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Effects of Protein Level in Concentrate and Urea-Treated Corn Silage on Rumen Ecology and Milk Production in Lactating Dairy Cows

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Abstract: Four, lactating dairy cows were randomly assigned according to a 2x2 Factorial arrangement in a 4x4 Latin square design to study supplementation of concentrate containing different level of protein at 14 and 18% CP and urea-treated corn silage at 2 and 5% respectively. The treatments were as follows by concentrate containing protein at 14% CP + 2% urea-treated corn silage (T1); concentrate containing protein at 14% CP + 5% urea-treated corn silage (T2); concentrate containing protein at 18% CP + 2% urea-treated corn silage (T3) and concentrate containing protein at 18% CP + 5% urea-treated corn silage (T4), respectively. The animals were offered the treatment concentrate at a ratio to milk yields at 1:2 and urea-treated corn silage were fed *ad libitum*. The results have revealed that total DM intake (%BW) and ruminal pH were not affected (p>0.05). Likewise, the concentration of ammonia-nitrogen (NH_3-N) and Blood Urea Nitrogen (BUN) were significantly different affected by protein levels in concentrate with urea levels treated corn silage. In addition, rumen microorganism populations such as bacteria, protozoa and fungal zoospores were affected (p<0.05) by different by protein levels in concentrate with urea level treated corn silage. Moreover, the differences of protein levels in concentrate and urea level treated in corn silage were affected to milk yield and composition (p<0.05), especially income over feed highest in dairy cows were received a concentrate containing protein at 18% CP + 5% urea treated corn silage (T4). Therefore, results from this experiment indicated that the differences of protein levels in concentrate and urea level treated corn silage affected on rumen ecology and milk production in lactating dairy cows.

Key words: Corn silage, protein, urea, concentrate, rumen ecology, dairy cows

INTRODUCTION

Ruminants are able to use plant cell wall constituents and non-amino nitrogen through the biotransformation of these substances by rumen microbes into products that can be directly assimilated by the animal. Corn silage is an excellent forage for lactating cows because of its high content of digestible energy. However, corn silage contains considerably less RDP compared with other high quality forages (NRC, 2001). Consequently, the use of corn silage may require greater supplementation of diets with RDP. Urea has long been known to be a successful replacement for some of the degradable true protein in corn silage based diets (Van Horn *et al.*, 1969). Several studies have shown that milk and milk protein yields were improved and milk fat content was decreased with an increased degree of corn processing (Theurer *et al.*, 1999; Dhiman *et al.*, 2002). The extrusion method for processing corn has been used to improve digestibility of grains and starch gelatinization in livestock feeds, but has not been widely used in dairy rations (Ryu *et al.*, 1999). Extrusion differs from other processing methods in that grain is exposed to steam, high temperature and significant changes in pressure causing expansion of the processed grain (Castells *et al.*, 2005). However, the use of protein levels in concentrate containing cassava based-diets with urea-treated corn silage has not yet been investigated in tropics. Therefore, the objective of this experiment was to investigate the supplementation of protein level in concentrate containing cassava based-diets with urea-treated corn silage as a basal roughage on ruminal ecology and milk production in lactating dairy cows.

MATERIALS AND METHODS

Animals, diets and experimental design: Four, Holstein-Friesian crossbred cows (75%) in the third lactation were used in experiment. Milk yield pre-experiment was 15 ±2 kg/day and the body weight were 400±10 kg. Cows were randomly assigned according to a 2x2 Factorial arrangement in a 4x4 Latin square design to study supplementation of concentrate containing different level of protein at 14 and 18% CP and urea-treated corn silage at 2 and 5% respectively. The treatments were as follows by concentrate containing protein at 14% CP + 2% urea- treated corn silage (T1); concentrate containing protein at 14% CP + 5% urea-treated corn silage (T2); concentrate containing protein at 18% CP + 2% urea-treated corn silage (T3) and concentrate containing protein at 18% CP + 5% urea-treated corn silage (T4), respectively. The composition of dietary treatments and Urea-treated Corn Silage (UTCS) used are shown in Table 1.

Cows were housed in individual pens and individually fed concentrate at a ration to milk yield of 1:2, twice daily at 0600 a.m. and 1600 p.m. after milking. All cows were fed *ad libitum* of UTCS with water and a mineral-salt block. Feed intake of concentrate and roughage were measured separately and refusals recorded. The experiment was run in four periods, each experimental period lasted for 21 days, the first 14 days for treatment adaptation and for feed intake measurements whist the last 7 days were for sample collections of rumen fluid and faeces. Body weights were measured daily during the sampling period prior to feeding. Milk yield was recorded during the 21 day-period and samples were collected during the last 7 day of each period.

Urea-treated Corn Silage (UTCS) was prepared by using 2 and 5% (W/W) urea mixed with 100 kg of water in 100 kg of Corn Silage (CS) batches (50:50, water to corn) and poured over a stack of corn and then covered with a plastic sheet for a minimum of 10 days before feeding to animals.

Data collection and sampling procedures: UTCS and concentrate were sampled daily during the collection period and were composted by period prior to analyses. Composites samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden) and then analyzed for DM, ether extract, ash and CP content (AOAC, 1985), NDF and ADF (Goering and Van Soest, 1970).

Cows were milked twice daily and milk weights were recorded at each milking of each period. Milk samples were composite daily, according to yield, for both the a.m. and p.m. milking, preserved with 2-bromo-2 nitropropane-1, 3-dial and stored at 4°C until analysis for fat, protein, lactose, totals solids and solids-not-fat content by infrared methods using Milko-Scan 33 (Foss Electric, Hillerod, Demark).

Rumen fluid samples were collected at 0 and 4 h post-feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by a stomach tube connected with a vacuum pump at each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using (HANNA instruments HI 8424 microcomputer) after withdrawal. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions. One portion was used for NH_3-N analyses where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture was centrifuged at 16,000 g for 15 min and the supernatant stored at -20°C prior to NH_3-N analysis using the micro Kjeldahl methods (AOAC, 1985).

Table 1: Chemical composition of concentrates and urea-treated corn silage used in the experiment (% DM basis)

Item	Dietary treatments: Concentrate I	Concentrate II	2% urea -treated corn silage	5% urea -treated corn silage
Cassava chip	55	50	-	-
Rice bran	9.0	5.0	-	-
Brewer's grain	7.0	7.0	-	-
Palm meal	6.0	6.0	-	-
Soybean meal	7.0	15	-	-
Cassava hay	5.0	5.0	-	-
Coconut oil	2.0	2.0	-	-
Urea	2.0	3.0	-	-
Sulfur	0.5	0.5	-	-
Mollasses	5.0	5.0	-	-
Mineral mix	1.0	1.0	-	-
Salt	0.5	0.5	-	-
Chemical compositions (%)				
DM	88.2	89.1	55.2	55.7
OM	91.3	91.2	92.3	91.9
CP	14.1	18.2	10.6	13.8
NDF	21.2	20.5	47.8	46.2
ADF	10.2	10.9	31.1	30.4
TDN	79.9	80.2	65.3	66.1
ME, Mcal/kg (DM)[1]	3.2	3.1	2.4	2.4

[1]Estimated: Metabolizable energy (ME, Mcal/kg, DM)=TDNx0.04409x0.82.

The total count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haematocytometer (Boeco). A blood sample (about 10 ml) was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 5000 g for 10 minutes and stored at -20°C until analysis of Blood Urea Nitrogen (BUN) according to the method of Crocker (1967).

Statistical analysis: All data obtained from the experiment were subjected to ANOVA for a 4x4 Latin square design with 2x2 Factorial arrangement of treatments using the General Linear Models (GLM) procedures of the Statistical Analysis System Institute (SAS, 1998). Treatment means were compared by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition of feeds: The chemical composition of roughage and concentrate diets fed in dairy cows are presented in Table 1. Concentrate diets contained similar concentrations of DM, OM, NDF, ADF and TDN. Furthermore, the chemical composition of Urea-treated Corn Silage (UTCS) is presented in Table 1.

Effect on feed intake and digestibility: The effects of protein level and urea-treated corn silage on feed-intake of lactating dairy cows are presented in Table 2. Feed intakes were not significantly affected by protein level

Table 2: Influence of protein levels and urea-treated corn silage on feed-intake and rumen ecology in lactating cows

	Treatments[1]				Contrast[2]			
Item	T1	T2	T3	T4	SEM	CP	U	CPxU
DM intake (%BW)								
Roughage	1.5	1.6	1.6	1.7	0.11	NS	NS	NS
Concentrate	1.2	1.3	1.3	1.4	0.08	NS	NS	NS
Total	2.7	2.9	2.9	3.1	0.16	NS	NS	NS
Ruminal pH	6.8	6.9	6.8	7.0	0.121	NS	NS	NS
NH_3-N (mg/dl)	16.8[a]	18.6[ab]	18.8[ab]	20.5[b]	0.499	*	*	NS
BUN (mg/dl)	8.0[a]	12.0[b]	12.9[bc]	15.1[c]	0.726	*	*	NS
Total direct counts (cell/ml)								
Bacteria ($x10^{11}$)	5.5[a]	7.7[b]	7.8[b]	8.4[b]	0.406	NS	*	NS
Protozoa								
Holotrich ($x10^4$)	3.5[a]	2.3[b]	2.4[b]	1.7[c]	0.185	*	*	NS
Entodiniomorph ($x10^5$)	9.8[a]	6.4[b]	6.3[b]	2.6[c]	0.661	*	*	NS
Fungal zoospores ($x10^6$)	3.4[a]	5.2[b]	5.1[b]	7.6[c]	0.411	*	*	NS

[a,b,c]Values on the same row with different superscripts differ (p<0.05). [1]T1 = Concentrate containing protein at 14% CP + 2 % urea-treated corn silage, T2 = Concentrate containing protein at 14% CP + 5 % urea-treated corn silage, T3 = Concentrate containing protein at 18% CP + 2 % urea-treated corn silage, T4 = Concentrate containing protein at 18% CP + 5 % urea-treated corn silage. [2]Probability of main effects of level protein in concentrates (CP) (14 vs 18 %), levels of urea-treated corn silage (U) (2 vs 5%), or the CPxU interaction. * = p<0.05, ** = p<0.01, NS = p>0.05.

Table 3: Effects of protein levels and urea-treated corn silage on milk yield and composition in lactating dairy cows

	Treatments				Contrast			
Item	T1	T2	T3	T4	SEM	CP	U	CPxU
Production								
Milk yield (kg/d)	17.1[a]	19.5[b]	20.1[b]	24.5[c]	0.738	*	*	NS
3.5%FCM (kg/d)	18.1[a]	23.4[b]	23.7[b]	31.1[c]	1.262	*	*	NS
Milk composition (%)Milk fat	3.7[a]	4.1[bc]	4.2[bc]	4.4[c]	0.078	**	*	NS
Milk protein	3.1[a]	3.3[b]	3.4[b]	3.5[b]	0.049	**	*	NS
Lactose	4.2[a]	4.5[b]	4.6[b]	4.8[c]	0.058	*	*	NS
Solids not fat	8.4	8.5	8.6	8.7	0.251	NS	NS	NS
Total solids	13.3[a]	13.4[a]	12.3[b]	12.4[b]	0.138	**	NS	NS

[a,b,c] Values on the same row with different superscripts differ (p<0.05).

and urea-treated corn silage supplementation (2.7-3.1% BW). This data indicated that protein level with urea-treated corn silage supplementation had no effect on feed-intake in lactating dairy cows.

Characteristics of ruminal fermentation and blood metabolism: Rumen ecology parameters were measured for temperature, pH, NH_3-N and microorganisms (Table 2). In addition, BUN was determined to investigate their relationships with rumen NH_3-N and protein utilization. Rumen pH at 0 and 4 h post-feeding were unchanged by dietary treatments and the values were quite stable at 6.8-7.0, but all treatment means were within the normal range which has been reported as optimal for microbial digestion of fiber and also digestion of protein (6.0-7.0) (Hoover, 1986).

Ruminal NH_3-N and BUN concentrations were significantly different by protein level and urea-treated corn silage supplement in diets. As NH_3-N is regarded as the most important nitrogen source for microbial protein synthesis in the rumen (Bryant, 1974). In addition, the result obtained was closer to optimal ruminal NH_3-N (15-30 mg%, Wanapat and Pimpa, 1999; Chanjula *et al*., 2004) for increasing microbial protein synthesis, feed digestibility and voluntary feed intake in ruminant fed on low-quality roughages.

Rumen microorganisms populations: The populations of rumen microorganisms such as Table 2 presents rumen microorganism populations. The fungal zoospores, protozoa and total bacteria direct counts were significantly different and populations of bacteria had higher numbers in lactating cows receiving T4 and those cows fed T3, T2 and T1 (8.4, 7.8, 7.7 and $5.5x10^{11}$ cell/ml, respectively). In contrast, the present number of protozoa in the rumen was decreased by protein level and urea-treated corn silage supplementation in high cassava-based diets. The decrease in protozoa count may attribute the increase in fungal zoospores per ml rumen fluid, as removal of protozoa has been associated with an increase in the concentration of fungi (Demeyer, 1981).

Milk production and composition: The influences of protein level and urea-treated corn silage supplementation in lactating dairy cows receiving cassava-based diets are shown in Table 3. All cows were able to maintain levels of milk yield during the 80 days of experiment. Yield of milk and 3.5%FCM were greatest in cows fed cassava-based diets with concentrate containing protein at 18% CP with 5% urea-treated corn silage, but were lowest ($p<0.05$) when receiving concentrate containing protein at 14% CP with 2% urea-treated corn silage in diets. In addition, the supplementation of protein level in concentrate and urea-treated corn silage was significantly different affected on milk compositions especially milk fat, milk protein, lactose and total solid especially were higher in dairy cows receiving concentrate containing protein at 18 % CP with 5% urea-treated corn silage (T4) than T3, T2 and T1, respectively (Table 3).

Conclusion: Based on this experiment, it could be concluded that supplementation of protein level in concentrate containing cassava chip based diets with urea-treated corn silage could improved ruminal fermentation efficiency and increase populations of bacteria, but decreased protozoal populations in lactating cows. These results suggest that the combined use of concentrates containing protein at 18% CP with 5 % urea-treated corn silage could highest improved rumen ecology and milk production in lactating cows.

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