

2.3.4 Chemical analyses

In both trials, analyses for proximate composition as well as fiber fractions, *in vitro*-digestibility, and ME contents (in feed samples only) were carried out in two of the three feed, urine, and feces subsamples. If the results for these two subsamples deviated from the mean by > 5 % of the mean value, the third subsample was additionally analyzed. Concentrations of DM, crude ash, crude lipid, and crude fiber of feed and feces (DM and crude ash only) were determined in duplicate according to Naumann and Bassler (1997). Concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), both with residual ash, and acid detergent lignin in feed were analyzed in duplicate using an Ankom²⁰⁰ Fiber Analyzer (Ankom Technology, Macedon NY, USA) according to procedures described by Van Soest et al. (1991). A heat-stable α -amylase was used in the NDF procedure. The *in vitro*-digestibility of organic matter (OM) and ME concentrations of the feed were estimated from gas production during *in vitro*-incubations according to Menke and Steingass (1987). For this, three replicates of each feed subsample were incubated on two days each. Starch concentrations of the concentrate feeds were determined in triplicate according to Brandt et al. (1987) after enzymatic oxidation by glucose oxidase using Chinonimin as colorimetric indicator. In the pre-trial, N concentrations of feed, feces, and urine were analyzed in triplicate using a TruSpec[®]N analyzer (LECO Corporation, St. Joseph MI, USA). In the main trial, subsamples were analyzed by the Kjeldahl procedure in a single determination (Naumann and Bassler, 1997). Concentrations of crude protein (CP) were calculated from mean N concentrations of two or three subsamples using a conversion factor of $CP = N * 6.25$.

For PD analysis, only concentrations (mmol/l) of allantoin and uric acid were determined following the procedures described by Chen et al. (1993) and Balcells et al. (1992), respectively, because xanthine and hypoxanthine are reported to be almost absent in cattle urine (Verbic et al., 1990; Martín-Orúe et al., 2000; González-Ronquillo et al., 2004). For this, the diluted urine samples (1:5) were further diluted with distilled water to 1:50 for allantoin and to 1:20 for uric acid analysis. The PD analyses were conducted using high-performance reversed-phase liquid chromatography (JASCO[®] HSS-1500, Groß-Umstadt, Germany; column: Kromasil C18, 5 μ m, 250 * 4.0 mm) followed by peak area analysis with BORWIN[™] chromatography software. Allantoin and uric acid concentrations were determined in triplicate and duplicate in the pre-trial and main trial, respectively. Analyses were repeated, if the deviation between individual determinations and their mean exceeded 6 % of the mean concentrations. Daily urinary allantoin and uric acid excretions (mmol/d)

were calculated by multiplying the molar concentrations (mmol/l) by the respective urine volume (l/d). Total urinary PD excretion was calculated as the sum of daily allantoin and uric acid excretions.

2.3.5 Calculations and statistical analyses

Apparent total tract DM and OM digestibilities were calculated without considering daily glucose dosage as intake because glucose was assumed to be completely digestible. Since increasing glucose infusions were expected to increase rumen microbial growth and fecal excretions of microbial N, apparent total tract digestibility of CP-free organic matter (CPFOM) was additionally calculated as an indicator for the effects of glucose on the total tract digestibility of carbohydrates in the basal diet:

$$\text{CPFOM digestibility} = \frac{\text{OM intake} - \text{CP intake} - \text{Fecal OM} - \text{Fecal CP}}{\text{OM intake} - \text{CP intake}} \quad (1)$$

where intakes and fecal excretions of OM and CP are expressed in g/d.

All statistical analyses were conducted using the software R version 3.1.0 (the R foundation for Statistical Computing, Vienna, Austria). To determine the number of days after the onset of glucose and urea infusion until urinary PD excretion reached a new plateau level, data were subjected to a regression analysis (Equation 2). The model comprised the following parameters to identify mean PD excretion (mmol/d) without (a , days 11–20) and with infusion of glucose and urea (days 21–37), the slope s of the increase in PD excretion after onset of glucose and urea infusion at day 21 (mmol/d), and the breakpoint (x_1 , in d) at which urinary PD excretion reached a new plateau level.

$$f(t) = \begin{cases} a & t < 21 \\ a + s(t - 21) & \text{, if } 21 \leq t < x_1 \\ a + s(x_1 - 21) & x_1 \leq t \end{cases} \quad (2)$$

a , basal PD excretion; s , slope of the increase in PD excretion with glucose and urea infusion; x_1 , breakpoint (d); t , time (d).

According to the results of this regression analysis, the breakpoint x_1 was at day 23.6 (see Results section). To determine the effects of the glucose and urea infusion in the pre-trial, arithmetic means of all measured parameters were calculated for each animal for days 11–20 without and for days 25–34 with glucose and urea infusion, respectively. Similarly, PD excretion during Control II of the main trial did not decrease even after 9 d without glucose infusion, so that arithmetic means for all parameters were calculated across the five sampling days.

To identify treatment effects, data of both trials were then analyzed by mixed model procedure (lme) and analysis of variance. The model included treatment as fixed effect and animal as random factor. An autoregressive correlation structure was chosen. Least squares means and standard errors of the means (SEM) were calculated for all measured and calculated parameters. Significant differences ($P < 0.05$) between least squares means were determined by the Tukey-Kramer test. Additionally, data of the main trial were used to determine the linear relationships between glucose dosage (independent variable; g/d) and measured and calculated parameters (dependent variables), omitting data of Control II (see Results section).

2.4 Results

2.4.1 Pre-trial

During both trials, all animals remained in good health. In the pre-trial, the breakpoint x_1 was at day 23.6 (standard error 1.12; $P < 0.001$) according to the regression analysis (Figure 3). Hence, PD excretion reached a new plateau level about 4 d after the onset of glucose and urea infusion at day 21. Comparing the two phases (without (days 11–20) versus with glucose and urea infusion (days 25–34)), mean daily urine volumes increased with glucose and urea infusion from 6.8 l to 9.1 l (SEM 0.86; $P = 0.037$), most likely due to the increased intake of N (Bannink et al., 1999; Table 2). Mean daily PD excretion via urine increased by 29 mmol/d from 70.8 mmol/d to 99.8 mmol/d (SEM 2.36; $P = 0.006$) with glucose and urea infusions. Irrespective of the glucose and urea dosage, allantoin accounted for 0.90 (SEM 0.01) of total urinary PD concentrations ($P = 0.297$).

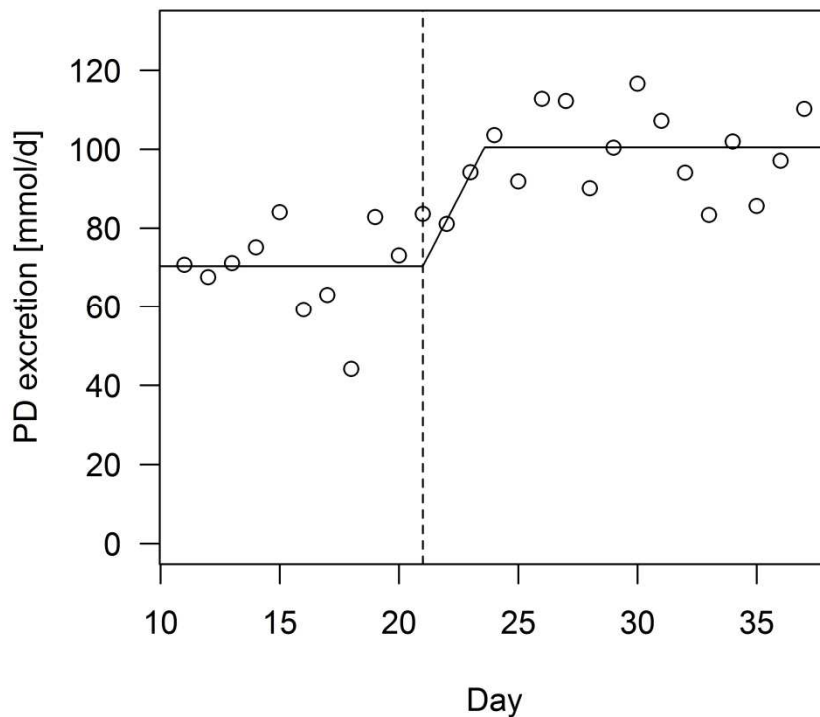


Figure 3 Mean daily urinary purine derivatives (PD) excretion in heifers (n=3) during the pre-trial. The continuous line represents the estimated regression equation, and the dotted line marks the onset of intraruminal infusion of glucose (1000 g/d) and urea (90 g/d) at day 21.

Fecal and urinary N excretions increased with glucose and urea infusion, whereas the absolute increases were greater for urinary N (31 g/d; $P=0.010$) than fecal N excretion (7 g/d; $P=0.005$). While apparent total tract digestibilities of DM, OM, and CPFOM were lower with than without infusion ($P\leq 0.047$), apparent CP digestibility increased when glucose and urea were administered ($P=0.004$).

Table 2 Urinary purine derivatives (PD) excretion, intake and excretion of nitrogen (N), and apparent total tract nutrient digestibilities in heifers (n=3) without (basal) and with 1000 g/d glucose and 90 g/d urea infusion during the pre-trial (least squares means and standard errors of the means (SEM)).

Parameter		Basal	With infusion	SEM	P-Value
PD excretion	[mmol/d]				
Allantoin		64.0	89.8	2.91	0.010
Uric acid		6.7	10.0	0.71	0.013
Total PD		70.8	99.8	2.36	0.006
N intake	[g/d]	79.7	121.6		
N excretion	[g/d]				
Fecal N		29.8	37.1	1.04	0.005
Urinary N		47.5	78.5	1.76	0.010
Total N		76.3	115.6	1.96	0.009
Apparent total tract digestibility					
DM ^a		0.657	0.618	0.51	0.047
OM ^a		0.674	0.634	0.47	0.045
CPFOM ^b		0.687	0.622	0.59	0.022
CP		0.626	0.695	1.04	0.004

DM, dry matter; OM, organic matter; CPFOM, crude-protein-free organic matter; CP, crude protein.

^a Calculated without glucose intake.

^b Calculated by subtracting CP (N*6.25) intake and excretion from intake and fecal excretion of OM, respectively.

2.4.2 Main trial

Daily urine volumes ranged between 6.3 l and 24.8 l at minimum and maximum water intakes of 11.7 l/d to 34.4 l/d, respectively. Neither parameter was affected by glucose infusion level (Table 3; $P \geq 0.109$). Without glucose infusion, mean PD excretion at Control I was lower than the PD excretion in the pre-trial when animals were fed on the basal diet. However, the relative increase with 1000 g/d of glucose compared to urinary PD excretion without glucose infusion was similar in the pre-trial and main trial (29.1 % and 27.4 %, respectively). According to linear regression analysis, the relationship between glucose dosage and urinary PD excretion was significant at $P=0.032$. However, as illustrated in Figure 4, a linear model would underestimate PD excretion at G₁₂₅, while overestimating PD excretion at highest glucose dosages. Hence, the relationship between glucose dosage and urinary PD excretion

was rather non-linear. Compared with Control I, mean PD excretions were higher at G_{125} , G_{1000} , and Control II ($P \leq 0.011$). The greatest increase in urinary PD excretion was observed at G_{125} with 17.5 mmol/d above the Control I level ($P = 0.012$). At Control II, PD excretion did not decline to the level of Control I ($P = 0.001$). The average proportion of allantoin in the total PD was 0.89 (SEM 0.01; $P < 0.001$) which was similar to that in the pre-trial. The proportion was higher at G_{125} than at Control I, G_{250} , and G_{500} ($P \leq 0.008$).

Table 3 Urinary purine derivatives (PD) excretion in heifers (n=4) without glucose infusion (Control I and II) and with infusion of glucose at 125 g/d (G₁₂₅), 250 g/d (G₂₅₀), 500 g/d (G₅₀₀), and 1000 g/d (G₁₀₀₀) during the main trial (least squares means and standard errors of the means (SEM)).

Parameter	Control I	G ₁₂₅	G ₂₅₀	G ₅₀₀	G ₁₀₀₀	Control II	SEM	P-value
Water intake [l/d]	20.9	23.2	21.1	17.2	16.8	15.3	2.69	0.109
Urine excretion [l/d]	11.3	15.2	13.1	8.8	9.4	9.4	2.17	0.189
PD excretion [mmol/d]								
Allantoin	47.7 ^A	64.7 ^B	58.5 ^{AB}	58.0 ^{AB}	66.2 ^B	70.2 ^B	2.96	0.001
Uric acid	6.2 ^A	6.7 ^{AB}	8.5 ^B	7.6 ^{AB}	7.9 ^{AB}	8.7 ^B	0.75	0.016
Total PD	53.9 ^A	71.4 ^B	67.0 ^{AB}	65.5 ^{AB}	74.2 ^B	79.0 ^B	3.42	0.002
Allantoin : total PD	0.886 ^{AB}	0.905 ^C	0.875 ^A	0.885 ^{AB}	0.893 ^{BC}	0.890 ^{ABC}	0.008	<0.001

^{A,B,C} Means within the same row with different superscripts differ at $P < 0.05$.

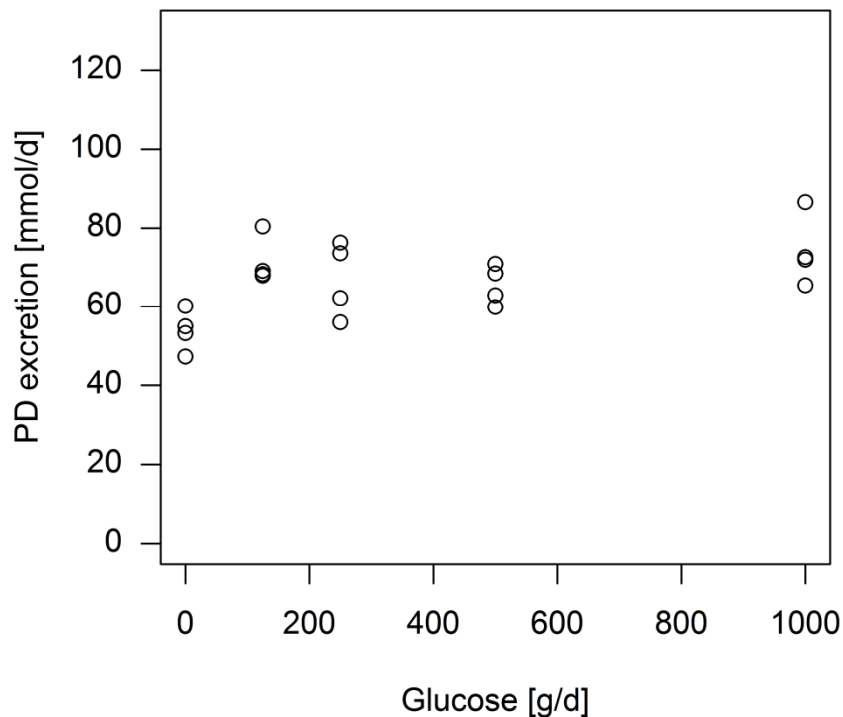


Figure 4 Urinary purine derivatives (PD) excretion in heifers (n=4) in response to incremental intraruminal glucose infusion during the main trial.

Fecal N excretions ranged between 31.2 g/d and 40.5 g/d and linearly increased with increasing glucose infusion ($P < 0.001$) due to increasing fecal DM excretion ($P = 0.021$; Table 4) and N concentrations in feces ($P < 0.001$). Accordingly, a linear decrease with increasing glucose infusion was observed for apparent total tract digestibility of CP ($P < 0.001$). At Control II, fecal N excretion returned to the level measured at Control I. Hence, data for this period were omitted from regression analyses. Apparent total tract digestibilities of DM and OM were lower at G_{500} than at Control II ($P = 0.018$). Glucose infusion did not affect apparent total tract digestibility of CPFOM ($P = 0.095$).

Table 4 Nitrogen (N) excretion and apparent total tract nutrient digestibilities in heifers (n=4) without glucose infusion (Control I and II) and with infusion of glucose at 125 g/d (G₁₂₅), 250 g/d (G₂₅₀), 500 g/d (G₅₀₀), and 1000 g/d (G₁₀₀₀) during the main trial (least squares means and standard errors of the means (SEM)).

Parameter	Control I	G ₁₂₅	G ₂₅₀	G ₅₀₀	G ₁₀₀₀	Control II	SEM	P-value
N concentration								
Fecal N [% DM]	1.75 ^{AB}	1.70 ^A	1.76 ^A	1.82 ^B	1.97 ^C	1.67 ^A	0.03	<0.001
Urinary N [%]	0.56	0.46	0.55	0.69	0.69	0.75	0.11	0.118
N excretion ^a [g/d]								
Fecal N	33.9 ^{AB}	33.0 ^{AB}	34.8 ^B	37.2 ^C	39.7 ^D	31.8 ^A	0.50	<0.001
Urinary N	61.0	62.4	67.5	60.8	62.5	69.6	4.16	0.580
Total N	94.9	95.3	102.3	98.2	102.2	101.5	4.06	0.513
N-retention [g/d]	19.1	18.7	11.7	15.8	11.8	12.5	4.06	0.513
Fecal N/urine N	0.61	0.57	0.54	0.66	0.66	0.47	0.05	0.161
Fecal excretion [g/d]								
DM	1995 ^{AB}	1992 ^{AB}	2029 ^{AB}	2099 ^B	2061 ^{AB}	1960 ^A	30.32	0.021
OM	1680 ^{AB}	1678 ^{AB}	1709 ^{AB}	1776 ^B	1745 ^{AB}	1661 ^A	24.02	0.015

To be continued

Table 4 continued

Parameter	Control I	G ₁₂₅	G ₂₅₀	G ₅₀₀	G ₁₀₀₀	Control II	SEM	P-value
Apparent total tract digestibility coefficient								
DM ^b	0.602 ^{AB}	0.603 ^{AB}	0.596 ^{AB}	0.581 ^A	0.589 ^{AB}	0.609 ^B	0.01	0.021
OM ^b	0.631 ^{AB}	0.632 ^{AB}	0.625 ^{AB}	0.610 ^A	0.617 ^{AB}	0.636 ^B	0.01	0.015
CPFOM ^c	0.618	0.617	0.612	0.599	0.611	0.620	0.01	0.095
CP	0.703 ^{CD}	0.711 ^{CD}	0.695 ^C	0.674 ^B	0.652 ^A	0.721 ^D	0.00	<0.001

^{A,B,C,D} Means within the same row with different superscripts differ at $P < 0.05$.

DM, dry matter; OM, organic matter; CPFOM, crude-protein-free organic matter; CP, crude protein.

^a N intake was 114 g/d.

^b Calculated without glucose intakes.

^c Calculated by subtracting CP (N * 6.25) intake and excretion from intake and fecal excretion of organic matter, respectively.

2.5 Discussion

2.5.1 Urinary purine derivatives excretion level

Urinary PD excretions of heifers when they did not receive any glucose infusion were higher in the pre-trial than in the main trial, although the same animals and diets of similar composition were used. This difference was most likely due to small differences in dietary ME concentrations and hence, animals' ME intakes (46.5 and 42.3 MJ/d in the pre-trial and main trial, respectively). According to the equations of Chen and Ørskov (2003), a urinary PD excretion of 120 mmol/d¹ is expected during Control I which is more than twice as high as the measured PD excretion during this period. Similarly low urinary PD excretions were described by Wang et al. (2009) in cattle of comparable BW fed at maintenance energy level. Estimated endogenous PD excretion of 40 mmol/d would already account for 74 % of the measured PD excretion (53.9 mmol/d) during Control I, suggesting that endogenous PD excretion in urine proposed by Chen and Ørskov (2003) overestimates that of heifers in our study. Furthermore, overestimation of the efficiency of MCP synthesis, the purine content in microbial mass, and/or the proportion of the microbial purines that are excreted via the urine might explain the discrepancy between expected and measured PD (see also section 2.5.2).

2.5.2 Effect of glucose infusion on urinary purine derivatives excretion

Intraruminal glucose infusion increased urinary PD excretion. The absolute increase in urinary PD excretion with infusion of 1000 g/d of glucose was 29.0 mmol and 20.3 mmol/d during the pre-trial and main trial, respectively. These values are similar or only slightly lower than the expected increase of 27 mmol/d which was calculated using the equations of Chen and Ørskov (2003) assuming a ME content of glucose of 14 MJ/kg and the efficiency of MCP synthesis of 10.1 g/MJ ME intake (GfE, 2001). Several studies have reported a positive linear increase in urinary PD excretion with increasing intake of digestible OM (Lindberg, 1985; Han et al., 1992) or energy (Giesecke et al., 1994; Moorby et al., 2006). Moreover, González-Ronquillo et al. (2004) found that PD excretion in urine decreased with declining feed intake. Nevertheless, PD excretion did not linearly increase with incremental glucose infusion.

¹ Theoretical basis of the calculation of urinary PD excretion: 1) efficiency of MCP synthesis = 10.1 g MCP/MJ ME intake (GfE, 2001); 2) proportion of N in purines = 0.116 g/g of microbial N; 3) praecaeal digestibility of MCP = 0.83; 4) renal clearance rate of absorbed PD = 0.85; 4) endogenous PD excretion in cattle = 0.385 mmol/kg^{0.75} BW (Chen and Ørskov, 2003).

Possible reasons for the non-linear response in PD excretion to increasing glucose infusion are discussed in the following and might be related to:

- (1) the experimental design and methodology,
- (2) changes in the apparent digestibility of the basal diet,
- (3) treatment effects on the efficiency of MCP synthesis, and
- (4) changes in the urinary recovery of absorbed PD, endogenous PD excretion, praecaecal digestibility of microbial nucleic acids, and purine concentration in microbial matter, all of which are assumed to be constant and independent of diet (Chen and Ørskov, 2003).

In the main trial, all animals simultaneously received the same amount of glucose in every period with 4 d of adaptation. We believe that this experimental design did not affect the relationship between PD excretion and level of glucose infusion because fully grown heifers were used whose feed intake was constant across all periods and only covered their ME and uCP requirements for maintenance. Moreover, the animals were kept in a closed building and were thus unaffected by seasonal changes in climatic conditions. Urinary PD excretion reached a constant level within 4 d after onset of glucose infusion during the pre-trial, suggesting that it had likely stabilized over the same time span in the main trial when increments in glucose dosages were even smaller. Glucose and urea are rapidly degradable carbohydrate and N sources, respectively, and were administered to the solid phase of rumen contents in two (pre-trial) and three (main trial) portions throughout the day. It therefore appears unlikely that considerable proportions of glucose and urea reached the duodenum and digested post-ruminally. The ruminal N balance (GfE, 2001) was close to 0 at G_{1000} , if an efficiency of MCP synthesis of 10.1 g/MJ ME intake and an approximate ME content of glucose of 14 MJ/kg are assumed, indicating that N availability was not limiting microbial growth at any glucose dosage level. Hence, we conclude that the methodological factors mentioned above do not explain the non-linear effects of glucose infusion on urinary PD excretion.

Any effects of glucose infusion on apparent total tract OM digestibility would have altered energy supply to rumen microbes and therefore urinary PD excretion. Apparent total tract digestibility of CPFOM was not affected by glucose infusion in the main trial, indicating that the extent of fermentation of the (structural) carbohydrate fraction of the basal diet was similar across all infusion levels. Furthermore, during health monitoring rumen pH was

measured in two animals at G_{1000} (data not shown) which consistently stayed above 6.2, suggesting that activity of cellulolytic microbes was not hampered by a drop in pH (Grant and Mertens, 1992). Hence, differences in OM digestibility of the basal diet and their effects on PD excretion are precluded.

Glucose infusion might have affected the efficiency of MCP synthesis. Although liquid-associated microbes have higher maintenance energy requirements than particle-associated microbes (Russell et al., 1992), the efficiency of MCP synthesis is higher in diets that favour the proliferation of liquid-associated microbes due to higher rumen outflow rates of the liquid phase (Clark et al., 1992; Rodríguez et al., 2000). Hence, an enhanced efficiency of MCP synthesis due to the supply of a readily fermentable carbohydrate compared to Control I may explain the disproportionately high response of urinary PD excretion at low glucose dosages.

Urinary recovery of absorbed PD is relatively constant with a mean recovery of 0.85 for cattle (Verbic et al., 1990; Chen and Ørskov, 2003). Urinary excretion of endogenous PD from nucleic acid degradation in animal tissue depends on the physiological conditions (Laurent and Vignon, 1983). Nevertheless, although energy intake increased as a result of the glucose infusions, profound variations in their physiological status of our experimental animals and thus endogenous PD excretion appear unlikely. Shifts in the proportion of protozoa and gram-positive or gram-negative bacteria in the post-ruminal MCP flow might have affected praecaecal digestibility of microbial nucleic acids because true digestibility is higher for protozoa (0.87–0.91) than for bacteria (0.74–0.79; Owens and Zinn, 1988). However, protozoa are selectively retained within the rumen (Punia et al., 1992), contribute only 11 % to the abomasal digesta CP flow (Shabi et al., 2000), and their total nucleic acid content is only slightly higher than that of bacteria (6.8 mg versus 6.1 mg/100 mg; Czerkawski, 1976). Moreover, although gram-positive bacteria such as *Streptococcus bovis* likely proliferated in response to glucose infusion (Russell and Hino, 1985) and praecaecal digestibility of their protein is lower than that of gram-negative bacteria (Wallace, 1983), the effects of this proliferation on praecaecal MCP digestibility from mixed microbial matter are considered to be small (Wallace, 1983).

Several studies have shown that the ratios of purine-N to total-N in (mixed) rumen microbial matter differ from the constant ratio of 0.116 proposed by Chen and Ørskov (2003) and vary between 0.080 and 0.120 (Dewhurst, 1989; cited in Dewhurst et al., 2000). They are higher in

bacteria than in protozoa (Firkins et al., 1987) and in liquid- than in particle-associated microbes (Martín-Orúe et al., 2000; Rodríguez et al., 2000). Moreover, ratios increase with higher microbial growth (Bates et al., 1985) and rumen outflow rates (Rodríguez et al., 2000) and are higher with concentrate than with roughage feeding (Pérez et al., 1998). Purine-N to total-N ratio in microbial matter in our study might have increased with glucose infusion due to enhanced growth rates and/or a shift towards a greater proportion of liquid-associated microbes. For instance, assuming only a slightly higher purine-N concentration in microbial matter of 0.120 g /g MCP (instead of 0.116; Chen and Ørskov, 2003) would result in a lower urinary PD excretion estimated from ME intake at G_{125} of 72 mmol/d, which equals the measured PD excretion at this glucose dosage level (71.4 mmol/d). Hence, a change in purine concentration in microbial matter is most likely the main reason for the pronounced increase in the urinary PD excretion at G_{125} compared to Control I.

2.5.3 Fecal nitrogen excretion

In both trials, fecal N excretion increased with glucose and urea infusion which in turn reduced apparent total tract DM, OM, and CP digestibilities in the main trial, when urea was also administered during the control periods. Although changes in the proportion of different N fractions in feces towards a lower proportion of microbial N might hamper the sensitivity of this indicator, these effects are most likely due to a greater synthesis and thus fecal excretion of MCP (Kebreab et al., 2002). Additionally, a lower praecaecal MCP digestibility due to shifts towards microbial species and/or groups that are more resistant to praecaecal digestion (Wallace, 1983) in response to glucose infusion might have contributed to higher fecal N excretions with increasing glucose dosages (see section above). In contrast to urinary PD, the increase in fecal N excretion was linear which supports the assumption that changes occurred in the ratio between purine-N to total-N in microbial mass at low glucose dosages.

2.5.4 Purine derivatives excretion at Control II

Urinary PD excretion during Control II remained at the level measured during G_{1000} and was therefore higher than during Control I. In contrast, fecal N excretion during Control II returned to almost the same level as that of Control I, which suggests that fecal excretion of microbial N decreased rapidly after the end of glucose infusion. Similarly, a study conducted by Grubb and Dehority (1975) indicated that bacterial numbers (in colony forming units per g of rumen contents) had still not stabilized after a diet change from a 100 % hay to 60 % cracked corn and 40 % hay diet after 14 d, even though the apparent total tract digestibility of

DM and cellulose adjusted to the new diet after 5 d within the same experiment (Potter and Dehority, 1973). During Control II, shifts in the rumen microbial consortium resulting in a higher concentration of purines in the microbial matter after the end of glucose infusion may have taken longer than the 9 d of the adaptation and sampling owing to differences in the lag phases of the microbial species involved. This shift in lag phases might provide one explanation for the different results observed for fecal N and urinary PD excretion.

2.6 Conclusions

The present results have clearly shown that the provision of incremental amounts of readily fermentable carbohydrates to ruminants does not result in a linear increase in urinary PD excretion and that the response may differ depending on the dietary treatment (i.e. increase or decrease of fermentable carbohydrate supply). Moreover, shifts in the composition of the rumen microbial consortium towards lower or higher purine concentrations in microbial matter appear to be the main reason for these unexpected results and considerably reduce the accuracy and sensitivity of the urinary PD excretion as a non-invasive indicator.

2.7 References

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3 Influence of ruminal Quebracho tannin extract infusion on apparent nutrient digestibility, nitrogen balance, and urinary purine derivatives excretion in heifers.

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3.1 Abstract

Six fully grown heifers (491 kg body weight, standard deviation 35) fitted with ruminal cannulae received incremental dosages of a commercial Quebracho tannin extract (QTE) to investigate its effects on apparent total tract nutrient digestibilities, nitrogen balance, and purine derivatives excretion as an indicator of duodenal microbial crude protein flow. The basal diet comprised 2.6 kg/d grass hay, 2.6 kg/d concentrate feed, and 60 g/d of a mineral premix (as-fed basis) and was offered in two equal meals. The QTE was administered at 0 (CON 1), 1, 2, 4, or 6 % of the daily dry matter (DM) intake from the basal diet (i.e., 45 g, 90 g, 180 g, and 270 g/d; as-fed basis). For this, half of the daily QTE dosage was suspended in water and intraruminally infused during each feeding. All animals simultaneously received the same QTE dosage. Every period comprised 9 d of adaptation and 6 d of total urine and feces collection. Subsequent to highest QTE dosage, infusion was ceased and after 14 d of adaptation, urine and feces were collected again for 10 d (CON 2). The QTE infusions at ≥ 4 % lowered apparent total tract organic matter digestibility ($P \leq 0.045$). The effects were more pronounced for neutral detergent and acid detergent fiber digestibilities which decreased from 0.718 and 0.626 without QTE to 0.590 and 0.493 at 6 % QTE, respectively ($P < 0.001$). Nitrogen excretion linearly decreased in urine ($P = 0.003$) and linearly increased in feces ($P < 0.001$) with incremental QTE dosages. Irrespective of the dosage level, nitrogen retention was higher with than without QTE infusion ($P \leq 0.035$). Urinary purine derivatives excretion declined from 103 mmol/d at 1 % QTE to 80 mmol/d at 6 % QTE ($P < 0.001$), indicating a 36 % decrease in estimated duodenal microbial crude protein flow. At QTE dosages of ≥ 4 % of DM intake, reduced carbohydrate digestibility will lower energy supply to the host animal. The pronounced decrease in estimated microbial protein synthesis even at moderate QTE levels is unlikely to be compensated by the increase in rumen-escape protein. The QTE addition to ruminant diets might not improve protein supply and performance of cattle.

3.2 Introduction

Protein is one of the most expensive dietary components in ruminant nutrition. Low to moderate concentrations (2-4 %) of condensed tannins in ruminant diets are considered to increase post-ruminal flow of non-ammonia nitrogen (N) due to their ability to form reversible complexes with feed proteins and thereby protect them from rumen degradation and enhance animals' protein supply (Jones and Mangan, 1977; Barry and Manley, 1984; Min et al., 2003). Hence, there is much interest in the strategic use of condensed tannins in

commercial ruminant feeding to enhance protein use efficiency. However, although condensed tannins primarily form complexes with proteins, they may also bind to minerals and polysaccharides such as starch, cellulose, hemicellulose, and pectin, thereby retarding their fermentation (Jansman, 1993; Bravo, 1998; Smith et al., 2005). Moreover, they may inhibit rumen microbial growth depending on the type of tannin, its source and dietary concentration, and the composition of the rumen microbial consortium (McSweeney et al., 2001; Smith et al., 2005). Slightly reducing the rate of protein degradation may increase efficiency of microbial crude protein (MCP) synthesis due to a synchronization of nutrient release (Makkar, 2003) as well as the inhibition of certain microbial species that are mainly responsible for protein degradation (Jones et al., 1994; Min et al., 2002; Vasta et al., 2010).

Quebracho tannin extract (QTE) is extracted from the heartwood of Quebracho tree (*Schinopsis lorentzii* (Engl.) and *Schinopsis balansae* (Engl.) found in the forests in the *Chaco* region of Argentina, Bolivia, and Paraguay. The QTE has frequently been used as a condensed tannin source in animal studies investigating its ability for e.g. improving body weight gain (Min et al., 2006), milk production (Dschaak et al., 2011), or efficiency of N utilization (Mezzomo et al., 2011) as well as reducing methane emissions (Beauchemin et al., 2007). Only a few studies investigated effects of QTE covering a broad range of dietary concentrations to examine its dose-dependency.

The present study aimed at analyzing the influence of low to high concentrations of QTE in the diet on rumen carbohydrate digestibility, N turnover and partitioning, and urinary purine derivatives (PD) excretion as an indicator of duodenal MCP flow. We hypothesized that QTE effects are dose-dependent and that even low QTE dosages will decrease ruminal protein degradation without affecting MCP synthesis and carbohydrate fermentation. It is expected, that moderate to high QTE dosages will reduce organic matter and especially protein and structural carbohydrate fermentation, thus limiting rumen microbial growth and reducing overall MCP supply to the host animal.

3.3 Material and methods

3.3.1 Animals, feeding, and experimental design

Six adult heifers fitted with ruminal cannulae were used (Jersey x German Black Pied Lowland). The animals were non-lactating, non-pregnant and housed in individual tie stalls with free access to fresh drinking water. The animals were offered a basal diet (as-fed basis; Table 5) of 2.6 kg/d of hay, 2.6 kg/d of concentrate feed, and 60 g/d of a mineral premix that

met their energy requirements for maintenance and utilizable crude protein (uCP) according to the GfE (2001). The composition of the concentrate feedstuff was as follows (on fresh matter basis): barley grain (35 %), wheat grain (35 %), sugar beet pulp (15 %), and rapeseed meal (15 %). Prior to the study, the grass hay was chopped (10-15 cm length), mixed well, and stored until feeding. At the same time, concentrations of dry matter (DM) were measured in grass hay and concentrate to calculate DM intakes of the experimental animals and hence, to determine the required QTE dosages. The basal diet was offered in two equal meals at 7:30 AM and 5:30 PM. The QTE product was a spray-dried moistened powder (Indusol ATO) produced in Argentina (Silvateam S.p.a., San Michele M.vì, Italy) containing 68.6 % total tannins and 15.9 % condensed tannins (on fresh matter basis; determined with butanol-HCL method after Terrill et al. (1992).

The study consisted of six periods, starting with a control period without QTE infusion (CON 1), followed by four consecutive periods in which all animals simultaneously received the same amount of QTE. Daily QTE dosages were 1, 2, 4, and 6 % of animals' DM intake (i.e. 45 g, 90 g, 180 g, and 270 g/d; as-fed basis). At each feeding, half of the daily QTE dosage was suspended in 1 l of lukewarm water and administered via the rumen cannula. Each period comprised 9 d of treatment adaptation followed by 6 d of total urine and feces collection. Subsequent to the 6 % QTE dosage, a second control period (CON 2) was conducted and urine and feces were collected again for 10 d, after 14 d of adaptation to the basal diet to minimize any carry-over effects.

This experimental design does not allow for exclusion of possible cumulative or residual effects of the previous QTE dosage. However, any effects of the period (i.e. advancing time of experiment) are unlikely since i) all meals had been prepared before the experiment started and their chemical composition did not differ between periods; ii) non-growing and non-pregnant heifers were used and fed at maintenance energy and protein level with a constant DM intake across all periods; and iii) animal and feeding management and climatic conditions were very similar during all periods. Furthermore, due to the high turnover rates of the microbial consortium, it is expected that the microbial ecosystem and with it protein and carbohydrate degradation pattern reached a steady state to the new tannin dosages after 9 d irrespective of the previous treatment.

Individual water intakes were measured by water meters attached to the animals' drinking troughs. The heifers were weighed prior to the study, once on d 9 of every period, and after the study to determine possible changes in body weight. Ambient air temperature and relative

air humidity in the stall were continuously recorded using a data logger (EL-USB-2-LCD, Lascar Electronics Ltd., Salisbury, United Kingdom; Table 6).

Table 5 Chemical composition of the grass hay and the concentrate feed (arithmetic means \pm standard deviations; n=6 samples).

		Hay			Concentrate	
DM	[% FM]	86.1	\pm	1.1	87.3	\pm 0.5
OM	[% DM]	94.4	\pm	0.6	96.0	\pm 0.0
CP		10.0	\pm	0.4	16.5	\pm 0.2
CL		1.2	\pm	0.2	2.3	\pm 0.1
NDF		77.6	\pm	3.9	27.8	\pm 3.5
ADF		38.0	\pm	0.8	10.8	\pm 0.6
ADL		3.0	\pm	0.2	1.7	\pm 0.2
Starch		n.d.			41.4	\pm 0.6
ME	[MJ/kg DM]	9.2	\pm	0.2	11.7	\pm 0.1
IvdOM	[%]	65.4	\pm	0.9	78.5	\pm 0.8

FM, fresh matter; DM, dry matter; OM, organic matter; CP, crude protein; CL, crude lipid; NDF, neutral detergent fiber (inclusive residual ash) ; ADF, acid detergent fiber (inclusive residual ash); ADL, acid detergent lignin; ME, metabolizable energy; IvdOM, *in vitro*- digestibility of organic matter; n.d., not determined.

Table 6 Minimum (min), maximum (max), and mean daily ambient air temperatures and relative air humidity during the collection phase at control periods (CON 1 and 2) and at different levels of intraruminal Quebracho tannin extract (QTE) infusion in heifers.

Period	QTE dosage (% of daily DM intake)	Temperature [°C]			Relative humidity [%]		
		min	max	mean	min	max	mean
1	CON 1	17.0	26.5	21.0	51.5	85.5	70.1
2	1	17.5	23.0	20.5	50.0	83.0	70.5
3	2	17.0	22.0	19.1	66.0	91.0	81.2
4	4	16.5	21.0	18.1	67.5	86.5	79.3
5	6	16.5	18.5	17.5	68.0	87.5	79.4
6	CON 2	14.5	17.5	16.1	49.5	73.5	61.5

DM, dry matter

3.3.2 Sample collection and preparation

Samples of hay and concentrate feed (~ 750 g fresh matter) were collected once during each period, ground to pass a 1-mm sieve (Retsch ZM1 mill), and each divided into three subsamples (~ 100 g fresh matter). For urine collection, a harness was attached to the vulva of each heifer that drained the urine directly into a canister. The canister contained about 150 ml of 20 % (v/v) of sulfuric acid to reduce pH to < 3 and to prevent bacterial degradation of PD and ammonia losses. Feces were manually collected from rubber mats placed behind the animals. The urine and feces were collected twice daily at each feeding and individually weighed. Additionally, urine volumes were recorded. Total feces excreted by each animal between two subsequent morning feedings were pooled and mixed well. A 10- %- aliquot of the feces was stored frozen until the end of each period. Similarly, total urine excreted between the two morning feedings were pooled and homogenized. For allantoin and uric acid determination, 500 ml of the pooled urine were filtered (Macherey-Nagel, average pore size 4 – 12 μm). Of this filtrate, 20 ml were diluted with distilled water to 100 ml (1:5, v/v), and three aliquots of approximately 10 ml of the diluted filtrate were taken and stored at -20°C until PD analysis. Of the remaining non-filtered urine, a 2- %- aliquot of urine was stored frozen until the end of each period. Then, all urine aliquots were thawed, pooled, and homogenized. Similarly, aliquots of feces were pooled. Three subsamples each of the urine (~ 500 ml) and feces (~ 500 g) pool samples were taken and stored at -20°C until chemical analysis. For determinations of fecal N, neutral and acid detergent fiber (NDF and ADF) concentrations aliquots (~ 300 g FM) of the frozen fecal subsamples were lyophilized (for 72 h) and ground to 1 mm before analysis (VirTis Freezemobile, Modell 12EL, Warminster, USA).

3.3.3 Laboratory analyses

All analyses were performed on two of the three feed, feces, and urine subsamples. If measured values between these two subsamples differed by > 5 %, the third subsample was additionally analyzed. Concentrations of DM, organic matter (OM), and crude lipid of feed and feces (DM and OM only) subsamples were determined in duplicate according to Naumann and Bassler (1997). Feed and feces subsamples were analyzed in duplicate for concentrations of NDF (using α -amylase for feed analysis) and ADF, both inclusive of residual ash, as well as acid detergent lignin (not in feces samples) according to procedures by Van Soest et al. (1991). For total N determination, feed, feces, and urine subsamples were analyzed in single determination following the Kjeldahl procedure (Naumann and Bassler,

1997). Mean N concentrations of two or three subsamples were calculated and concentrations of crude protein (CP) of feeds and feces were determined ($CP = N * 6.25$). Additionally, the acid detergent insoluble nitrogen (ADIN) concentrations in feed and feces were determined in duplicate by measuring the N content of the residues after ADF analysis. Starch concentrations of the concentrate feed were determined in triplicate determinations according to Brandt et al. (1989) after enzymatic oxidation by glucose oxidase using Chinonimin as colorimetric indicator. *In vitro*- digestible OM and metabolizable energy concentrations were estimated from gas production during *in vitro*- incubations using the Hohenheim gas test (Menke and Steingass, 1987). For this, three replicates of each feed subsample were incubated on two days each.

The urinary excretion of PD is commonly used as a non-invasive microbial marker for predicting the duodenal flow of MCP (Topps and Elliott, 1965; Verbic et al., 1990; Chen and Ørskov, 2003). The underlying principle is that purine bases reaching the small intestine are mainly of microbial origin (McAllan, 1982), are efficiently absorbed and their derivatives are mainly excreted via the urine (Verbic et al., 1990; Chen and Gomes, 1992). For PD analysis two samples of the filtered and diluted urine of day 1 and 2, 3 and 4, and 5 and 6 of each period were combined to three pooled. The amount of urine taken from each sample corresponded to the urine volume of the respective day. Around 20 ml of the pooled sample was stored. During CON 2, two additional samples were generated from day 7 and 8 and d 9 and 10. From these pooled samples, aliquots of 10 ml each were taken for analysis of allantoin and uric acid, respectively. Samples from animals on days when considerable urine losses occurred were excluded from pooling and data analysis (in 18 incidents of 240 cases (=6 periods * 6 animals * 6 d (except CON 2: 10 d)). The PD analysis was conducted using high-performance reversed-phase liquid chromatography (HPLC; JASCO[®] HSS-1500, Groß-Umstadt, Germany). Allantoin and uric acid concentrations (mmol/l) were determined following the procedures described by Chen et al. (1993) and Balcells et al. (1992), respectively. For this, the diluted aliquots (1:5) were further diluted with distilled water to 1:25 for allantoin and 1:20 for uric acid analysis. Allantoin and uric acid concentrations were quantified in duplicate by peak area analyses using JASCO BORWIN[™] chromatography software. Urine aliquots were analyzed in duplicate and analyses were repeated, if the discrepancy exceeded 6 %. Urinary allantoin and uric acid excretions (mmol/d) were calculated by multiplying the molar concentrations by the respective daily urine volume. Total urinary PD excretion was calculated as the sum of allantoin and uric acid excretion.

3.3.4 Calculations and statistical analyses

For calculation of apparent total tract digestibilities (ATTD) of DM, OM, NDF, ADF, and ADIN, daily QTE dosages were not considered as intake. Daily QTE dosages (on DM and OM basis) were subtracted from fecal DM and OM excretions, respectively, because QTE was taken as indigestible (Mueller-Harvey, 2006; López-Andrés et al., 2013). Daily QTE dosages were not subtracted from fecal NDF, ADF, and ADIN excretions because concentrations of NDF and ADF of QTE were very low (i.e. about 10 g ADF/kg DM). Duodenal MCP flow was estimated from urinary PD excretion according to the equations of Chen and Ørskov (2003).

All statistical analyses were conducted using the software R version 3.1.0 (the R foundation for statistical computing, Vienna, Austria). To identify effects of QTE infusion data were analyzed using the mixed model procedure (lme) and analysis of variance (anova). The model included treatment as a fixed effect and the animal as a random factor. An autoregressive correlation structure was chosen. Least squares means and standard errors of the means (SEM) were calculated for all measured and calculated parameters. Significant differences ($P < 0.05$) between least squares means were determined by the Tukey-Kramer test (mcp). Additionally, a linear regression model (lme) was applied to determine the dose-response relationship for all measured and calculated parameters, respectively. No significant differences were observed between parameters at CON 1 and 2, except for ATTD of ADIN (see Results section). Hence, mean values across both control periods of all parameters except ADIN were calculated for each animal and used for regression analysis.

3.4 Results

Except for the fact that some mucus was observed in the feces of all animals at 6 % QTE dosage, all heifers remained clinical healthy. No feed refusals occurred throughout the entire study. Mean body weight increased linearly with incremental QTE infusion ($P < 0.001$) from 475 kg (standard deviation (SD) 33) at CON 1 to 504 kg (SD 35) at 6 % QTE ($P = 0.045$). Thereafter, it decreased again to 484 kg (SD 33) at CON 2 ($P = 0.009$).

The ATTD of DM ($P \leq 0.045$), OM ($P \leq 0.044$), and NDF ($P \leq 0.020$) were lower at 4 and 6 % QTE compared to CON 1 and 2, and 1 and 2 % QTE (Table 7). Moreover, ATTD of ADF was lower at 4 and 6 % QTE than at CON 1 and 2 and 2 % QTE (SEM 0.02; $P \leq 0.026$). This negative effect of QTE on ATTD of carbohydrates was more pronounced for NDF and ADF than for total OM. A linear increase in fecal N excretion with increasing QTE dosage was found ($P < 0.001$; Table 8), whereas urinary N excretion linearly decreased with increasing

QTE dosage ($P<0.001$). With each additional %-unit of QTE infused, fecal N excretion increased by 2.0 g/d and excretion of urinary N decreased by 2.5 g/d. The absolute decrease in urinary N excretions (g/d) exceeded the increase in fecal N excretion, in particular at 1 and 2 % QTE. The partitioning of fecal N to urinary N linearly increased from 0.50 at CON 1 to 0.96 at 6 % QTE ($P<0.001$). The ATTD of CP decreased by 17 % from 0.698 at CON 1 to 0.576 at 6 % QTE (SEM 0.01; $P<0.001$). At 4 % QTE fecal excretion of ADIN was slightly higher than ADIN intake resulting in a negative coefficient of ATTD. Moreover, ATTD of ADIN was much higher at CON 2 than at CON 1 ($P=0.002$). These results may not be due to any real biological variability between heifers or between treatments, but are more likely due to the fact that the analytical methods available to us were not very accurate at the levels of ADIN found in the feces samples in this experiment.

Water intakes were higher at 1 % QTE than at 2, 4, and 6 % QTE, and CON 2 (SEM 3.6; $P\leq 0.029$; Table 9). Urine volume was not affected by QTE infusion ($P=0.107$). Urinary excretion of PD declined linearly with increasing QTE level ($P<0.001$). The infusion of QTE at 4 and 6 % reduced urinary PD excretion by about 9 and 19 %, respectively, compared to CON 1 (SEM 4.00; $P\leq 0.028$). Except for ATTD of ADIN, all measured and calculated parameters returned their CON 1 levels during CON 2.

Table 7 Nitrogen (N) excretion and apparent total tract nutrient digestibilities during control periods (CON 1 and 2) and at different levels of intraruminal Quebracho tannin extract (QTE) infusion in heifers (least squares means, standard errors of the means (SEM), n=6).

Parameter	Unit	QTE dosage (% of daily DM intake)						SEM	P-value
		CON 1	1	2	4	6	CON 2		
N excretion ^a	[g/d]								
Urinary		63 ^B	53 ^A	49 ^A	45 ^A	46 ^A	64 ^B	2.19	<0.001
Fecal		29 ^{AB}	31 ^{BC}	33 ^C	38 ^D	41 ^E	28 ^A	0.68	<0.001
Total		92 ^C	84 ^{AB}	82 ^A	84 ^A	87 ^{ABC}	92 ^{BC}	2.00	0.002
N retention	[g/d]	4.0 ^C	11.6 ^{AB}	13.7 ^A	12.5 ^A	9.4 ^{ABC}	4.3 ^{BC}	2.00	0.002
Fecal N/Urinary N		0.50 ^A	0.63 ^{AB}	0.72 ^B	0.91 ^C	0.96 ^C	0.48 ^A	0.04	<0.001
Fecal DM ^b	[g/d]	1143 ^A	1186 ^A	1170 ^A	1309 ^B	1307 ^B	1158 ^A	28.50	<0.001
Fecal OM ^b		964 ^A	996 ^A	1005 ^A	1092 ^B	1082 ^B	968 ^A	21.74	<0.001

To be continued

Table 7 continued

Parameter	QTE dosage (% of daily DM intake)							SEM	P-value
	Unit	CON 1	1	2	4	6	CON 2		
Apparent total tract digestibility coefficient									
DM ^c		0.750 ^B	0.741 ^B	0.744 ^B	0.714 ^A	0.714 ^A	0.747 ^B	0.01	<0.001
OM ^c		0.776 ^B	0.769 ^B	0.767 ^B	0.746 ^A	0.749 ^A	0.775 ^B	0.01	<0.001
NDF ^d		0.718 ^B	0.700 ^B	0.707 ^B	0.625 ^A	0.590 ^A	0.710 ^B	0.02	<0.001
ADF ^d		0.623 ^B	0.608 ^{BC}	0.628 ^B	0.539 ^{AC}	0.493 ^A	0.634 ^B	0.02	<0.001
CP		0.698 ^{DE}	0.672 ^{CD}	0.655 ^C	0.604 ^B	0.576 ^A	0.710 ^E	0.01	<0.001
ADIN		0.112 ^B	0.092 ^B	0.095 ^B	-0.001 ^A	0.060 ^{AB}	0.232 ^C	0.03	<0.001

^{A,B,C,D,E} Means within the same row with different superscripts differ at $P < 0.05$.

DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein; ADIN, acid detergent insoluble nitrogen.

^a N intake = 96 g/d

^b The respective QTE dosage was subtracted from fecal excretion.

^c Digestibility of DM and OM determined by excluding QTE from intake and subtracting respective QTE dosage from fecal excretion.

^d QTE dosage was excluded from intake. For details see section material and methods.

Table 8 Linear regression equations ($y = ax + b$) between urinary purine derivatives excretion (PD), nitrogen (N) excretion, and apparent total tract nutrient digestibilities (y, dependent variables) and Quebracho tannin extract dosage (x, independent variable, in % DM intake) (Coefficient value and standard errors (SE), n=6).

Parameter	Unit	a		b			
		Value	SE	P-value	Value	SE	P-value
Total PD excretion	[mmol/d]	-3.4	-0.4	<0.001	102.0	3.909	<0.001
N excretion ^a	[g/d]						
Urinary		-2.5	0.6	<0.001	58.6	2.064	<0.001
Fecal		2.0	0.2	<0.001	29.0	0.566	<0.001
Total		-0.5	0.5	0.337	87.5	1.846	<0.001
Apparent total tract digestibility coefficient							
DM ^b		-0.006	0.001	<0.001	0.749	0.004	<0.001
OM ^b		-0.005	0.001	<0.001	0.774	0.004	<0.001
NDF ^c		-0.023	0.004	<0.001	0.724	0.016	<0.001
ADF ^c		-0.024	0.004	<0.001	0.641	0.013	<0.001
CP		-0.021	0.002	<0.001	0.698	0.006	<0.001

DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; ADF, acid detergent fiber; CP, crude protein.

^a N intake = 96 g/d

^b Digestibility of DM and OM determined by excluding QTE from intake and subtracting respective QTE dosage from fecal excretion.

^c QTE dosage was excluded from intake. For details see section material and methods.

Table 9 Urinary purine derivatives excretion (PD) during control periods (CON 1 and 2) and at different levels of intraruminal Quebracho tannin extract (QTE) infusion in heifers (least squares means, standard errors of the means (SEM), n=6).

Parameter	Unit	QTE dosage (% of daily DM intake)						SEM	P-value
		CON 1	1	2	4	6	CON 2		
Water intake	[l/d]	22.4 ^{AB}	29.6 ^B	21.7 ^A	16.5 ^A	15.0 ^A	14.4 ^A	3.63	0.003
Urine volume	[l/d]	15.9	20.4	16.6	12.9	9.3	12.3	3.96	0.107
Urinary excretion	[mmol/d]								
Allantoin		87 ^{BC}	91 ^C	84 ^B	80 ^B	72 ^A	86 ^{BC}	3.52	<0.001
Uric acid		13 ^C	11 ^{BC}	11 ^{BC}	10 ^{AB}	9 ^A	11 ^{BC}	1.10	<0.001
Total PD		99 ^{CD}	103 ^D	95 ^{BC}	90 ^B	80 ^A	98 ^{BCD}	4.00	<0.001
Allantoin : total PD		0.87 ^A	0.89 ^B	0.89 ^{AB}	0.89 ^B	0.89 ^B	0.89 ^B	0.01	0.003

^{A,B,C,D} Means within the same row with different superscripts differ at $P < 0.05$.

3.5 Discussion

3.5.1 Apparent total tract nutrient digestibility

Compared to both control periods, feed intakes of animals were not reduced at any QTE dosage most likely due to the restricted feeding level. Moreover, the intraruminal QTE application might not have interfered with diet palatability. Besides the pronounced ability of condensed tannins to form complexes with feed proteins, they may impair microbial degradation of other polymers such as cellulose, hemicellulose, pectin, and starch (Jansman, 1993; Bravo, 1998; Smith et al., 2005). Similar to Komolong et al. (2001), Beauchemin et al. (2007), and Al-Dobaib (2009), the effect of QTE was much greater on the ATTD of CP compared to OM. While ATTD of CP was already affected even at low QTE dosages, ATTD of DM, OM, NDF, and ADF was only reduced at dosages ≥ 4 %. The decrease in NDF and ADF digestibilities were most pronounced. The greater N contents in fecal ADF at moderate to high QTE dosages may overestimate their negative effects on true ADF (and likely also NDF) digestibilities. However, the absolute increase in fecal excretion of ADF was greater than of ADIN, suggesting that ATTD of fiber decreased with increasing QTE dosage. The negative effect of QTE on ruminal fiber degradability is consistent with the shift in short-chain fatty acid concentrations in rumen fluid towards lower proportions of acetate and higher proportions of propionate and butyrate with increasing QTE dosages that was observed within the frame of our study (Dickhoefer et al., 2014) supports the negative effect of QTE on ruminal fiber degradability. Additionally, Kardel (2013) determined lower *in situ*-degradabilities of NDF from hay at 6 % QTE compared to CON 1 in our study. Other studies with sheep also reported lower NDF digestibilities with QTE at dietary concentrations (on DM basis) of 3 % (Al-Dobaib, 2009) and 5 % (Dawson et al., 1999; Salawu et al., 1997). On the one hand, the reduced ruminal carbohydrate fermentation is attributed to a complex formation of QTE- molecules with cell wall carbohydrates that prevents microbial attachment making the substrates unavailable for digestion (McAllister et al., 1994; McSweeney et al., 2001; Smith et al., 2005). On the other hand, condensed tannins might have directly inhibited fibrolytic microbes, by, for instance, binding to their cell surface (Bae et al., 1993; Scalbert, 1991) and/or reducing activities of their extracellular enzymes (Bae et al., 1993; Jones et al., 1994). The lower structural carbohydrate digestibility at high QTE dosages may have reduced the short-chain fatty acids supply to the animals as well as the availability of energy for MCP synthesis. Therefore, the strong decline in duodenal MCP flow as estimated from urinary PD

excretion can at least be partly explained by this reduced structural carbohydrate digestibility (see below).

3.5.2 Nitrogen excretion and retention

Infusion of incremental QTE dosages greatly shifted partitioning in N from urine to feces which is in line with results of the majority of studies on QTE effects (Dawson et al., 1999; Komolong et al., 2001; Al-Dobaib, 2009) and could contribute to lower ammonia emissions from manure in ruminant production (Powell et al., 2011). The strong decrease in urinary N excretion can be attributed to the protein-binding property of QTE and the consequently lower ruminal protein degradation and ammonia-N release (Dawson et al., 1999; Frutos et al., 2000; Dschaak et al., 2011). This is confirmed by the reduced *in situ*-effective degradability of CP from hay and rapeseed meal at 4 and 6 % of QTE compared to CON 1 observed in our study (Kardel, 2013). At high QTE dosages of 4 and 6 %, urinary N excretion did not further decline compared to 1 and 2 % QTE which might be attributed to the fact that there was less scope for a reduction in ruminal ammonia-N release and/or urinary N excretion. In addition to that, the decrease in N excretion from lower estimated duodenal MCP flow at 4 and 6 % QTE might have been compensated by increasing rumen-escape protein leading to a higher quantity of amino acids being absorbed in the small intestine. Also, the linear increase in fecal N excretion with increasing QTE dosage was likely related to an increased outflow of rumen-escape protein (including increased ADIN) as well as to higher losses of endogenous proteins (i.e. salivary proteins, digestive enzymes, mucus). As highlighted in a review by Waghorn (2008), it still remains unclear, if tannin-protein complexes fully dissociate at low pH in the abomasum and, if not, they also account for higher fecal N excretion. Additionally, there is evidence in the literature that free tannin molecules reaching the small intestine might reduce the activity of intestinal enzymes (Barry and Duncan, 1984; Silanikove et al., 1994) and/or rebind with feed or endogenous proteins after their release in the abomasum (McNabb et al., 1998) thereby reducing post-ruminal amino acid absorption (Dawson et al., 1999; Waghorn, 2008). Hence, assuming a lower precaecal digestibility of (feed) protein with QTE in the present study would have accounted for higher fecal N excretions as well as lower urinary N excretions and would negate any positive effect of increased rumen-escape protein flow.

Irrespective of the QTE dosage, N retention was higher with QTE infusion than at CON 1 and 2 which contradicts results of other studies with growing lambs that observed no (Dawson et al., 1999; Komolong et al., 2001) or negative effects of QTE on N retention (Al-Dobaib,

2009). The higher N retention with than without QTE in the our study cannot be fully explained because study animals were fully grown, fed at maintenance energy level, and QTE infusion markedly reduced nutrient digestibilities. However, we suspect that animals utilized protein for compensatory mechanisms such as to protect the intestinal epithelium against tannins. Feces of animals were covered with mucus at highest QTE dosage in our study which agrees with findings of Hervás et al. (2003). Also, Dawson et al. (1999) observed focal surface epithelial ulceration and increased mucosal histiocytes in the jejunum and ileum of sheep dosed with 5 % QTE (on DM basis). Beside these defence mechanisms, animals might have retained protein for compensation of endogenous losses such as increased secretion of salivary glycoproteins and digestive enzymes, higher regeneration of epithelial cells, and higher mucus production in the gastro-intestinal tract with increasing QTE infusion. The increase in body weight with QTE in our study is considered to arise at least partly from these defence mechanisms rather than being due to a higher muscle growth. Besides, greater gut fill of the gastro intestinal tract due to lower ATTD of OM may furthermore explain the body weight increase as well as its decline back to CON 1 level after QTE infusion was ceased at CON 2.

3.5.3 Urinary purine derivatives excretion and estimated microbial protein synthesis

Condensed tannins are considered to have adverse effects on rumen microbial growth (McSweeney et al., 2001), depending on, amongst other factors, the microbial group or species (Jones et al., 1994; O'Donovan and Brooker, 2001; Vasta et al., 2010) and the type, source, and concentration of tannins in the diet (Min et al., 2003; Smith et al., 2005). In the present study, urinary PD excretion decreased linearly with increasing QTE dosage levels. Similarly, alfalfa hay with 3 % QTE (on DM basis) reduced urinary PD excretion in sheep in a study by Al-Dobaib (2009). Conversely, Komolong et al. (2001) found no effect of up to 6 % QTE (on DM basis) on urinary PD excretion in sheep. According to the equation of Chen and Ørskov (2003), the lower urinary PD excretions of our study animals suggest a decrease in duodenal MCP flow from 318 g/d at Con 1 to 293 g, 269 g, and 215 g/d at 2, 4, and 6 % QTE. However, higher ratios of purine-N to total-N with increasing QTE dosages were determined in liquid-associated microbes in the solid phase within the framework of our study (Dickhoefer et al., 2014). Estimated MCP flow using these purine-N to total-N ratios are much lower than those derived according to Chen and Ørskov (2003), indicating that the negative linear effects of QTE on MCP synthesis were even more pronounced. Moreover, infusion of QTE might have reduced praecaecal digestibility of microbial purines (see chapter

N excretion and retention), although changes do not greatly alter MCP estimates from urinary PD excretion.

A lower estimated duodenal MCP flow in our study could be ascribed to direct inhibitory effects of QTE on rumen MCP synthesis such as the inhibition of extracellular enzymes (Bae et al., 1993; Jones et al., 1994), binding of QTE molecules to the microbial cell wall and membrane causing morphological changes (Bae et al., 1993; Scalbert, 1991), and/or a reduced availability of essential metal ions (Scalbert, 1991). A lack of rumen degradable N for microbes due to a lower protein degradability associated with QTE is precluded because ammonia-N concentrations in rumen liquid in our study (Dickhoefer et al., 2014) were within the optimal range for microbial growth (Satter and Slyter, 1974). The impaired carbohydrate digestibility, however, probably reduced energy availability for rumen microbes and thus MCP synthesis (see section above).

Altogether, the reduced ATTD digestibility of CP, lower duodenal flow of MCP estimated from urinary PD excretion, and the increasing endogenous protein losses at moderate to high QTE infusion levels most likely decreased rather than increased protein availability for the host animal.

3.6 Conclusions

Dosing cattle with moderate QTE levels (≥ 4 % DM) has detrimental effects on ruminal carbohydrate degradability, lowering energy supply to the host and reducing duodenal MCP flow estimated from urinary PD excretion. The QTE infusion may greatly shift N excretion towards feces, which can be desired from an environmental perspective. However, it is unlikely that the increase in rumen-escape protein will lead to greater post-ruminal amino acid absorption and improve cattle performance at moderate to high QTE dosages mainly due to pronounced reductions in estimated duodenal MCP flow and increase in fecal N excretions.

3.7 References

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4 General discussion

The microbial crude protein (MCP) is of great nutritional importance in ruminants and its correct quantification is a key issue in order to improve the accuracy of protein feeding to ruminants (Shingfield and Offer, 1998; Reynal et al., 2005). The urinary purine derivatives (PD) excretion is a non-invasive indicator for prediction of MCP supply in ruminant animals and has been frequently used in feeding studies because the traditional measurement of duodenal MCP flow is associated with difficulties including low statistical power and the need for (double) cannulated animals (Titgemeyer, 1997). Using urinary PD excretion as a method for prediction of MCP supply is based on the assumption that purine bases reaching the duodenum originate mainly from rumen microbes (McAllan and Smith, 1973; McAllan, 1982), are extensively metabolized, and their derivatives are largely excreted via the urine (Verbic et al., 1990; Balcells et al., 1991; Chen and Gomes, 1992). Hence, the urinary PD excretion could serve as method for detecting relative changes in duodenal MCP flow due to dietary modifications and for evaluation of protein supply under practical conditions. One of the main focuses of the present thesis is to evaluate the potential of the urinary PD approach to reflect changes in duodenal MCP flow induced by intraruminal infusion of increasing dosages of glucose, a rapidly degradable carbohydrate, and Quebracho tannin extract (QTE), a condensed tannin source.

4.1 Validation of urinary purine derivatives excretion as an indicator for estimation of duodenal microbial protein flow

4.1.1 Accuracy of urinary purine derivatives excretion to quantify microbial protein flow

Several methods have been applied to differentiate between dietary protein and MCP in duodenal digesta flow (Broderick and Merchen, 1992; Stern et al., 1994). The variability of MCP flow estimates obtained with different markers is quite large (Siddons et al., 1982; Carro and Miller, 2002; Reynal et al., 2005). There is no absolute standard marker method that accurately predicts duodenal MCP flow which is attributed to the complexity of the rumen microbial consortium and its metabolism on the one hand and technical difficulties involved with measurements using cannulated animals on the other hand (Stern et al., 1994; Dewhurst et al., 2000). To evaluate a microbial marker, researches attempted to find the most suitable microbial marker for estimating duodenal MCP flow by simultaneously using different markers (Stern et al., 1994). Several studies aimed at validating the method of urinary PD

excretion by comparing estimated duodenal MCP flow from urinary PD excretion with other internal (total purines, amino acid profiles) or external (^{15}N) markers, however, results presented are contradictory. In some studies, estimates of duodenal MCP flow based on urinary PD excretion were 31 % (Reynal et al., 2005) and 48 % (Martín-Orúe et al., 2000) lower compared to MCP flow estimates based on total purine bases flow in the duodenum. Likewise, other experiments reported underestimation of duodenal MCP flow when using urinary PD excretion compared to estimates using ^{15}N as microbial marker (Pérez et al., 1997; Brito et al., 2007). In contrast, in a study by Hristov et al. (2005), duodenal MCP flow estimates were about 21 and 52 % higher using urinary PD excretion compared to estimations based on ^{15}N or total purine bases as microbial marker, respectively. Although there was no other microbial marker measured in the present studies in order to validate the urinary PD excretion method, a comparison to calculated values of duodenal MCP flow based on the GfE (2001) was carried out. Estimated duodenal MCP flow based on urinary PD excretion without infusion of glucose (Control I; main trial; Chapter 2) and QTE (CON 1; Chapter 3) were 76 and 318 g MCP/day, respectively. However, a duodenal MCP flow of 450 and 522 g MCP/d was expected during the glucose and QTE study, respectively, based on digestible organic matter intake (DOMI) and an average efficiency of MCP synthesis of 156 g MCP/kg DOMI (GfE, 2001) which reveals in a discrepancy of 83 and 39 % in prediction of duodenal MCP flow estimates. Instead, De Boever et al. (1998) observed a good agreement between estimated duodenal MCP flow in bulls based on urinary PD excretion and MCP estimates derived from different protein evaluation systems currently in use (i.e. NRC, 1996; Madsen, 1985; Alderman and Cottrill, 1993; Tamminga et al., 1994). Besides, it should be noted that the pronounced underestimation of duodenal MCP flow derived from urinary PD excretion in the present studies might also result from an overestimation of the calculated value based on GfE (2001). The underestimation of duodenal MCP flow when using purine bases and urinary PD excretion compared to ^{15}N as microbial marker is mainly attributed to the unrepresentative nature of the isolated microbial sample (Pérez et al., 1997). Microbes associated with the liquid phase have generally higher purine-nitrogen (N) to total-N ratios than solid-associated microbes (Firkins et al., 1987; Obispo and Dehority, 1999; Martín-Orúe et al., 2000). Therefore, if liquid-associated microbes in the isolated microbial reference sample are over-represented compared to solid-associated microbes, duodenal MCP flow will be underestimated (Broudiscou and Jouany, 1995, Hristov et al., 2005). Besides, the purine-N to total-N ratios in mixed rumen microbes were shown to vary with i) microbial growth rates (Bates et al., 1985), ii) rumen outflow rates (Rodríguez et al., 2000), and iii) forage to

concentrate ratio (Pérez et al., 1998). Hence, the accuracy of estimating MCP supply from urinary PD excretion or other internal and external microbial marker largely depends on obtaining a microbial sample that is representative for the mixed rumen microbial consortium (Dewhurst, 2000; Shingfield, 2000; Gonzáles-Ronquillo et al., 2004). Purine-N to total-N ratios of mixed rumen microbes were determined within the frame of our second study (Dickhoefer et al., *submitted*). Results indicate that microbial purine concentration was actually lower compared to the value proposed by Chen and Ørskov (2003) and using the determined values will result in higher MCP estimates. Hence, the underestimation of duodenal MCP flow partly results from lower microbial purine concentration in the second study and probably in the first study, too, although there is no information on the microbial purine concentration. Besides, further reasons for the discrepancy between estimated and predicted duodenal MCP flow might be a difference in the contribution of endogenous PD, the digestibility of microbial purine bases, and/or the proportion of microbial purines that are excreted via the urine from the proposed value in the model of Chen and Ørskov (2003). Considering the former aspect, Verbic et al. (1990) mentioned that the basal endogenous PD excretion measured in steers using the “intra-gastric nutrition technique” where animals are totally nourished by volatile fatty acids and casein might not represent the true endogenous PD excretion when animals receive normal feed. Thus, it can be assumed that endogenous PD excretion of our study animals, fed at maintenance requirements, might have been lower compared to Verbic et al. (1990) and would have also caused the underestimation in duodenal MCP flow since values of these authors were used.

4.1.2 Reliability of urinary purine derivatives excretion to reflect relative changes in duodenal microbial protein flow

Although estimates of duodenal MCP flow were often lower using urinary PD excretion, the excretion of PD in urine appears to highly correlate with the flow of purine bases (Martín-Orúe et al., 2000), microbial non-ammonia-N flow (Reynal et al., 2005), dietary rumen degradable protein content (Martín-Orúe et al., 2000; Reynal et al., 2005), dry matter intake (Stefanon et al., 1995; Moorby et al., 2006), and forage to concentrate ratios (Perez et al., 1997). Chen and Ørskov (2003) emphasized that the urinary PD excretion should rather be used to evaluate relative differences in duodenal MCP flow than for estimating the absolute duodenal MCP flow. In contrast thereto, results presented in this thesis indicate that the urinary PD excretion is unable to reflect small changes in duodenal MCP flow with increasing dosages of glucose and unreliable to predict the absolute extent of decrease in duodenal MCP

flow with increasing QTE infusion. In the first study (main trial; Chapter 2), the intraruminal infusion of incremental glucose dosages increased urinary PD excretion, suggesting an increased duodenal MCP flow (Rooke et al., 1987; Chamberlain et al., 1993; Kim et al., 1999a). The extent of increase in urinary PD excretion at 1000 g/d of glucose compared to that without glucose administration (Control I) is only slightly lower than the predicted increase in urinary PD excretion assuming the efficiency of MCP synthesis given by GfE (2001; Chapter 2, subchapter 2.5.2) and using the equations provided by Chen and Ørskov (2003). However, contrary to our hypothesis, the relationship between glucose dosage and urinary PD excretion was not linear. A pronounced increase in PD excretion was observed at lowest glucose dosage level of 125 g/d. Higher dosages up to 1000 g/d did not lead to any further increases in urinary PD excretion compared to glucose at 125 g/d. Therefore, it can be assumed that the PD excretion failed to reflect the responses of rumen MCP synthesis to even greater increments in intraruminal glucose dosages. It appears likely that glucose induced changes in the species composition of the microbial consortium and the efficiency of MCP synthesis as well as changes of the microbial growth rates that in turn altered the purine concentration in mixed microbial matter. It is concluded, that these shifts are mainly responsible for the non-linear response in urinary PD excretion and they may thus hamper the accuracy of MCP estimates. However, although fecal N excretion increased linearly with incremental glucose infusion, which is assumed to arise from higher duodenal MCP flow, it cannot be excluded that MCP synthesis at high glucose dosages was lower than expected and hence, would also give an explanation for the non-linear response in PD excretion.

In the second study (Chapter 3), urinary PD excretion declined linearly with increasing levels of intraruminal QTE infusion, reflecting a decrease in duodenal MCP flow which is mainly attributed to direct and indirect inhibiting effects of QTE on total microbial consortium or certain microbial species within the rumen. This decrease in urinary PD excretion at moderate to high QTE dosages is in line with the effects observed for apparent total tract digestibility of OM and urinary and fecal N excretions and confirms our hypothesis. This suggests that urinary PD excretion sensitively reflected the adverse effects of tannins on MCP supply. However, the observed shifts towards higher purine-N to total-N ratio in liquid-associated microbes with increasing levels of QTE infusion within the frame of our study (Dickhoefer et al., 2014) has revealed a relevant shortcoming. Estimated duodenal MCP flow using actually measured purine-N to total-N ratios were much lower than those estimates derived using the purine concentration in microbial mass given by Chen and Ørskov (2003) which indicates that the negative linear effects of QTE on MCP synthesis might have been even more pronounced.

As the extent of inhibition of condensed tannins is reported to depend, amongst other factors, on the microbial group or species (Jones et al., 1994; O'Donovan and Brooker, 2001; Vasta et al., 2010), the infusion of QTE in our study likely altered species composition towards those with higher purine concentrations and/or higher growth rates. Also, Castro-Montoya et al. (2011) have shown increment in purine base content and a decreasing crude protein to purine ratio in *in vitro*-incubations of different purified QTE levels and attributed this to shifts in microbial consortium. Hence, these results suggest that estimates of duodenal MCP flow from urinary PD excretion in response to QTE in ruminant diets may be inaccurate due to shifts in the composition of the microbial consortium.

The results of both studies demonstrate that the prediction of MCP supply is subjected to considerable inaccuracy using urinary PD excretion. Assuming a constant purine-N to total-N ratio in microbial matter represents a potential source of error which may mask changes in duodenal MCP flow when estimated from urinary PD excretion. Although the absolute ratio is not important when the aim is to reflect relative differences in duodenal MCP flow caused by the treatment (e.g. glucose or QTE), shifts in purine concentration between different treatments of a study (e.g. dosage of glucose or QTE) will result in highly inaccurate MCP estimates, rendering the urinary PD excretion an unreliable indicator. In order to enhance the accuracy of prediction when using urinary PD excretion, research is needed that focus on the purine-N to total-N ratio under various feeding conditions.

4.1.3 Equations to predict the duodenal purine bases flow

Several studies established predictions equations derived from measuring the urinary recovery of post-ruminally infused nucleic acids (steers, Verbic et al., 1990), purines (dry and lactating cows, Vagnoni et al., 1997), or yeast ribonucleic acid (bulls, Beckers and Thewis, 1994; dry cows, Orellana Boero et al., 2001; lactating cows, González-Ronquillo et al., 2003). All the established prediction equations are available for use, however, estimated values of endogenous contribution and recovery of absorbed purines differ between 0.236 (Orellana Boero et al., 2001) and 0.531 mmol/kg metabolic body weight ($BW^{0.75}$) (Beckers and Thewis, 1994), and 0.56 (González-Ronquillo et al., 2003) and 0.86 (Vagnoni et al., 1997; Orellana Boero et al., 2001), respectively. Different estimates of endogenous PD losses can partially be attributed to differences in measurement techniques. While the prediction equation of Verbic et al. (1990) incorporates estimates of endogenous PD losses derived from direct measurements of basal PD excretion using the gastric nutrition technique, other studies

calculated endogenous PD losses from the intercepts of the equation (Beckers and Thewis, 1994; Vagnoni et al., 1997) or used a combination with ^{15}N -labelled microbial purine bases (Orellana Boero et al., 2001; González-Ronquillo et al., 2003). Differences in recovery rate are mainly attributed to differences in duodenal digestibility of infused purines (Orellana Boero et al., 2001) and, in dairy cows, recovery of PD was shown to differ between early and late lactation (González-Ronquillo et al., 2003). It was hypothesized, that the extent of endogenous contribution and excretion of PD via non-renal routes, which determines the recovery of absorbed purines, may vary depending on the physiological and nutritional status of the animal (Chen et al., 1990; Balcells et al., 1991; Johnson et al., 1998; González-Ronquillo et al., 2003). For instance, Reynal et al. (2005) observed an underestimation of duodenal MCP flow based on urinary PD excretion and attributed this underestimation partly to the prediction equation used because this equation was developed with dry and late lactation cows (Vagnoni et al., 1997) compared to the early lactation cows used in their study. Therefore, use of equations that are derived from experiments where conditions considerably differ to those conditions of the actual study may lead to inaccuracy in MCP estimates (Reynal et al., 2005). Additionally, Firkins et al. (2006) emphasized that the prediction equations are based on direct measurements of duodenal flow of purine bases and any errors in estimation of the intercept will affect accuracy of the prediction equation since intercept and slope are highly correlated.

In the present studies, the model after Chen and Ørskov (2003) was used for estimating duodenal MCP flow which is based on the prediction equation for duodenal flow of purine bases established by Verbic et al. (1990). This prediction equation assumes a value for endogenous contribution of $0.385 \text{ mmol/kg BW}^{0.75}$ and a recovery rate of 0.85 ($y = 0.85x + 0.385 \cdot \text{BW}^{0.75}$). In the following, a comparison between the prediction equation of Verbic et al. (1990) and the equations of Beckers and Thewis (bulls, 1994; $y = 0.72x + 0.531 \cdot \text{BW}^{0.75}$) and Orellana Boero (dry cows, 2001; $y = 0.86x + 0.236 \cdot \text{BW}^{0.75}$) will be conducted to highlight differences in estimating duodenal MCP flow using different prediction equations. Applying the model proposed by Beckers and Thewis (1994) to the measured urinary PD excretion in the present studies reduces estimated duodenal MCP flow without glucose infusion (Control I; Chapter 2) to -4 g MCP/d (instead of 76 g MCP/d) and without QTE infusion (CON 1; Chapter 3) to 278 g MCP/d (instead 318 g MCP/d), respectively. Applying the response model developed by Orellana Boero (2001), would result in higher estimates of duodenal MCP flow being at 160 g MCP/d (instead of 76 g MCP/d) without glucose infusion (Control I) and at 404 g MCP/d (instead 318 g MCP/d) without QTE infusion (CON 1).

Regarding relative differences in estimated duodenal MCP flow, the extent of increase with 1000 g/d of glucose and decrease at 6 % QTE in the diet, respectively, were similar between models. Therefore, even if estimated values for endogenous contribution and recovery of absorbed purine bases differ between prediction equations, relative changes in duodenal MCP flow will be reflected. However, for quantifying duodenal MCP flow, this comparison of prediction models clearly demonstrates that different assumptions about endogenous contribution and recovery of absorbed purine bases will cause considerable under- or overestimations depending on the equation used.

4.1.4 Implications of using the urinary purine derivatives excretion as indicator for microbial protein flow

The urinary PD excretion as an indicator for duodenal MCP flow is based on a number of parameters that are assumed to be constant, including the ratio of purine-N to total-N ratio in microbial matter, duodenal purine base digestibility, recovery rate of absorbed purines, and endogenous contribution to urinary PD excretion. Although the majority of studies that aim at validating the quantitative relationship between urinary PD excretion and duodenal MCP flow concluded that it is a sensitive indicator for animals' MCP supply (Vagnoni and Broderick, 1997; Chen and Ørskov, 2003; Moorby et al., 2006), results presented in this thesis (Chapter 2 and 3) indicate that this conclusion might not be valid for situations when considerable amounts of rapidly fermentable carbohydrates and condensed tannins are fed. Treatment-related changes in microbial purine concentrations might not only result in considerable under- or overestimations of duodenal MCP flow, but also mask potential treatment effects on urinary PD excretion. Moreover, the ability of the urinary PD approach to account for potential variations in recovery rates of absorbed PD in urine or, vice versa their diversion into non-renal routes as well as the excretion of endogenous PD strongly influences the accuracy of the results derived from it. Besides, as mentioned above, the use of prediction equations to estimate duodenal MCP flow from urinary PD excretion that were established under different conditions may potentially increase the inaccuracy of MCP estimates.

In conclusion, the urinary PD excretion as an indicator of duodenal MCP flow has potential, however, there are still limitations making it unreliable for being applied as reference measurements for ruminal MCP synthesis within protein evaluation systems. For improving the accuracy and sensitivity of predicting duodenal MCP flow, further research is required that focus on dietary and animal factors that lead to considerable variations in, for instance, microbial purine concentration, true digestibility and recovery rate of absorbed purines, and

endogenous PD losses. Consequently, a profound database should be created in order to establish prediction equations for different feeding situations (i.e. various concentrates and forages, secondary plant metabolites) and animals (i.e. breeds, physiological state).

4.2 Strategies to modify microbial protein synthesis and efficiency of nitrogen utilization in the rumen

The maximization of microbial growth and the amount of rumen degradable protein that is incorporated into microbial biomass is one of the main goals of ruminant nutritionists in order to improve the amino acid flow to the small intestine and to reduce fermentation losses of N (Bach et al., 2005). The rumen metabolism is considered as the most important factor which contributes to the lower efficiency of N utilization in ruminants (Tamminga, 1992). The microbial protein degradation in the rumen often leads to a net loss of N when protein and carbohydrates are imbalanced and cannot be used efficiently for MCP synthesis (Tamminga, 1996). The traditional assessment of the efficiency of MCP synthesis is determined in g MCP/ unit of ruminal available energy (i.e. kg digestible organic matter intake (DOMI) or metabolizable energy (ME)) since energy is assumed to be the most limiting factor for MCP synthesis (Clark et al., 1992; Stern et al., 1994). Therefore, the efficiency of MCP synthesis is an indicator of the efficiency of utilization of available energy for microbial growth, however, it does not indicate the efficiency of N utilization by ruminal microbes (Bach et al., 2005; Calsamiglia et al., 2010). Bach et al. (1999) proposed a rumen N use efficiency in terms of g of bacterial N/ g of available N. Although this concept may improve our understanding of efficiency of N capture by rumen microbes and would contribute to reduce N excretion by ruminants, it is difficult to apply because it relies on estimating the availability of endogenous N in the rumen (Calsamiglia et al., 2010). At the animal level, efficiency of N utilization (ENU) is commonly expressed in terms of g N in product/ g N intake. There is a large variation in ENU (15 - 40 %, Calsamiglia et al., 2010) indicating the opportunity for dietary modifications to improve ENU (Dijkstra et al., 2013). Numerous studies investigated strategies for modifying rumen metabolism in view of optimizing rumen microbial fermentation and duodenal protein flow. Most effective strategies to reduce N losses are decreasing the concentration of dietary N, manipulating the rumen protein degradation, and/or manipulating the efficiency of N utilization by rumen microbes (Tamminga, 1996). The present thesis aimed at investigating the influence of nutritional factors on ruminal MCP synthesis in view of their potential to increase animals' protein supply by increasing duodenal MCP flow and/or reducing ruminal protein degradation, and to thereby improve ENU.

4.2.1 Feeding rapidly fermentable carbohydrates

In ruminant diets, feeding rapidly fermentable carbohydrates (i.e. sugars) aims at increasing energy availability for rumen microbial growth and thereby enhance MCP synthesis (Nocek and Russell, 1988) and N capture by rumen microbes (Sniffen et al., 1992). Silage-based diets often contain high levels of non-protein N and rumen degradable protein and ammonia-N will be rapidly released in the rumen (Givens and Rulquin, 2004). In such cases, providing rapidly fermentable carbohydrates is believed to increase MCP synthesis because availability of fermentable energy and N in the rumen will be more synchronized (Dewhurst et al, 2000).

Regarding the effects of feeding sugars on MCP synthesis, results in the literature are inconsistent. In the first study, intraruminal infusion of glucose increased duodenal MCP flow (Chapter 2). Likewise, several studies observed increased flow of MCP at the duodenum with increasing amounts of rapidly fermentable carbohydrates including glucose (Rooke et al., 1987), sucrose (Kim et al., 1999 a, 2000; Chamberlain et al., 1993), and maltodextrin (Kim et al., 1999b). This increase in ruminal MCP synthesis is mainly attributed to be a direct result of increased energy supply with sugar supplementation that enables higher MCP synthesis. However, the partial replacement of dietary corn with sucrose decreased ruminal MCP synthesis (Sannes et al., 2002) and MCP synthesis was unchanged when replacing dietary starch with sucrose (Broderick et al., 2008). Oba (2011) concluded that feeding sugars may not increase duodenal MCP flow if it is replaced for starch. This can partially be explained by the fact that the fermentation of mono- and disaccharides (sugars) yields less adenosine triphosphate per g fermented substrate compared to the maximum value achieved by polysaccharides (Hall and Hereijk, 2001; VanDuinkerken et al., 2011).

Some studies have shown that feeding sugars decreased ammonia-N concentration in ruminal fluid (Chamberlain et al., 1993; Sannes et al., 2002; Broderick et al., 2008) and urinary N excretion (Sannes et al., 2002; Broderick et al., 2008). However, ENU for milk protein production was shown to decrease with inclusion of sucrose (Broderick et al., 2008). It was suggested that feeding high-sugar diets may not improve ENU when replaced for starch (Oba, 2011). The data of the first study (Chapter 2) cannot contribute to a better understanding regarding effects of sugar infusion on ENU because ruminal ammonia-N concentrations were not measured and animals used were non-producing and fed at maintenance energy level. Although fecal N excretion increased with incremental glucose infusion which was attributed to higher ruminal MCP synthesis, urinary N excretion remained at a constant level, irrespective of the glucose dosage level. It is suggested that the effect of N capture of rumen

microbes due to glucose was not high enough to provoke any reduction in urinary N excretion and hence, decrease volatile N losses. If N intake from the basal diet and urea would have been lower, it can be assumed that urinary N excretion would have decreased with increasing glucose dosages. Besides, Heldt et al. (1999) emphasized that feeding sugars (glucose and fructose) are superior to starch at improving the utilization of low quality forages owing to increased fiber digestion and feed intake if ruminal degradable protein is sufficient.

4.2.2 The use of Quebracho tannin extract in modification of rumen protein turnover

Some of the most important factors determining protein degradation by rumen microbes include the solubility and structure of the dietary protein (Yu, 2006), interactions with other feed compounds, and the prevailing microbial consortium which in turn greatly depends on the type of ration, passage rate, and rumen pH (Bach et al., 2005). High-quality forages often contain high proportions of rapidly soluble protein that are degraded in the rumen, releasing ammonia-N that will, if not incorporated into microbial matter, result in inevitable losses of N from the rumen and reduce ENU (Calsamiglia et al., 2010). Therefore, protecting feed proteins from microbial breakdown will decrease their excess degradation beyond the microbial requirements for N and thereby improve the ENU (Tamminga, 1992; Reynolds and Kristensen, 2008). For instance, low to moderate concentrations of condensed tannins are considered to increase rumen-escape protein due to reduced ruminal protein degradation because condensed tannins are able to bind (dietary) protein and thus improve protein supply at the small intestine (Jones and Mangan, 1977; Min et al., 2003). In the second study of this thesis (Chapter 3), the decreasing urinary N and increasing fecal N excretions with increasing QTE dosages are primarily attributed to reduced ruminal protein degradation and a greater outflow of rumen-escape protein. Ammonia-N concentrations in ruminal fluid were not altered by QTE infusion in our study (Dickhoefer et al., 2014). This was likely related to a lower ammonia-N release (from reduced protein degradation) along with lower incorporation of N compounds in microbial matter due to decrease in rumen MCP synthesis with increasing QTE dosages. Considering another reason, ruminal protein degradation to peptides can be reduced without affecting ruminal ammonia-N concentration (Bach et al., 2005). Ferme et al. (2004) showed that *Prevotella ruminantium* and *Prevotella bryantii* are important ammonia-producing bacteria and reduction in their numbers reduced ammonia-N concentration in rumen fluid in the study of these authors. Therefore, lack of QTE effect on major ammonia-producing microbes and their deaminase activity could also explain the similar ammonia-N concentrations in rumen fluid across QTE infusion levels. Besides, regarding effects on rumen

MCP synthesis, an incomplete ruminal protein degradation to peptides could have caused a shortage of peptides and amino acids, limiting growth of certain microbial species that require such N sources (Maeng et al., 1976; Griswold et al., 1996). This effect might have additionally hampered rumen MCP synthesis besides the other direct (e.g. inhibition of extracellular enzymes) and other indirect effects (e.g. reduced energy availability through impaired carbohydrate fermentation) of QTE on rumen MCP synthesis discussed in Chapter 3.

Although the reduction in urinary N excretion with increasing QTE dosages can be regarded as beneficial because it could contribute to lower ammonia-N emissions from manure (Powell et al., 2011), the decrease in duodenal MCP flow, decrease in efficiency of MCP synthesis, and hence, the reduction in animals protein supply at moderate to high QTE dosages will negate any positive effects of reduced urinary N excretion. Therefore, it is concluded that QTE addition appears unable to improve ENU. Other studies investigating effects of QTE addition in producing dairy cows (Dschaak et al., 2011) and growing lambs (Komolong et al., 2001) could not observe positive effects on ENU.

4.3 Conclusions

The results of the present thesis demonstrate that the intraruminal infusion of glucose increases and QTE decreases the urinary excretion of PD. Therefore, using the urinary PD excretion as a non-invasive indicator appears to be able to reflect changes in duodenal MCP flow. However, urinary PD excretion probably underestimates duodenal MCP flow compared to calculated values (GfE, 2001). Furthermore, the intraruminal infusion of increasing dosages of glucose does not result in a linear response in urinary PD excretion. This non-linear response in urinary PD excretion to incremental dosages of glucose infusion is at least partly attributed to shifts in the species composition of rumen microbial consortium causing changes in the purine-N to total-N ratio of mixed rumen microbes. Urinary PD excretion declined linearly with increasing QTE dosage reflecting a decrease in duodenal MCP flow which is mainly attributed to inhibiting effects of QTE infusion on rumen microbial growth. Shifts in the purine concentration with increasing QTE dosages show that the urinary PD excretion is unreliable to predict the absolute extent of decrease in duodenal MCP flow with increasing QTE dosages. The QTE infusion greatly shifted N excretion towards feces, which can be desired from an environmental perspective. However, the QTE addition to ruminant diets might not be able to improve protein supply and performance of cattle.

Hence, the urinary PD excretion as an indicator of duodenal MCP flow has potential, however, there are still limitations making it unreliable for being applied as reference measurements for duodenal MCP flow within protein evaluation systems. Treatment related changes in i.e. microbial purine concentrations might not only result in considerable under- or overestimations of duodenal MCP flow, but also mask potential treatment effects on urinary PD excretion and hence, limiting the accuracy and validity of the urinary PD excretion as a non-invasive indicator. Therefore, extensive research is required that focus on various dietary and animal factors that lead to considerable variations of the parameters (i.e. purine concentration, endogenous PD excretion) required for using this indicator.

4.4 References

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5 Summary

The protein synthesis by rumen microbes plays a central role in ruminant nutrition, providing more than half of the essential amino acids that are available for absorption at the small intestine. Therefore, the accurate quantification of duodenal microbial crude protein (MCP) flow is a key issue in ruminant nutrition in order to provide especially high producing ruminants with adequate amounts of protein and to improve production performance and efficiency of nitrogen utilization. The excretion of purine derivatives (PD) via the urine is a non-invasive indicator for estimating duodenal MCP flow in ruminant animals and has the potential to be used even under on-farm conditions. The overall aim of this thesis was to investigate the response of urinary PD excretion to nutritional factors affecting ruminal MCP synthesis in two animal studies in order to evaluate the accuracy and validity of this indicator to reflect changes in MCP supply.

In the first study, four rumen-cannulated heifers were infused intraruminally with increasing dosages of glucose, a rapidly fermentable carbohydrate source, to stimulate MCP synthesis and to verify the linearity between glucose dosage and urinary PD excretion. Urinary PD excretion increased with glucose infusion, however, against our expectations, the relationship between glucose dosage and urinary PD excretion was not linear. A pronounced increase in PD excretion was observed at lowest glucose dosage and any increment in glucose dosage did not lead to further increments. In contrast, fecal nitrogen excretion linearly increased with increasing glucose dosage which is most likely due to a greater excretion of MCP synthesised in the rumen. This contradicting response between urinary PD and fecal nitrogen excretion to incremental dosages of glucose infusion is at least attributed to shifts in the species composition of rumen microbial consortium causing changes in the purine concentration of the microbial biomass, limiting the accuracy of the urinary PD excretion as a non-invasive indicator.

In the second study, six rumen-cannulated heifers were used to investigate the effects of intraruminal infusion of increasing dosages of commercial Quebracho tannin extract (QTE), a condensed tannin source, on apparent total tract nutrient digestibilities, nitrogen balance, and urinary PD excretion. Moderate to high QTE dosages (≥ 4 % of dry matter intake) reduced apparent total tract digestibility of organic matter especially that of structural carbohydrates, lowering energy supply to the host. The QTE infusion greatly shifted nitrogen excretion towards feces, which is desired from an environmental perspective. Urinary PD excretion

declined linearly with increasing QTE dosage reflecting a decrease in duodenal MCP flow which is mainly attributed to inhibiting effects of QTE infusion on rumen microbial growth. At moderate to high QTE dosages, it is unlikely that the increase in rumen-escape protein will lead to greater post-ruminal amino acid absorption due to pronounced reductions in duodenal MCP flow. According to these findings, the addition of QTE to ruminant diets does not improve protein supply and performance of cattle.

The results of the present thesis indicate that the urinary PD excretion as a non-invasive indicator appears to be able to reflect changes in duodenal MCP flow. However, for situations when considerable amounts of rapidly fermentable carbohydrates and condensed tannins are part of the diet, the accuracy of estimating duodenal MCP flow from urinary PD excretion is limited.

6 Zusammenfassung

Die Synthese des mikrobiellen Rohproteins im Pansen spielt in der Ernährung von Wiederkäuern eine zentrale Rolle, da sie mehr als die Hälfte der essentiellen Aminosäuren für die Absorption am Dünndarm bereitstellt. Aus diesem Grund ist die genaue Quantifizierung des duodenalen mikrobiellen Proteinflusses von entscheidender Bedeutung, um vor allem hochleistende Wiederkäuer adäquat mit Protein zu versorgen und dabei die Tierleistung und Effizienz der Stickstoffverwertung zu verbessern. Die Ausscheidung von Purinderivaten im Urin ist ein nicht-invasiver Indikator zur Schätzung des duodenalen mikrobiellen Proteinflusses und hat zudem das Potential, auch unter praktischen Haltungsbedingungen Anwendung zu finden. Die Zielsetzung der vorliegenden Arbeit lag darin, die Reaktion der Purinderivatausscheidung im Urin auf nutritive Faktoren, welche die ruminale mikrobielle Proteinsynthese beeinflussen, im Rahmen von zwei Tierversuchen zu untersuchen. Dabei stand die Bewertung dieses Markers hinsichtlich seiner Genauigkeit und Validität, Veränderungen des duodenalen mikrobiellen Proteinflusses zu erkennen, im Vordergrund.

Im ersten Tierversuch wurde vier fistulierten Färsen steigende Mengen Glukose intraruminal appliziert. Ziel war es dabei, die mikrobielle Proteinsynthese zu stimulieren und zu prüfen, ob die Beziehung zwischen Glukosemenge und Purinderivatausscheidung im Urin linear ist. Die Purinderivatausscheidung im Urin nahm mit steigender Glukosemenge zu, jedoch war die Beziehung zwischen renaler Purinderivatausscheidung und verabreichter Glukosemenge nicht linear. Der größte Anstieg in der Purinderivatausscheidung trat bei der geringsten Glukosezulage (125 g/Tag) auf. Ein weiterer Anstieg in der Purinderivatausscheidung konnte auch mit steigenden Glukosemengen (bis 1000 g/Tag) nicht beobachtet werden. Die Kotstickstoffausscheidung nahm hingegen mit steigender Glukosemenge linear zu, was hauptsächlich auf eine erhöhte Ausscheidung von mikrobiellem Protein, aufgrund einer erhöhten mikrobiellen Proteinsynthese im Pansen, zurückgeführt werden kann. Der Widerspruch zwischen der Purinderivatausscheidung im Urin und der Kotstickstoffausscheidung mit steigender Glukosemenge liegt teilweise in einer Verschiebung der Zusammensetzung der mikrobiellen Population im Pansen begründet, welche zu einer Veränderung der Konzentration an Purinbasen in der mikrobiellen Biomasse führt. Da im Modell von einer konstanten Purinbasenkonzentration ausgegangen wird, reduzieren Veränderungen die Genauigkeit der Purinderivatausscheidung als Indikator.

Im zweiten Tierversuch mit sechs fistulierten Färsen wurde der Einfluss von steigenden intraruminal applizierten Dosierungen von Quebracho Tannin Extrakt (QTE), einer

kondensierten Tanninquelle, auf die scheinbaren Nährstoffverdaulichkeiten, die Stickstoffbilanz und die Purinderivatausscheidung, untersucht. Dosierungen von $\geq 4\%$ QTE (der Trockenmasseaufnahme) reduzierten die scheinbare Gesamtraktverdaulichkeit der organischen Substanz und vor allem die der Strukturkohlenhydrate, was zu einer Verringerung der Energieversorgung der Tiere führte. Durch das QTE kam es zu einer deutlichen Verschiebung der Stickstoffausscheidung vom Urin zum Kot, was unter dem Gesichtspunkt des Umweltschutzes einen positiven Effekt darstellen könnte. Die Purinderivatausscheidung im Urin nahm linear mit steigender QTE Dosierung ab, was auf eine Abnahme des duodenalen mikrobiellen Proteinflusses hindeutet und vor allem auf eine hemmende Wirkung des QTE auf die Pansenmikroben zurückgeführt werden kann. Es erscheint unwahrscheinlich, dass der erhöhte Fluss von unabgebautem Protein aus dem Pansen bei mittlerer bis hoher QTE Dosierung die Reduktion des mikrobiellen Proteinflusses am Duodenum ausgleichen kann. Es konnte somit kein Hinweis auf eine Verbesserung der Proteinversorgung und Leistung bei Wiederkäuern durch die Zugabe von QTE gefunden werden.

Die Ergebnisse dieser Versuche deuten darauf hin, dass die renale Purinderivatausscheidung geeignet ist, Veränderungen des duodenalen mikrobiellen Proteinflusses zu erkennen. Bei Rationen, welche hohe Gehalte an schnell fermentierbaren Kohlenhydraten und kondensierten Tanninen enthalten, kann es zu Ungenauigkeiten in der Schätzung des mikrobiellen Proteinflusses am Duodenum kommen.

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