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Effect of Diets Containing Different Ratio of Effective Rumen Degradable Protein to Fermentable Metabolizable Energy on Early Lactating Holstein Cow Responses

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Abstract: The effect of different Effective Rumen Degradable Protein (ERDP) to Fermentable Metabolizable Energy (FME) on early lactating cow responses was investigated. Total tract digestibility of organic matter of various feedstuffs including barely grain, corn grain, wheat bran, cottonseed meal, soybean meal, sugar beet pulp, alfalfa hay, cotton seeds, corn silage and fish meal was determined using *in situ* technique. These data were used to predict FME of the feedstuffs. Two diets were provided with different ERDP/FME ratio (9.7 and 10.7 g MJ^{-1}). The diets fed to fourteen early lactating Holstein cows averaging 21±16 Days In Milk (DIM) and 32±9 Kg d^{-1} milk yield for seven weeks, using a completely randomized design. Dry matter intake, milk yield and milk composition were measured weekly. Blood metabolites including glucose and urea nitrogen were measured in weeks 4 and 7. Rumen fluid NH_3-N was recorded in the 3rd week of the experiment. Dry matter intake was significantly ($p< 0.05$) lower for cows fed diet with ERDP/FME = 9.7 g MJ^{-1} than those fed ERDP/FME = 10.7 g MJ^{-1} (21 vs. 21.6, respectively). Milk yield was significantly ($p< 0.05$) higher in cows fed ERDP/FME = 9.7 g MJ^{-1} compared with those fed ERDP/FME = 10.7 g MJ^{-1} (35.2 vs. 32.3, respectively). Milk composition, blood metabolites and rumen fluid NH_3-N were not significantly affected by the treatments ($p>0.05$).

Key words: Effective rumen degradable protein, fermentable metabolizable energy, milk, cow

INTRODUCTION

Ruminants are provided with two major sources of protein, microbial protein and Rumen Undegraded Protein (RUP) in the intestine (Broderick *et al*., 1991). Microbial protein is a good quality protein because of its Amino Acids (AA) content and post-ruminal digestibility (Broderick *et al*., 1991). However, RUP value is completely related to its nitrogen fractions and relative AA profile (Taghizadeh *et al*., 2005). Post-ruminal digestion of this mixture of microbial protein and RUP in the abomasum and small intestine yields the AA supply to the host animal (Broderick *et al*., 1991). In view of the importance of microbial protein to the ruminant nutrition, the understanding and control of microbial protein synthesis in the rumen is essential for optimizing the production performance of the animal (Dijkstra *et al*., 1998). In the other hand, animal industry, including dairy industry, may contribute considerably to the environmental problems (Tamminga, 1992). Nutrition management can be used as a tool to help the environmental pollution control (Tamminga, 1992). Under some feeding regimens, 75 to 85% of the N ingested by dairy cows is estimated to excrete in feces and urine (Charmley *et al*., 1988). A 650 Kg dairy cow is estimated to excreted 116 Kg N/yr (Smith and Frost, 2000) 12 % of which could be lost by ammonia volatilization (Lockyer and Whitehead, 1990). At high Rumen-degradable Protein (RDP), more N would be absorbed as ammonia or more AA deaminated, which might increase N excretion in urine (Castillo *et al*., 2001). Modern protein systems for dairy cows (such as AFRC, 1993; NRC, 2001) involve feeding protein first to meet the requirements of rumen microbes and then supply DUP to meet the metabolizable protein requirements of the animal. In metabolizable protein system the microbial nitrogen synthesis is not limited only by dietary degradable nitrogen, but the providing of fermentable energy could be also affected on it (AFRC, 1993). Maximizing the utilization of RDP and conversion into microbial protein is a key object of protein feeding strategies (Cabrita *et al*., 2003). Increasing the efficiency of microbial protein synthesis in the rumen will increase the utilization of the nutrients supplied to the host animal and the supply of essential AA at a relatively lower cost than dietary RUP. Furthermore, improving the efficiency of dietary Crude Protein (CP) is important for both

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increasing profitability and reducing potential pollution of dairy farms (Cabrita *et al*., 2003). Results of some experiments have shown the importance of balancing the supply of RDP to the Fermentable Metabolizable Energy (FME) (Casper *et al*., 1999; Mabjeesh *et al*., 1997). In metabolizable protein system, Quickly Degradable Protein (QDP) is used less efficiently than Slowly Degradable Protein (SDP). This system considers QDP to be used with 0.8 efficiency and defines the term effective RDP (ERDP) as 0.8 of QDP plus 1.0 of SDP. If FME is not limit, efficiency of converting ERDP to microbial protein is one, when the ratio of ERDP/FME (g MJ^{-1}day) would be 10.5-11 in lactating dairy cows (AFRC, 1993). However, some workers showed a lower efficient ratio in dairy cow nutrition (Cabrita *et al*., 2003). The aim of this experiment was to evaluate the response of Holstein lactating cows to the rations containing different ERDP/FME (9.7 vs. 10.7 g MJ^{-1}).

MATERIALS AND METHODS

***In situ* organic matter digestibility:** Digestible Organic Matter in total Dry Matter (DOMD) and Dry Matter Digestibility (DMD) of barely grain, corn grain, wheat bran, sugar beet pulp, cotton seeds, cottonseed meal, soybean meal, fish meal, corn silage and alfalfa hay were determined using mobile nylon bag technique in four Holstein steers. Animals were fitted with ruminal fistulae and intestinal T-shape cannulate. Steers were fed with a diet to meet their maintenance requirements. Animals were housed individually in stalls and had free access to water. About 6 g dry matter of each feed (grounded through 2-mm screen) was placed in each polyester bag (4 bags per each feed, 12×17 cm, 48 μm pore size) and incubated in the rumen for 12 h. After removal from the rumen, the bags were washed using cold water and dried (65°C, 48 h) then weighted. Samples from each bag were taken and analyzed for Organic Matter (OM). A part of each rumen incubated sample was placed in a polyester bag (n = 4, 3×6 cm, 48 μm pore size). These bags were used for post-ruminal digestibility. Bags were inserted into the small intestine via the cannulae at the rate of one bag every 30 min and removed from the voided feces and rinsed in cold running water. Finally, the bags were dried in a forced air oven (65°C, 48 h), then weighted to determine the dry matter disappearance (Danesh Mesgaran and Stern, 2005). Samples were taken to determine the organic matter value of the residual. Metabolizable energy of the samples was performed from the total tract DOMD value of each feed.

Feeding trial: Multiparous (n = 11) and primiparous (n = 3) early lactating Holstein cows averaging 21±16 days in milk (DIM) 32±9 Kg d^{-1} of milk were used in this experiment. Cows were assigned to a completely randomized design employing 2 treatments for 7 weeks. Animals were kept in tie stalls with individual feed bins in an animal house and had free access to water. Two diets with 9.7 and 10.7 (g MJ^{-1}) Effective Rumen Degradable Protein (ERDP) to Fermentable Metabolizable Energy (FME) were provided (Table 1). Rations were formulated according to AFRC (1993) recommendation for early lactating cows. In the present experiment, FME values of the feeds were calculated from ME values obtained from the *in situ* digestibility trial. Feed ERDP were calculated using protein degradability coefficients reported by Heravi *et al*. (2004). The diets were fed as a TMR for *ad libitum* intake twice a day (08:30 and 18:00 h). Banks were cleaned out each morning and orts were collected and weighted. Cows were milked three times per day at 05:00; 12:00 and 20:00 h. Dry matter intake and milk production were recorded daily. Milk samples were taken at each milking during each week and mixed daily for each cow in proportion to the amount of milk produced at the 05:00, 12:00 and 20:00 h milking. Blood samples were collected into heparinized tubes from the jugular vein of each cow

Table 1: Ingredients and chemical composition of the experimental diets containing different ratios of effective rumen degradable protein to fermentable metabolizable energy (9.7 and 10.7 g MJ^{-1})

	Effective rumen degradable protein/ fermentable metabolizable energy ratios	
Item	9.7 g MJ^{-1}	10.7 g MJ^{-1}
Corn silage (kg DM)	3.45	3.45
Alfalfa hay (kg DM)	7.18	7.18
Concentrate (kg DM)	16.27	16.27
Corn grain (% of concentrate)	23.35	27.47
Barely grain (% of concentrate)	22.13	22.13
Cotton seed (% of concentrate)	15.98	15.98
Soybean meal (% of concentrate)	14.14	14.14
Wheat bran (% of concentrate)	10.45	10.45
Cottonseed meal (% of concentrate)	5.78	5.78
Fish meal (% of concentrate)	5.10	0.00
Urea(% of concentrate)	0.00	0.98
Di calcium phosphate (% of concentrate)	0.30	0.30
Limestone (% of concentrate)	0.25	0.25
Sodium bicarbonate(% of concentrate)	0.86	0.86
Magnesium oxide (% of concentrate)	0.43	0.43
Mineral and vitamin premix (% of concentrate)*	1.23	1.23
CP (%)	17.2	17.6
ME (MJ Kg^{-1})	10.9	10.7
FME (MJ Kg^{-1})	9.4	9.4
NDF (%)	33.5	33.8
ADF (%)	16.2	16.3
Starch (%)	23.3	25.1
ERDP (g cow)	2363	2604
DUP (g cow)	1444	1278
ERDP/FME (g MJ^{-1})	9.7	10.7

*: Premix contained (DM basis): 190000 mg Kg^{-1} Ca, 90000 mg Kg^{-1} P, 50000 mg Kg^{-1} Na, 9000 mg Kg^{-1} Mg, 3000 mg Kg^{-1} Fe, 3000 mg Kg^{-1} Zn, 2000 mg Kg^{-1} Mn, 100 mg Kg^{-1} Co, 300 mg Kg^{-1}, Cu, 100 mg kg^{-1} I, 1 mg kg^{-1} Se, 500,000 IU kg^{-1} vitamin A, 100,000 IU kg^{-1} vitamin D3, 100 mg kg^{-1} vitamin E, 3000 mg Kg^{-1} antioxidant (B.H.T)

Table 2: Metabolizable Energy (ME) and fermentable metabolizable energy (FME), MJ Kg^{-1}, values of various feedstuffs determined by in situ technique

Ingredient	FME* (MJ Kg^{-1})	ME€ (MJ Kg^{-1})	TOMD¥ (g Kg^{-1} DM)	POMDƒ (g Kg^{-1})	ROMD‡ (g Kg^{-1})	EE† (g Kg^{-1})
Barely grain	12.1	12.9	826	254.5	831.6	25.0
Corn grain	12.6	13.8	882	678.4	726.2	35.0
Wheat bran	8.8	10.1	642	117.5	685.9	37.5
Cottonseed meal	5.1	7.1	454	283.2	314.2	57.5
Soybean meal	11.4	12.2	777	716.7	630.0	22.5
Sugar beet pulp	11.0	11.6	740	468.3	649.1	17.5
Alfalfa hay	8.0	8.4	534	150.3	613.5	10.0
Cotton seed	2.0	8.5	540	185.3	510.4	185.0
Corn silage	7.4	7.9	430	129.1	445.0	15.0
Fish meal	4.4	10.0	637	781.3	529.5	160.9

† EE: Ether Extract, ‡ ROMD: Ruminal Organic Matter Digestibility ƒ POMD: Post-ruminal Organic Matter Digestibility, ¥ TOMD: Total Organic Matter Digestibility, €ME(MJ Kg^{-1}) = 0/0157 [DOMD] (g Kg^{-1}), * FME (MJ Kg^{-1}) = ME - [Oil (Kg) × 35]

at 0.0, 3 and 6 h after the morning feeding in weeks 4 and 7. Samples were immediately centrifuged (3000 RPM, 10 min) and plasma was analyzed for glucose and urea nitrogen. Ruminal fluid was collected by ruminothensis (3 h after the morning feeding) in mid period of the experiment and passed from cheese cloth. Samples of rumen fluid (5 mL) were mixed with 5 mL HCl (0.2 N), then analyzed for NH_3-N.

Analytical procedure: Dry Matter (DM) was determined by drying the samples at 105°C for 24 h and Organic Matter (OM) by ashing at 550°C for 6 h. Fat content of each feed was determined using standard procedure (Tecator Soxtex 1043 Extraction, Swede). Milk samples were used to determine fat, protein, lactose and Solid Not Fat (SNF) using milko-tester (Foss Electric, conveyor 4000). For Milk Urea Nitrogen (MUN), milk serum was prepared and MUN was determined using enzymatic procedure (CECIL 1021 spectrophotometer). Ruminal NH_3-N was determined using distillation method (Kjeltec Auto, 1300). Blood urea nitrogen and glucose were determined using enzymatic procedure (Selectra E).

Calculations and statistical analysis: Fermentable metabolizable energy was calculated using the following equations (AFRC, 1993).

$FME = ME - ME_{fat}$

Where:

ME = 0.0157 DOMD and ME_{fat} = 35 MJ Kg^{-1} fat

Statistical analysis was performed as a repeated measurement Analysis of Variance (ANOVA) using MIXED procedure of Statistic Analytical Software (Version 8e, SAS Inst. Inc., Cary, NC). The following variables were included in the model:

$$Y_{ijk} = \mu + T_i + A_{ij} + D_k + (T \times D)_{ik} + \varepsilon_{ijk}$$

Where:

Y_{ijk} = Dependent variable
μ = The overall mean
T_i = Treatment effect
A_{ij} = Cow in treatment
D_k = Time effect
$(T \times D)_{ik}$ = Treatment and time interaction
ε_{ijk} = Error

RESULTS AND DISCUSSION

Data of Ruminal Organic Matter Digestibility (ROMD), Intestinal Organic Matter Digestibility (IOMD) and Total Tract Organic Matter Digestibility (TOMD) of the feeds measured by *in situ* technique are reported in Table 2. ME and FME values of each feed samples are also presented in Table 2. The ME values of the feeds were in the range of 7.1 (cottonseed meal) to 13.8 (corn grain) and predicted FME values were in the range of 2.01 (cotton seeds) to 12.62 (corn grain). In the present experiment, the predicted ME and FME (Table 2) were similar to those reported by AFRC, except for feed samples that had high amount of fat (cotton seeds, cottonseed meal and fish meal which had 185, 57.5 and 160.9 g fat kg^{-1} DM, respectively).

Dry Matter Intake (DMI), milk production and milk composition are presented in Table 3. Dry matter intake was higher in cows fed higher ERDP/FME ratio than the other cows (21.6 vs. 21 for ERDP/FME= 10.7 vs. 9.7 (g MJ^{-1}), respectively). Lower DMI along with reducing ERDP/FME ratio has been reported by Cabrita *et al.* (2003) who showed that the reduction of ERDP/FME to 6.7 (g MJ^{-1}) led to significantly lower DMI than diets contain 10.1 and 11.2 ERDP/FME (g MJ^{-1}). Reducing DMI in lower ERDP/FME ratio might indicate that ruminal microbial growth may be limited when rumen degraded protein was decreased (Cabrita *et al.*, 2003; Dhiman and Satter, 1993). Therefore, ruminal degradation of ingested feeds caused to decrease out flow rate and DMI.

Cows offered lower ERDP/FME (g MJ^{-1}) ration had higher milk production [35.2 vs. 32.3 kg d^{-1}, for ERDP/FME = 9.7 vs. 10.7 (g MJ^{-1}), respectively] than the other animals ($p<0.05$). Time effect on milk production was

Table 3: Feed intake, milk production and milk composition in early lactating Holstein cows fed diets containing different ratios of effective rumen degradable protein to fermentable metabolizable energy (9.7 and 10.7 g MJ^{-1})

	Effective rumen degradable protein/ fermentable metabolizable energy		Treatment effect		Time effect	
Item	9.7 g MJ^{-1}	10.7 g MJ^{-1}	SEM†	*P*‡	SEM	*P*
Dry matter intake (kg d^{-1})	21.0	21.6	0.28	0.03	0.48	0.01
Milk Yield (kg d^{-1})	35.2	32.3	0.44	0.01	0.81	0.01
Fat (g Kg^{-1})	27.4	25.7	0.15	0.34	0.17	0.72
Protein (g Kg^{-1})	29.3	29.4	0.05	0.73	0.05	0.04
Lactose (g Kg^{-1})	44.9	44.7	0.07	0.47	0.07	0.99
Solids non fat (g Kg^{-1})	84.8	84.9	0.12	0.96	0.12	0.85
Milk urea nitrogen (mg dL^{-1})	20.47	21.59	0.67	0.44	0.84	0.04

SEM: Standard Error of Mean, ‡ *P*: Probability

Table 4: Blood glucose (mg dL^{-1}) and urea nitrogen of early lactating Holstein cows (mid and end of the experimental period) fed diets containing different ratios of effective rumen degradable protein to fermentable metabolizable energy (9.7 and 10.7 g MJ^{-1})

			Effective rumen degradable protein to fermentable metabolizable energy		Treatment effect*	
Blood metabolites	Sampling (week)	Time (hours after the morning feeding)	9.7 g MJ^{-1}	10.7 g MJ^{-1}	SEM†	*P*‡
Blood glucose	4	0	63.7	67.7	2.2	0.07
		3	59.4	65.8		
		6	63.4	71.2		
	7	0	69.3	70.8	1.16	0.63
		3	68.8	71.4		
		6	74.0	72.4		
Blood urea nitrogen	4	0	20.1	20.0	2.2	0.16
		3	21.0	22.5		
		6	19.6	21.8		
	7	0	21.6	22.0	1.94	0.3
		3	22.4	23.2		
		6	20.4	21.5		

† SEM: Standard Error of Mean, ‡ *P*: Probability

significant ($p<0.05$). This result was in consistent with previous publications (Dijkstra *et al*., 1998; Kang-Meznarich and Broderick, 1981) which showed lower RDP in diet might increase milk production. In the present experiment, altering dietary ERDP content did not affect milk protein percentage. This result is consistent with those obtained by Christensen *et al*. (1993). However, the results of Rodriguez *et al*. (1997) showed that increasing dietary RUP from 29 to 41% decreased the percentage of milk protein and suggested it was due to ammonia production and microbial protein which decreased using ration with low level of dietary RUP. Nocek and Russell (1988) postulated that the failure of ruminally insoluble or non degradable protein to increase milk protein in some trials might have resulted because of:

- Dietary protein exceeded the cows requirements for protein,
- RUP was poorly digested in the small intestine,
- Unforeseen interactions occurred with mobilization or utilization of nutrients from body tissue, or
- A depression in microbial protein synthesis in the rumen resulted from lowered concentration of ruminal ammonia.

In the present study, milk protein yield was higher in cows fed higher RUP diet (1032 vs. 950 g d^{-1}, for ERDP/FME= 9.7 vs. 10.7 (g MJ^{-1}), respectively). This result confirmed the results of Seymour *et al*. (1990) which observed total milk protein production and essential and branch chain amino acids in blood plasma increased, when casein injected post ruminal. Decreasing the ERDP/FME (g MJ^{-1}) ratio had no effect on percentage of milk fat. This result is in contrast to previous experiments (Spain *et al*., 1990; Zerbini *et al*., 1988) which fish meal was replaced with soybean meal and percentage of milk fat was decreased. Mantysaari *et al*. (1989) suggested that high concentration of high molecular weight polyunsaturated fatty acids in fish oil might alter microbial flora in the rumen, resulting in decreased acetate to propionate ratio or a decrease in uptake of plasma fatty acids by the mammary glands. Milk lactose and SNF percentages were similar among diets. MUN in cows fed higher ERDP/FME (g MJ^{-1}) tended to be increased. MUN primarily reflects blood urea concentration and blood urea originate mainly from exceed produced ammonia in the rumen and amino acid catabolism in liver (Dhiman and Satter, 1993). In this experiment, RDP content in diet with ERDP/FME = 10.7 (g MJ^{-1}), might be higher than what has consumed by microbial population. Therefore, it absorbed from ruminal wall and converted to urea in the liver. Any increases in blood non ammonia nitrogen might increase MUN (Broderick and Clayton, 1997). Altering ERDP/FME (g MJ^{-1}) ratio had no effect on blood glucose concentration. Similarly, dietary treatments had no effect

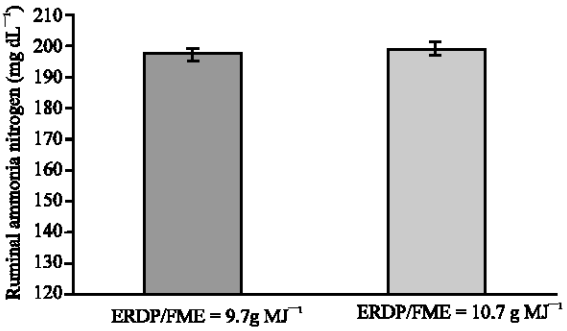

Fig. 1: Effect of different ratio ERDP/FME (g MJ^{-1}) on ruminal ammonia nitrogen (mg dL^{-1}) 3h after the morning feeding in early lactating Holstein cows

on blood urea nitrogen concentration in each sampling (0.0, 3 and 6 h after the morning feeding) (Table 4). These results confirmed some previous reports (Cabrita *et al*., 2003; Christensen *et al*., 1993; Stern *et al*., 1983) however, they were in contrast to Rodriquez *et al*. (1997) observations which showed increasing in RUP caused to increase blood urea nitrogen, while decreased ruminal ammonia. Therefore, plasma urea nitrogen concentration might not be merely associated with ruminal nitrogen degradation and it might be affected by synchronization between energy and NH_3-N in the rumen (Mabjeesh *et al*., 1997).

Ruminal NH_3-N concentration tended to be higher with higher ERDP/FME (g MJ^{-1}) diet (Fig. 1). Results of previous experiments indicated that ruminal ammonia concentration was not affected by diet when different amount of urea from 0.0 to 0.5 or 1 % were offered to steers (Kang-Meznarich and Broderick, 1981; Milton *et al*., 1997). However, Rodriquez *et al*. (1997) reported ruminal ammonia concentration decreased by 34.3%, when dietary RUP decreased from 41 to 29%. Martin-Orue *et al*. (2000) indicated that ruminal ammonia concentration was appropriate with ruminal injected ERDP. These differences between studies might be explained by different nitrogen sources or the effects of other dietary ingredients (Martín-Orue *et al*., 2000). Ruminal NH_3-N concentration throughout the day serves as a good indicator of both CP degradability and energy availability (Mabjeesh *et al*., 1997). When dietary RDP content is low, urea recycling to rumen from saliva or ruminal wall may provide considerable amount of nitrogen that can be used by ruminal microbial population (Dijkstra *et al*., 1998). Therefore, it can be postulated that urea recycling in cows fed diet with low RDP may be higher compared with cows fed higher RDP.

CONCLUSION

It might be suggested that diet with ERDP/FME of 9.7 might decrease DMI and increase milk production in Holstein lactating cows. Therefore, the results of the present experiment demonstrated that the proposed ERDP /FME (g MJ^{-1}) by AFRC (1993) might be overestimated regarding the requirement of protein to energy ratio in lactating cows. In addition, these results indicated that the milk composition and some blood metabolites were not affected by the ERDP/FME ratios. Therefore, ERDP/FME (g MJ^{-1}) ratio of 9.7 can be used to diet formulation for early lactating cows without any undesirable effects on cow performances.

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