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Effect of urea supplementation in diet based on barley grain or corn silage on performance, digestion, rumen fermentation and microbial protein synthesis in Holstein bull calves

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ABSTRACT

This study was aimed to evaluate the effect of non-protein nitrogen source of urea with barley grain (BG) or corn silage (CS) based diet in Holstein bull calves. Nutrient digestibility, rumen fermentation activities, microbial nitrogen yield (MN) and blood metabolites were determined. Holstein bull calves (14), weighing 126 ± 4.2 kg were allocated in 2 different diets based on BG or CS for 9 weeks. Diets were based on rolled barley grain (BG) and on finely chopped corn silage (CS). Both diets were supplemented with 7.5 g/kg urea on DM basis. Intake and performance of animal did not differ between diets, whereas digestibility of neutral detergent fiber (NDF) decreased in BG diet. Ruminal concentration of propionate and blood glucose concentration increased in BG calves, whereas ruminal pH and acetate concentration increased in CS fed calves. The ruminal NH_3-N was increased in CS diet, while excretions of allantoin, purine derivatives and estimated MN yield through urine were greater in BG diet vs. CS diet. In conclusion, the results suggested that increased urinary PD excretion and consequently increased estimated MN yield value for BG diet presented more potential for this diet to be synchronized with urea in calves' rumen digestion.

Key words: Bull calves, Carbohydrate sources, Microbial nitrogen, Rumen fermentation

Microbial yield (MY) in the rumen depends largely on the availability of carbohydrates and nitrogen and their synchronization (Ghasemi *et al.* 2014). However high-concentrate diets in growing animals which are mostly based on barley grain are rapidly fermented in the rumen, leading to high ruminal concentrations of volatile fatty acids and low ruminal pH and thereby decreasing fiber and protein degradation and the efficiency of MY synthesis (Beauchemin *et al.* 2001). Feeding well-processed corn silage would supply both the required NDF to maintain rumen pH and the valuable energy amount (Gandra *et al.* 2011). Reviewing the interaction of N and carbohydrate source metabolism in rumen revealed that ammonia is the preferred N source of fiber-degrading bacteria in the rumen (Firkins 1997). Little information is available regarding the MY and performance in growing calves fed different carbohydrate sources when supplemented with urea.

MATERIALS AND METHODS

Calves management and digestibility: Holstein bull calves (14), 3.6 ± 0.1 month-old, weighing 126 ± 4.2 kg, were assigned to a completely randomized design with 2 different diets based on barley grain (BG) and finely chopped corn silage (CS) (7 calves for each diet) (Table 1). The vaccinations and other farm practices were done for all calves routinely as done in rearing system. Basal diets were contained 7.5 g/kg urea on DM basis and fed to calves for 9 weeks. The animals were kept in individual stanchions and had free access to water. Orts were collected and weights recorded once daily at 07:30 h and the feeding rates were adjusted daily to yield orts of about 5–10 % of intake. The DM was determined in composites of feed by drying at 60°C for 48 h (AOAC 1995). Samples were analyzed for total nitrogen, DM and ash (AOAC 1995), sequentially for neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al.* 1991). Body weights, were recorded at the first day of experiment and by 7 days intervals thereafter until the end of experiment. The weighing of animals was conducted before offering morning meal. On the last five days of experiment 2 fecal grab samples were collected daily on 6 and 18 h after feeding (10 samples / each calf). Equal DM from each fecal subsample was mixed to obtain one composite sample for each animal. Chemical analyses were performed for fecal samples same as to feed samples. Apparent total tract digestibility of nutrients was measured by using acid insoluble ash as an internal marker (Van Keulen and Young 1977).

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Table 1. Feed ingredients and chemical composition of experimental diets

	Diets[1]	
	BG	CS
Feed ingredients (g/kg of DM)		
Corn silage, finely chopped	150	300
Barley grain, rolled	300	150
Alfalfa hay, chopped	300	300
Wheat straw	100	100
Wheat bran	130	130
Urea	7.5	7.5
White salt (NaCl)	2.5	2.5
Min-Vit[2]	10	10
Chemical composition		
Metabolizable energy, Mcal/kg	2.30	2.27
Net energy for gain, Mcal/kg	1.12	1.09
Organic matter, %	93.1	92.7
Crude protein, % of DM	14.5	14.2
Degradable protein in rumen, % of CP	65.4	65.2
Non-fiber carbohydrate, % of DM	39.6	38.4
Neutral detergent fiber, % of DM	35.3	36.6
Acid detergent fiber, % of DM	26.5	29.5
Ether extract, % of DM	2.61	2.67

[1]Diets based on barely grain (BG) or corn silage (CS).[2]Contained per kilogram of supplement: 200,000 IU,vitamin A; 75,000 IU, vitamin D; 2.500 IU, vitamin E; 2g , Mn; 160 g, Ca; 7.2 g, Zn; 60 g, P; 21 g, Mg; 20 g, Na; 1.75 g, Fe; 20 mg, Co; 1 g, Cu; 100 mg, I; 3 mg, Se.

Blood metabolites: Blood was sampled at 4 h after morning feeding from the jugular vein of each animal two times throughout the study (on days 20 and 40 of experiment). Plasma samples were analyzed for glucose, non-esterified fatty acids, cholesterol, triglyceride and total protein.

Rumen fermentation and microbial protein synthesis: Rumen fluid samples were collected using the stomach tube 3 h after morning feeding on days 35 and 50 of experiment. The rumen samples were squeezed through four-layer cheesecloth and pH was measured immediately using portable pH meter. Two 8 mL aliquots were preserved with 0.2 mL sulfuric acid 50% and stored at –20°C for later analysis of ammonia nitrogen (NH_3-N) and volatile fatty acids (VFA). Before analysis, samples were thawed, centrifuged (10,000 × g, 4°C, 20 min) and analyzed for ammonia Crooke and Simpson 1971) and for VFA by using gas chromatography with a 15 m (0.53 mm i.d.) fused silica column.

Spot sampling technique of urine was used for estimation of daily urine volume using creatinine concentration and then for measuring daily PD excretion (allantoin + uric acid) (Chizzotti *et al.* 2008). The microbial N yield was estimated based on measured PD (Valadares *et al.* 1999). On days 36 and 51 of experiment 2, urine samples at morning (08:00–11:00 h) and at evening time (14:00–17:00 h) were collected from all calves when calves urinated spontaneously. Aliquots (10 mL) were diluted immediately with 90 ml of 0.036 N sulfuric acid and stored at –20°C for analysis. Later, urine samples were thawed at room temperature and were analyzed for urinary urea nitrogen by an automated colorimetric assay (Broderick and Clayton 1997), for creatinine, for uric acid and for allantoin using the high-performance liquid chromatography (HPLC-20A). One mean daily creatinine excretion rate that was computed with the data from all calves in our study (28.5 mg/kg of BW/d). Urine volumes used to compute daily excretion of allantoin, and uric acid from spot urine samples were estimated: BW × 28.5/creatinine concentration. Total excretion of creatinine, allantoin and acid uric was computed as the product of the urine volume obtained during 24 h and metabolite concentration. Total purine derivatives (PD) excretion was the sum of allantoin and uric acid excreted in urine. Endogenous PD excretion (mmol/d) was estimated from BW of individual calf as: 0.385 mmol/$BW^{0.75}$ per d (Chen and Gomes 1992).

Statistical analysis: Data were analyzed using PROC MIXED in SAS (1999–2000). The following model was used for variables which there were repeated measurements over time: $Y_{ijk}=\mu + C_i + T_j + Z_k + ZT_{jk} + \varepsilon_{ijk}$ where, Y_{ijk}, dependent variable; μ, overall mean; C_i, effect of calf i; T_j, effect of treatment j (BG *vs.* CS); Z_k, effect of sampling time k; ZT_{jk}, interaction between time k and treatment j; ε_{ijk}, residual error. All terms were considered fixed except for ε_{ijk} which was considered random.

RESULTS AND DISCUSSION

Intake and daily gain did not differ between treatments as well as feed to gain ratio (Table 2). This may be due to the intake and most nutrient digestibility was statistically similar between BG and CS diets. Digestibility of DM, OM, CP, ADF, and NFC were not differing between the two diets; however digestibility of NDF was improved in CS diet compared to BG diet. The previous works revealed that a basal concentration of NH_3-N in rumen fluid is necessary to prevent depression in rumen fermentation and fiber digestibility (Jones *et al.* 1998). Ruminal NH_3-N concentration was decreased ($P < 0.05$) in BG fed calves vs. CS diet (Table 3). Russell *et al*. (1992) found that structural carbohydrate-degrading bacteria use NH_3-N as their N requirement. Therefore probably high ruminal NH_3-N concentration caused to improve fiber digestion in CS diet. In addition to ruminal NH_3-N effect, decreased rumen pH in BG diet might be a probable reason in smaller fiber digestibility in same diet. The rumen pH in BG diet was 0.3 lower compared to that of CS diet ($P < 0.05$). It is stated that high-starch diets which are rapidly fermented in the rumen, leading to relatively low ruminal pH (Beauchemin *et al.* 2001). Rodrýguez-Prado *et al.* (2004) found that when the level of fiber in the diet decreased, ruminal pH, retention time and fiber digestibility were also decreased. Our results suggested that fiber digestibility probably affected negatively by lower rumen pH (BG diet) and positively affected by availability of NH_3-N (CS diet) in rumen fluid. Total VFA production did not differ between experimental

Table 2. Average daily gain, feed intake, nutrient digestibility and blood metabolites in calves fed experimental diets

	Diet[1]			
	BG	CS	SEM	*P*-value
Intake and daily gain				
Dry matter intake, kg/d	6.64	6.73	0.731	0.55
Average daily gain, kg/d	0.960	0.947	0.121	0.63
Feed: Gain (Feed conversion ratio)	6.91	7.10	0.842	0.19
Nutrient apparent digestibility (%)				
Dry matter	62.1	62.8	2.21	0.73
Organic matter	63.3	63.7	1.75	0.64
Crude protein	74.3	73.9	2.27	0.29
Neutral detergent fiber	48.7	50.5	1.48	0.021
Acid detergent fiber	43.3	44.5	2.08	0.14
Non-fiber carbohydrate	82.5	83.3	4.24	0.38
Blood metabolites				
Glucose, mM	3.88	3.59	0.12	0.01
Non-esterfied fatty acids, µM	92	95	4.74	0.53
Cholesterol, mM	2.57	2.49	0.291	0.61
Triglyceride, mM	0.28	0.29	0.031	0.82
Total protein, g/L	69.4	69.9	2.682	0.47

[1]Diets based on barely grain (BG) or corn silage (CS).

Table 3. Rumen fermentation pattern, purine derivatives excreted through urine and microbial nitrogen production in calves fed experimental diets

	Diet[1]			
	BG	CS	SEM	*P*-value
Rumen fermentation pattern				
Rumen pH	6.11	6.42	0.26	0.017
Volatile fatty acids, mM	90.1	89.7	3.13	0.56
Propionate (P), mM	20.4	16.9	2.21	0.003
Butyrate, mM	12.0	12.8	1.03	0.52
Acetate (A), mM	55.2	57.3	3.18	0.022
A:P ratio	2.70	3.39	0.21	0.001
Valerate, mM	1.12	1.19	0.08	0.36
Isovalerate, mM	1.46	1.54	0.20	0.48
Branched-chain VFA, mM	2.58	2.73	0.43	0.20
Ammonia nitrogen, mg/dL	8.12	10.08	1.24	0.031
Microbial nitrogen				
Urinary urea nitrogen, mg/dL	10.7	11.6	1.83	0.09
Estimated urine volume, L/d	7.8	8.4	0.86	0.43
Creatinine, mg/kg BW	28.3	28.7	1.61	0.61
Allantoin, mM/d	104.1	89.8	6.45	0.015
Uric acid, mM/d	8.2	7.9	0.76	0.67
Purine derivatives, mM/d	112.3	97.8	7.42	0.004
Microbial nitrogen yield, g/d	72.2	61.4	3.68	0.027[1]

Diets based on barely grain (BG) or corn silage (CS).

diets whereas acetic acid was increased ($P < 0.05$) in BG but propionic acid was increased ($P < 0.01$) in CS diet (Table 3). Consequently, the ratio of A: P was increased in CS compared to BG based diet. It has been cleared that increased starch in diet would cause to produce more propionate in rumen (Plascencia *et al.* 1996). However, increased acetate concentration in rumen fluid of CS showed that feeding fiber source could positively increase both acetate and A:P ratio in rumen. Rodrýguez-Prado *et al.* (2004) reported that high fiber diet produced more acetate (63.5%) in comparison with low fiber diet (58.2%) *in vitro*. The previous study resulted in difference for VFA production in low or high fiber diets which was related to the amount of starch fermented in rumen (Bourquin *et al.* 1994). They suggested that low fiber diets increased VFA production due to increased amount of starch fermented in rumen. Contradict with this statement Popova *et al.* (2011) by feeding feedlot bulls with high fiber *vs.* high starch diets

indicated that only acetate was increased in high fiber diet and no difference was found for propionate between treatments. In the present study, difference in carbohydrate source caused difference in individual VFA and not in total VFA production. It could be suggested that CS based diet could produce the same amount of VFA in comparison to BG based diet; but the individual VFA would differ between diets.

Blood glucose concentration was higher in BG fed calves in comparison with CS fed calves (Table 2). Typically ruminants obtain the majority of their glucose supply from hepatic gluconeogenesis, which is derived primarily from propionate. Therefore increased glucose concentration in BG may be due to starch rumen fermentation which produced more available propionate in comparison with CS diet. The concentration of ruminal NH_3-N was increased ($P < 0.05$) in CS *vs.* BG diet. It seems that increased ruminal NH_3-N concentration was more efficiently utilized in CS based diet and this may be due to the numerically increased urinary urea N concentration in CS ($P = 0.09$) from one side, and also due to increased estimated MN yield in CS diet ($P < 0.05$). Feeding the two experimental diets did not change concentrations of urinary creatinine and uric acid. However daily urinary excretion of allantoin increased ($P = 0.01$) in BG compared to CS diet, and this increased ($P < 0.05$) the estimated MN yield in BG *vs.* CS calves (Table 3). As the source of carbohydrate offered to rumen microbes, in our present study, as energy source was different, ammonia was the preferred N source for fiber-digesting bacteria and required by starch, sugar and secondary fermenters for microbial protein synthesis (Russell *et al.* 1992). Ammonia-N produced by urea inclusion in calves' diet did not improve MN yield in CS diet but it was improved with BG diet, this may be due to the better utilization of NH_3-N by ruminal microorganisms with BG diet which was reflected in lower rumen NH_3-N concentration as well as lower urinary N excretion as the released NH_3-N was utilized in the presence of readily available starch from barley grain in the rumen. Increased ruminal NH_3-N in CS diet was due to poor utilization in the absence of readily available carbohydrate source commensurate with the rate of NH_3-N production in the rumen which consequently affected urinary N excretion through urine suggested that an excess of NH_3-N than that of rumen microbe's requirements in CS calves. Provision of ruminally available N in excess of microbial needs for protein synthesis results in the generation of NH_3-N that is absorbed and converted to urea in the liver (Kohn *et al.* 2005) and resulted in increased urinary urea excretion (Broderick 2003, Brito and Broderick 2007). These results could be applied in ration formulation in growing calves diet not only for improved N efficiency towards optimum microbial protein synthesis, but also for concerns in environmental pollution which is resulted by animal manure.

The results indicated that synchronized urea degradation with corn silage in the rumen improved NDF digestion. However, it seems that barley grain based diet was more efficient by its smaller urinary urea N, higher urinary excretion of PD and subsequently increased estimated microbial N yield. Considering optimum synchronization of non-protein nitrogen source with favorable carbohydrate source in calves nutrition would result in improving nitrogen efficiency and changing the N metabolism toward increasing purine derivatives instead of excretion of nitrogen as urinary urea N.

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