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Effects of Fibrolytic Enzyme on Milk Yield, Blood Metabolites, Rumen Microbial Growth and pH of Holstein Cows in Early Lactation

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Abstract: The aim of this experiment was to study the effect of fibrolytic enzyme on milk yield and composition, rumen microbial count and pH and blood metabolites of fifteen multiparous lactating cows (DIM 30±10 days). The experimental diets include: Control (based diet with no enzyme), basal diet with 2.5 g enzyme/kg of DM and basal diet with 5 g enzyme/kg of DM. Change-over design were used with three period, three treatments and five cows allocated to each diet. Individual dry matter intake and milk yield daily recorded and milk samples were taken for milk constituents analyze in each period. Rumen fluid collected for determining of pH and microbial count from each cow in every period at 0 and 4 h after feeding. Results indicated that low level of enzyme (2.5 g kg^{-1} of DM) increased milk production and 3.5% FCM compared to the other groups ($p \leq 0.05$). However, milk compositions were not affected by enzyme. Cows consuming high level of enzyme (5 g kg^{-1} of DM) had higher dry matter intake compared to the other groups ($p \leq 0.01$). Cows consuming high level enzyme had significantly lower milk efficiency compared to the other groups ($p \leq 0.05$). Enzyme had no effect on rumen pH and microbial (bacteria and protozoa) growth at 0 and 4 h after morning feeding. Furthermore, diet with low level enzyme increased plasma glucose concentration of dairy cows ($p \leq 0.05$). Results showed that low level of enzyme (2.5 g kg^{-1} of DM) can be recommended in the diet of early lactation Holstein cows.

Key words: Enzyme, Holstein dairy cow, milk production and composition, rumen microbes, blood metabolites, rumen pH

INTRODUCTION

Supplementing ruminant diets with feed enzyme to improve forage utilization has attracted growing attention (Beauchemin *et al*., 2003). Fibrolytic enzyme applied to the feed of dairy cows at or only hours before feeding have caused variable response. Milk yield have generally increased but often not significant (Lewis *et al*., 1999; Beauchemin *et al*., 1999). Changes in milk fat and protein sometimes positive (Beauchemin *et al*., 1999) and in other research fibrolytic enzyme had no effect on milk fat and protein (Lewis *et al*., 1999; Yang *et al*., 1999). This variation in responses can be related to type, amount, application method and the fraction of diet that enzyme added (Sutton *et al*., 2003). Fibrolytic enzymes have been used to different parts of diet including concentrate, forages and TMR. Effect of fibrolytic enzymes on feed intake was small and inconsistent (Yang *et al*., 1999; Phipps *et al*., 2000; Vicini *et al*., 2003) and Lewis *et al*. (1999) reported a significant increase in DMI. When fibrolytic added to diet, rumen pH decreased (Lewis *et al*., 1996; Hristov *et al*., 2000) or unchanged (Yang *et al*., 1999; Beauchemin *et al*., 2000). Wang *et al*. (2001) reported that fibrolytic enzyme could increase fibrolytic and non fibrolytic bacteria in rumen fluid. Fibrolytic enzyme had no effect on BUN of cows' plasma (Hristov *et al*., 1998) and Bata and Suwandyastuti (2005) reported that glucose plasma increased in fattening Holestein cows consumed diet treated with fibrolytic enzyme. Fibrolytic enzyme decreased βHB (beta Hydroxy Butyrate) when added to TMR of dairy cows (Bilik *et al*., 2009). Objectives this research was to determine effect of fibrolytic enzyme on milk yield intake, rumen pH and microbial growth in early lactating dairy cows.

MATERIALS AND METHODS

The fibrolytic enzyme that used in experiment was Natuzyme (Bioproton, Australia) that consist of amylase, protease and mainly cellulase and xylanase activity. Fifteen Holestein dairy cows in early lactation period (30±10 days) were randomly assigned to three treatments

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Table 1: Ingredient and nutrient composition of diet

Ingredients	Percentage of DM
Corn silage	18.70
Alfalfa hay (Chopped)	21.30
Corn grain (Ground)	8.40
Beet pulp	2.50
Wheat grain	5.60
Corn gluten meal	0.58
Cottonseed	2.90
Barely grain (Ground)	15.00
Soybean meal	7.80
Canola meal	9.50
Wheat bran	1.53
Fat powder	1.80
Sodium bicarbonate	0.90
Calcium carbonate	0.19
Dicalcium-phosphate	0.37
White salt	0.26
Mineral and vitamin premix[1]	0.67
Zeolite	2.00
Chemical composition	
NE_l (Mcal kg^{-2})	1.66
CP	17.00
RDP (% of CP)	65.00
NDF	37.40
ADF	18.80
Ca	0.86
P	0.48

[1]Mineral and vitamin premix contained: 120 g kg^{-1} of Ca, 40 g kg^{-1} of P, 21 g kg^{-1} of Mg, 2000 mg kg^{-1} of Mn, 300 mg kg^{-1} of Fe, 300 mg kg^{-1} of Cu, 100 mg kg^{-1} of Co, 100 mg kg^{-1} of I, 35 mg kg^{-1} Se, 500,000 IU of vitamin A kg^{-1}, 200,000 IU of vitamin D_3 kg^{-1}, 2000 IU of vitamin E kg^{-1}, 500 mg of antioxidant/kg^2. Calculated using Amino Cow (2008) software

on the basis of DIM, the average milk production, parity and body weight. Treatments were: control: cows were fed a basal diet (Table 1), basal diet with 2.5 g of enzyme/kg of DM, basal diet with 5 g of enzyme/kg of DM. Basal diet was formulated with Amino Cow software. Diets were fed as TMR twice daily. Cows were housed individually in tie stall and were milked 3 times daily. The enzyme added to TMR as top dress before feeding every morning. The design of trial was change over with three periods, three treatments and five cows allocated to each diet and every period was 21 days. The 1st week considered for adaptation of cows to diet and 2 weeks later for recording and sampling in every period. Milk yield recorded daily and sampled for 1 week in each period. Milk composition was analyzed by Milk-O-Scan (Foss Electric, Denmark). Feed offered and refused was measured and recorded daily to determine DMI. Samples of feed and orts were collected and composited in each period. The samples were milled through a 1 mm screen and analyzed for DM, CP (AOAC, 2000) and NDF according to Van Soest *et al.* (1991). Rumen fluid collected by stomach tube from each cow at 0 (before morning feeding) and 4 h after feeding to measure pH and microbial count in end of each period. Rumen fluid was strained with 2 layer of cheesecloth and transferred to laboratory in flask contained warm water (39°C). The method for bacterial counting was Most Probable Number (MPN) method according to Dehority *et al.* (1989) and used direct counting method with microscope for protozoa. Blood samples were collected by puncture of the median coccygeal vein into evacuated tube containing heparin (Beckton Dickinson, Franklin lakes, N7). Blood samples centrifuged at 3000×g for 15 min for plasma separation. Plasma was analyzed for glucose, urea nitrogen, beta hydroxy butyrate and total protein by using kit and ABX Mira Auto Analyzer (ABX Mira, cedex4, France). The data were analyzed by the Mixed procedure of SAS (2001) with following model:

$$y_{ijkl} = \mu + T_i + B_k + SUB\ (B)_{jk} + P_l + \varepsilon_{ijkl}$$

Where:

μ = Overall mean
T_i = Treatment effect
B_k = Effect of order of treatment
$SUB\ (B)_{jk}$ = Effect of cow on order of treatment
P_l = Effect of period
ε_{ijk} = The random residual. The level of significance was set to $p \leq 0.05$

RESULTS AND DISCUSSION

Effect of enzyme on milk yield and milk composition is shown in Table 2. Low level of Enzyme (Enz1) treatment increased milk yield significantly ($p \leq 0.05$) compared to control and high level of Enzyme (Enz2) group. Dry matter intake increased significantly in Enz2 treatment ($p \leq 0.01$). Milk efficiency decreased significantly ($p \leq 0.05$) in Enz2 compared to Enz1 groups. Effect of fibrolytic enzyme on rumen microbial growth and pH at 2 times (0 and 4 h after feeding) is shown in Table 3. Fibrolytic enzyme had no significantly effect on rumen pH and bacteria and protozoa numbers. Treatment with low level of Enzyme (Enz1) improved significantly plasma glucose concentration ($p \leq 0.05$) of cows compared to control and Enz2 group (Table 4).

Low level of enzyme improved milk production that can be due to increasing availability of nutrients. This level of enzyme increased significantly glucose concentration (Table 4) and glucose is precursor for lactose synthesis in mammary gland and so in cows consumed low level of enzyme caused more milk yield. This finding is consistent to Lewis *et al.* (1999), Rode *et al.* (1999) and Kung *et al.* (2002). But is contrast to Sutton *et al.* (2003), Yang *et al.* (2000) and Bowman *et al.* (2002) that fibrolytic enzyme had no any effect on milk production. Kung *et al.* (2000) reported that low level of enzyme increased milk production that

Table 2: Least squares means of DMI, milk yield and composition, feed efficiency of cows fed experimental diets[1]

Parameters	Control	Enz1	Enz2	SEM[2]
DMI (kg day^{-1})	22.3[a]	23.90[a]	25.40[b]	1.20
Milk yield (kg day^{-1})	37.9[a]	39.10[b]	36.70[a]	1.30
FCM 3.5% (kg day^{-1})	35.7[a]	36.20[b]	34.00[a]	1.20
Milk composition				
Fat (%)	3.21	3.00	3.18	0.11
Protein (%)	2.85	2.91	2.90	0.90
Milk composition yield (kg day^{-1})				
Fat	1.21	1.17	1.16	0.10
Protein	1.08	1.13	1.06	0.07
Feed efficiency[3]	1.60[a]	1.51[a]	1.33[b]	0.02

[1]Treatment groups include: Basal diet (control), basal diet supplemented with 2.5 g of fibrolytic Enzyme/kg of DM (Enz1) and basal diet supplemented with 5 g of fibrolytic Enzyme/kg of DM (Enz2)[2]: Standard error of means[3]: kg of FCM 3.5% per kg of DM; [a, b]Means within a row lacking a common superscript letter differ ($p<0.05$)

Table 3: Least squares means of pH and microbial count at 2 times from rumen fluid of cows fed experimental diets[1]

Parameters	Time (h)	Control	Enz1	Enz2	SEM[2]
pH	-	6.3	6.4	6.2	0.08
Bacteria number ($\times10^{10}$)	0	2.5	0.13	10.1	3.40
Protozoa number ($\times10^{4}$)		6.4	9	6.5	1.40
pH		6.1	6/0	6.0	0.09
Bacteria number ($\times10^{10}$)	4	0.3	0.3	0.2	0.10
Protozoa number ($\times10^{4}$)		5.5	6.8	5.7	1.20

[1]Treatment groups include: Basal diet (control), basal diet supplemented with 2.5 g of fibrolytic Enzyme/kg of DM (Enz1) and basal diet supplemented with 5 g of fibrolytic Enzyme/kg of DM (Enz2)[2]: Standard error of means

Table 4: Least squares means of blood plasma chemistry of cows fed experimental diets[1]

Item	Control	Enz1	Enz2	SEM[2]
Glucose (mg dL^{-1})	53.000[a]	59.000[b]	56.000[ab]	1.60
Urea nitrogen (mg dL^{-1})	16.090	15.470	15.950	0.76
Total protein (g dL^{-1})	7.350	7.010	7.100	0.17
βhydroxy butyrate (mmol L^{-1})	0.538	0.422	0.480	0.03

[1]Treatment groups include1: Basal diet (control), basal diet supplemented with 2.5 g of fibrolytic Enzyme/kg of DM (Enz1) and basal diet supplemented with 5 g of fibrolytic Enzyme/kg of DM (Enz2)[2]: Standard error of means [a, b]Means within a row lacking a common superscript letter differ ($p<0.05$)

support the finding. Responses in enzyme levels were not linear (Beauchemin *et al*., 2000). Enzymes maybe compete with rumen bacteria for attachment to feed particles and so rumen bacteria activity decrease (Beauchemin *et al*., 2000). Dry matter intake increased in cows consumed high level of enzyme that is consistent with Luchini *et al*. (1997), Beauchemin *et al*. (2000) and Lewis *et al*. (1999). But is contrast with Schingoethe *et al*. (1999), Kung *et al*. (2000, 2002) and Phipps *et al*. (2000). This difference can be due to type and activity of enzyme, composition of diet and method applying of enzyme. Enzymes probably solubilize digestible fraction of NDF and ADF of diet and increased rate of passage of feed from the rumen (Feng *et al*., 1996). In addition, fibrolytic enzyme improved fiber digestion in rumen and so it increased DMI (Zinn and Salinas, 1999). Milk fat decreased numerically in cows received enzyme that probably related to decrease of effective fiber due to effect of cellulase and xylanase on NDF and ADF. Feed efficiency decreased in cows consumed high level of Enzyme (Enz2) compared to Enz1 group. Cows in Enz2 group produced less milk and had more DMI than Enz1 and so feed efficiency decreased. When fibrolytic enzyme adds to TMR can increase attachment of enzyme to feed and make more resistance to proteolysis in rumen (Beauchemin *et al*., 2000). Enzyme had no any effect on blood urea that is similar to result of Hristov *et al*. (1998) and Bilik *et al*. (2009). Although, BUN of dairy cows that consumed enzyme was decreased numerically compared to control that may be is due to effect of enzyme to better utilization of NH_3 in rumen by microorganism. Effect of enzyme on βHB (beta Hydroxyl Butyrate) between treatments was similar and it was lower numerically to groups that consumed enzyme.

This is consistent with Bilik *et al*. (2009) that fibrolytic enzyme decreased βHB in blood of dairy cows. It may be due to improvement of energy balance and evidence of lower energy deficit in cows that received enzyme. The higher plasma glucose levels in cows that consumed enzyme may be evidence that energy requirement of dairy cows has been met. Fibrolytic enzyme had no effect on rumen pH that is consistent with Yang *et al*. (1999) and Beauchemin *et al*. (2000) but is contrast to Lewis *et al*. (1996) and Hristov *et al*. (2000) that reported rumen pH decreased when fibrolytic enzyme added to diet. Fibrolytic enzyme degrade cell wall component to soluble sugar and rumen bacteria probably used sugars and so rumen pH unchanged. Fibrolytic enzyme had no effect on bacteria and protozoa number in rumen fluid of cows compared to control that is consistent with Nsereko *et al*. (2002) but is contrast to Wang *et al*. (2001) that fibrolytic enzyme increased fibrolytic and non fibrolytic bacteria with batch culture system in rumen fluid. It may be due to attachment of rumen bacteria to feed particle and washout of bacteria with rumen fluid after feeding the experimental diets. Overall, adding of fibrolytic enzyme to diet of dairy cows was effective in improvement of milk production, dry matter intake and energy balance in early lactation period.

CONCLUSION

Results of present research showed that low level of fibrolytic enzyme (2.5 g kg^{-1} of DM of TMR) increased milk yield and FCM 3.5% compared to control and had no any effect on milk composition. Dry matter intake increased in dairy cows that consumed high level of enzyme (5 g kg^{-1} of DM of TMR). Feeding of low level of enzyme increased plasma glucose concentration compared to other treatments.

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