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Effects of Using Monensin and Different Levels of Crude Protein on Milk Production, Blood Metabolites and Digestion of Dairy Cows

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ABSTRACT : Twenty-four Holstein dairy cows were used to evaluate the single and combined effects of different levels of crude protein (CP) and monensin treatment during early lactation on blood metabolites, milk yield and digestion of dairy cows. The experiment was designed as a completely randomized block with a 3×2 factorial arrangement of treatments. The factors were three concentrations of CP supplement (19.5, 21.4, and 23.4% of dry matter) and two levels of monensin (0 and 350 mg per cow per day). The experiment consisted of three phases and each phase was 3 wk in length. Monensin did not affect milk yield, lactose, solids-non-fat (SNF), blood glucose, triglyceride and DMI, but increased blood cholesterol, blood urea nitrogen (BUN), insulin and reduced blood β-hydroxybutyrate (BHBA), milk fat and protein percentage. Monensin premix significantly decreased rumen ammonia, but rumen pH and microbial protein synthesis were not affected by monensin treatment. Increasing dietary CP improved milk and protein production, but did not alter the other components of milk. Digestibility of NDF, ADF, CP were improved by increasing dietary CP. Increasing dietary CP from 19.5 to 21.4% had no significant effect on ruminal ammonia, but increasing CP to 23.4% significantly increased ruminal ammonia. There was a linear relationship between level of crude protein in the diet and volume of urine excretion. Microbial protein synthesis was affected by increasing CP level; in this way maximum protein synthesis was achieved at 23.4% CP. (**Key Words :** Monensin, Crude Protein, Blood Parameters, Milk Production, Dairy Cow)

INTRODUCTION

At the beginning of lactation, dairy cows have to cope with the high energy and protein demands for milk synthesis at a time when nutrient intake is low. Mobilizing energy and protein from body tissue stores and repartition of nutrients away from extra-mammary tissues are the primary alternatives to supply sufficient nutrients for milk production during the first weeks of lactation. Excessive body reserves, especially fat, can subject cows to a series of metabolic disorders and consequent production losses (Fourichon et al., 1999). Maltz and Silanikove (1996) determined that high-yielding dairy cows had a negative nitrogen balance of 52 and 40 g/d at 2 and 7 wk postpartum, respectively. Komaragiri and Erdman (1997) assumed that cows have a greater capacity to mobilize body fat than protein and that in high-yielding dairy cows both energy and protein are limiting. It is believed that a reduction of this negative balance will contribute to increased production and health and more efficient use of dietary protein in early lactation by dairy cows.

Monensin is an ionophore that affects gram-positive bacteria as it influences ruminal fermentation and causes a shift in the molar proportions of VFA from less acetate and butyrate to more propionate (Duffield et al., 2002). The shift in ruminal VFA production concurs with a reduction in methane losses and as a result, energy efficiency is assumed to be improved.

Studies indicated that monensin could decrease amino acid deamination and ammonia accumulation (Yang and Russell., 1993). Ruiz et al. (2001) demonstrated that monensin could inhibit previously unrecognized ruminal bacteria that had very high rates of ammonia production. Phipps et al. (2000) reported that feeding monensin at 150, 300, and 450 mg/d increased milk yield (27.8, 27.5 and 26.5 kg/d, respectively) compared to a control group (25.0 kg/d).

Phipps et al. (2000) found a lower feed intake in cows treated with monensin than in the control group. Milk production was increased, and milk fat content was decreased. The mechanism of lowered milk fat by monensin may also be mediated through interference with

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biohydrogenation of long-chain fatty acids within the rumen, possibly through its effects on rumen bacteria.

Cadorniga and Satter (1993) pointed out the importance of protein and energy adequacy in early lactation and the critical influence of high peak milk yield on total lactation performance. Broderick (2003) reported that milk protein percentage of dairy cows fed low protein diets was reduced slightly as were blood urea and albumin.

Zimmerman et al. (1991) reported an increase in milk protein percentage (3.45 to 3.62%) when CP of a diet that was low in fiber was increased from 14 to 22%.

The objective of the present experiment was to determine the response of lactating dairy cows to monensin premix and diets with different CP concentrations. Hence, diets were formulated to provide CP at three concentrations (19.5, 21.4, 23.4% DM).

MATERIALS AND METHODS

The present experiment was conducted during summer and fall of 2006 in the north-east of Iran (Azarnegin husbandary-Tabriz). Twenty-four individually fed Holstein-Friesian cows were used to investigate the effect of using monensin and different levels of CP on milk production, blood metabolites and digestion of dairy cows. Cows were milked daily at 0830, 1430 and 2030 h, and also fed daily at 0700, 1600 and 2200 h. All cows had free access to water throughout the trial. Two groups received 0 and 350 mg/d of monensin and each group received three levels of crude protein. Treatments were introduced at 3 days post-calving and continued for 9 wk. The experiment consisted of 3 phases and each phase was 21 days. For each cow, the mean value of each variable during the treatment phase was recorded.

Milk production and composition

Cows were milked three times a day. During the last week of each period, milk samples were taken from each cow and stored at +4°C. Milk samples were taken at three consecutive milkings once per three weeks and analyzed for milk fat, protein, lactose and solids-non-fat (SNF) concentration by an infrared milk analyzer (Foss Electric).

Urine and rumen fluid sampling

Urine volume and allantoin were estimated from urinary creatinine concentration assuming a creatinine excretion rate of 29 mg/kg BW (Valadares et al., 1999). Allantoin was measured according to the colorimetric method proposed by Fujihara et al. (1987).

Rumen fluid was collected every 3 weeks, 3 to 4 h after the morning feeding. A stomach tube was used to collect approximately 200 ml of rumen fluid. A 45-ml subsample was filtered, and the pH of the filtrate was immediately measured using a Fisher Accumet pH meter (model 610; Fisher Scientific, Fairlawn, NJ) calibrated with a buffer solution (pH 7.0; Fisher Scientific). Cows were weigh every 21 days before the morning feeding.

Table 1. Composition of diets (%)

Item	Diets[1]		
	A	B	C
Legume forage hay, mature	30.95	31.13	31.25
Corn silage, normal	10	10.05	10.11
Barley grain, rolled	10.84	8.82	7.09
Corn grain, ground, dry	9.45	7.92	6.36
Molasses, beet sugar	3.34	3.36	3.36
Vegetable oil	1.29	1.43	1.30
Cottonseed, meal, solv	5.24	4.39	3.53
Cottonseed, whole with lint	5.64	4.73	3.79
Meat and bone, renderd	6.49	12.62	16.97
Beet sugar pulp, dried	9.26	9.32	9.35
Calcium phosphate (Di-)	0.43	0.37	0.30
Sodium bicarbonate	0.77	0.67	0.53
Vitamin premix 2	0.64	0.53	0.43
Salt	0.43	0.37	0.30
Soybean, meal, solv. 44% CP	4.97	4.06	5.16
Calcium carbonate	0.26	0.23	0.17
Chemical composition			
CP (%)	19.5	21.4	23.4
NDF (%)	32.8	31.6	30.6
ADF (%)	22.1	21.4	20.8
RDP[2] (%)	12.4	12.5	13.2
RUP[2] (%)	7.1	8.9	10.2
NE_L[2] (Mcal/kg)	1.58	1.58	1.58

[1] Diets with different levels of crude protein (A = 19.5% CP, B = 21.4% CP and C = 23.4% CP).

[2] Computed using NRC (2001) model based on actual composition of feeds, least squares means of actual DMI, milk yield, and milk composition for each diet, and overall average BW (614 kg).

Blood collection and analysis

Blood samples were collected into 10-ml red top (plain) vacutainer tubes, left to clot at room temperature (17-24°C) for 1 h, and subsequently centrifuged at 3,000 rpm for 20 min. Serum was stored at -20°C and then analyzed using a BM/Hitachi 911 analyzer (Boehringer Mannhein, Mannhein, Germany) using BHBA reagent (procedure no. 310-UV, Sigma Diagnostics, St. Louis, MO; McMurray et al., 1984) and a glucose kit (Cat. no. 1 448 668, Boehringer Mannheim, Mannheim, Germany; Trinder, 1969). Serum samples were also analyzed for triglycerides using an enzymatic and colorimetric procedure (Kit 10-525, Ziestchem Diagnostic kit, Tehran, Iran), for blood urea nitrogen with a kit (Cat no. 1 489 321 Boehringer Mannheim; Talke and Schubert, 1965), and cholesterol (kit number C7510; Pointe Scientific). Insulin was analyzed by

radioimmunoassay using a solid phase commercial kit (Insulin Coat-a-Count, Diagnostics Products Co., Los Angeles).

Chemical analyses

From each cow for the last 3 d of each phase, TMR and orts were sampled once daily and fecal samples (approximately 250 g) were taken twice daily. All TMR, orts and fecal samples were frozen at -10°C and composited as collected for the 3-d period. All TMR, orts, and fecal samples were thawed at room temperature and thoroughly mixed; subsamples of these were then dried at 105°C for 48 h to determine DM. In preparation for chemical analyses, subsamples of TMR, orts, and feces were dried at 55°C in a forced draft oven for 48 h and ground through a Wiley mill (2-mm mesh; Arthur H. Thomas Co., Philadelphia, PA), and then ground through a cyclone mill (Udy Co., Fort Collins, CO) to pass a 1-mm screen.

DM content of TMR, orts, and feces samples was determined by oven drying at 105°C for 48 h (AOAC, 1990; Method 930.15). Ash content of TMR, orts and feces was determined by incineration at 550°C overnight and organic matter (OM) content was calculated by subtracting ash percentage from 100 (AOAC, 1990; Method 942.05). The total N content of TMR, orts, and feces was determined by thermal conductivity (LECO model FP-428 Nitrogen Determinator). Crude protein content of TMR was calculated by multiplying nitrogen content by 6.25. The concentration of NDF in TMR, orts, and feces was determined as described by Van Soest et al. (1991) without using sodium sulfite and with inclusion of heat-stable α-amylase. ADF content in TMR, orts, and feces was determined according to AOAC (1990; Method 973.18). The NDF and ADF procedures were adapted for use in an ANKOM200 Fiber Analyzer. Concentration of ammonia in ruminal fluid was measured by colorimetry using the phenyl-hypochlorite reaction (Weatherburn, 1967). Protein, fat, lactose and SNF of milk samples were analyzed by infrared spectrophotometer (System 4000 MilkoScan; Foss Electric, Hillerød, Denmark; AOAC, 1990).

Statistical analysis

The study was done in a completely randomized block design with 2×3 factorial arrangement. The difference between day of calving and entry to the experiment and lactation period were included in the statistical model as covariates. Cows were allocated to treatment according to lactation period; therefore each treatment had cows in the first to fourth lactation periods. The model used for each variable was:

$$Y_{ijklm} = \mu + m_i + p_j + (mp)_{ij} + \beta_1 (X_{ijl} + \overline{X}) + \beta_2 (A_{ijk} - \bar{A}) + P_m + e_{ijklm}$$

Where, Y_{ijklm}= average value for cow L in treatments, μ = overall mean, m_i = effect of monensin, p_j = effect of different level of protein treatment, β_1 = regression coefficient for difference of entry to the experiment, β_2 = regression coefficient for different lactation periods, $\overline{X}$ = average days of entry to the experiment, X_{ijl} = number of days from beginning of experiment, $\overline{A}$ = average lactation period, A_{ijk} = number of lactation, P_m= Phase of experiment, e_{ijklm}= residual error.

Data were analyzed as repeated measures in time using MIXED procedure of SAS (2000).

RESULTS

The results of the present study showed that the interaction of monensin and different level of CP was not significant for blood metabolites, milk production and dry matter intake. Therefore, the main treatment effects including monensin and different level of CP were considered.

Milk production and composition

There were no significant differences for milk yield, lactose and SNF among treatments ($p>0.05$), but significantly lower milk fat and protein percentage were observed in monensin-treated cows ($p<0.05$) (Table 2). Increasing dietary crude protein from 19.5 to 21.4%

Table 2. Production of milk and its components in cows treated with monensin and fed different dietary levels of crude protein

Observation	Monensin (mg/d)		SE	Dietary CP (% DM)			SE	Lactation				SE
	0	350		19.5	21.4	23.4		1	2	3	4	
Milk production												
Yield (kg/d)	37.10	37.42	0.46	35.79^{b}	37.84^{a,1}	36.66^{ab}	0.54	27.28^{c}	37.97^{b}	43.67^{a}	44.12^{a}	0.62
Fat (%)	3.47^{a}	3.38^{b}	0.01	3.46	3.44	3.43	0.01	3.47^{a}	3.43^{ab}	3.39^{b}	3.41^{b}	0.02
Protein (%)	3.43^{a}	3.38^{b}	0.08	3.31^{b}	3.46^{a}	3.47^{a}	0.04	3.47^{a}	3.42^{b}	3.42^{b}	3.39^{bc}	0.01
SNF[2] (%)	8.94	8.99	0.01	8.89	8.92	8.94	0.01	9.02^{a}	9.1^{a}	8.89^{b}	8.85^{b}	0.02
Lactose (%)	4.78	4.92	0.05	4.77	4.86	4.91	0.06	4.67^{b}	4.98^{a}	4.91^{ab}	4.7^{b}	0.07

[1] Means within rows followed by different letters are significantly different ($p<0.05$).
[2] SNF = Solids-non-fat.

Table 3. Rumen pH, ammonia, urine volume, allantoin, creatinine and ratio of (A) to (C) in cows treated with monensin and fed different dietary levels of crude protein

Observation	Monensin (mg/d)		SE	Dietary CP (% DM)			SE	Lactation				SE
	0	350		19.5	21.4	23.4		1	2	3	4	
Rumen												
pH	6.10	6.18	0.04	6.12	6.17	6.19	0.03	6.13	6.13	6.16	6.15	0.06
Ammonia (mg/L)	247.29^{a,1}	240.30^{b}	2.21	232.56^{b}	234.55^{b}	265.71^{a}	2.6	237.08^{b}	239.67^{ab}	251.34^{a}	248.99^{ab}	2.54
Urine												
Allantoin (mg/ml)	1.95	1.87	0.14	1.92	2.01	1.99	0.17	1.82	1.88	1.98	2.00	0.21
Creatinine (mg/ml)	0.62	0.53	0.04	0.62	0.57	0.56	0.06	0.61	0.59	0.62	0.62	0.06
A:C^{2}	3.14	3.52	0.55	3.09^{b}	3.52^{a}	3.55^{a}	0.56	2.98	3.18	3.19	3.22	0.61
Volume (L/d)	24.51	24.62	0.34	23.52^{b}	25.03^{ab}	25.14^{a}	0.4	22.31^{c}	23.87^{bc}	24.94^{b}	27.15^{a}	0.55

[1] Means within rows followed by different letters are significantly different ($p<0.05$).
[2] Allantoin:Creatinine.

significantly increased milk production and protein ($p<0.05$). However, increasing crude protein had no effect on milk fat, lactose and SNF. There was a significant difference between lactation periods for milk production and composition. Milk yields in the third and fourth lactation periods were highest and cows in first lactation had the highest milk fat, protein and SNF (Table 2).

Ruminal ammonia and urine characteristic

Statistical analysis showed that monensin significantly reduced the amount of ammonia in rumen liquid ($p<0.05$), but did not have a significant effect on rumen pH, urine volume excretion, allantoin, creatinine and the ratio of these components in urine ($p>0.05$) (Table 3).

The levels of ammonia in rumen liquid and urine volume excretion were changed by dietary crude protein, but rumen liquid pH and urinary allantoin and creatinine were not affected by crude protein treatment. The ratio of allantoin to creatinine, that is, the index of protein synthesis, was significantly affected by increasing dietary protein from 19.5 to 21.4%, but increasing it to 23.4% had no significant effect on microbial protein synthesis. Increasing dietary crude protein caused an increase in rumen ammonia and urine volume excretion which was the highest at 23% CP. Period of lactation of treated cows had a significant effect on these parameters. Thus, cows in third lactation had the highest level of ammonia and cows in fourth lactation showed the highest urine volume excretion. There were no interactions between monensin and different level of protein on ruminal ammonia and urine volume.

Blood metabolites

Monensin had no significant effect on blood glucose and triglycerides, but cholesterol, BUN and insulin were significantly higher and BHBA was significantly lower in cows treated with monensin than in the control group ($p<0.05$) (Table 4). Increasing crude protein in the diet of cows from 19.5 to 23.4%, had no significant effect on blood glucose, cholesterol and triglycerides ($p>0.05$) but BUN and insulin were significantly increased ($p<0.05$). Increasing protein from 19.5 to 21.4%, increased insulin concentration but there was no significant difference between 21.4% and 23.4% CP in insulin concentration. On the other hand, increasing crude protein in the diet significantly decreased BHBA concentration ($p<0.05$), but this reduction was not significant between 21.4 and 23.4% CP ($p>0.05$) (Table 4).

DMI and digestibility

Statistical analysis of the effects of monensin and different level of CP is shown in Table 5. Monensin had no significant effect on dry matter intake and digestibility of DM, NDF, ADF or CP ($p>0.05$), but dietary crude protein levels had significant effects on DMI and digestibility of

Table 4. Blood metabolites in cows treated with monensin and fed different dietary levels of crude protein

Blood parameters	Monensin (mg/d)		SE	Dietary CP (% DM)			SE	Lactation				SE
	0	350		19.5	21.4	23.4		1	2	3	4	
Glucose (mg/dl)	53.63	54.22	0.32	53.72	53.76	54.30	0.36	53.05b^{1}	53.78^{b}	54.24^{ab}	54.63^{a}	0.31
Cholesterol (mg/dl)	198.17^{b}	210.91^{a}	3.61	198.82	204.59	210.22	6.32	199.74^{ab}	211.93^{a}	190.20^{b}	216.27^{a}	4.85
Triglyceride (mg/dl)	11.68	11.90	0.18	11.72	11.77	11.88	0.12	11.57^{b}	11.34^{b}	12.00^{a}	12.26^{a}	0.11
BUN^{2} (mg/dl)	15.42^{b}	16.25^{a}	0.21	13.96^{c}	15.84^{b}	17.72^{a}	0.25	14.43^{c}	15.50^{b}	16.75^{a}	16.68^{a}	0.28
BHBA^{3} (mg/dl)	11.84^{a}	10.98^{b}	0.23	12.42^{a}	11.33^{b}	10.48^{b}	0.28	11.76	11.36	11.43	11.11	0.53
Insulin (Ng/dl)	0.50^{b}	0.52^{a}	0.004	0.49^{b}	0.51^{a}	0.52^{a}	0.009	0.49	0.51	0.51	0.51	0.015

[1] Means within rows followed by different letters are significantly different ($p<0.05$).
[2] BUN = Blood Urea Nitrogen. [3] BHBA = β-hydroxybutyrate.

Table 5. DMI and apparent digestibility in cows treated with monensin and fed different dietary levels of crude protein

Observation	Monensin (mg/d)		SE	Dietary CP (% DM)			SE	Lactation				SE
	0	350		19.5	21.4	23.4		1	2	3	4	
DMI(kg/d)	17.92	17.69	0.09	18.15^{a}	17.79^{ab}	17.47^{b}	0.11	16.79^{b}	17.10^{b}	18.86^{a}	18.47^{a}	0.15
Apparent total tract digestibility %												
DM	61.11	63.63	0.86	59.94	63.75	63.42	1.01	64.65	61.85	62.21	60.76	1.37
CP	62.82	63.17	0.77	61.20^{b}	65.06^{a}	62.72^{ab}	0.89	66.33^{a}	62.98^{ab}	61.11^{b}	61.55^{b}	1.03
NDF	39.29	38.54	0.37	37.76^{b}	39.68^{a}	39.30^{ab}	0.44	40.17	39.63	37.92	37.93	0.50
ADF	35.80	37.10	0.49	34.93^{b}	37.52^{a}	36.91^{ab}	0.58	39.32^{a}	37.85^{a}	34.80^{b}	33.85^{b}	0.66

Means within rows followed by different letters are significantly different ($p<0.05$).

NDF, ADF and CP ($p<0.05$). Increasing dietary crude protein limited DMI and increased NDF, ADF and CP digestibility in diets with 21.4 and 23.4% CP compared to 19.5% CP. The highest digestibility of NDF, ADF and CP was observed for treatment with 21% CP. The period of lactation had a significant effect on DMI and digestibility of ADF, but had no effect on digestibility of DM and NDF. There were no interactions between monensin and crude protein levels on DMI and digestibility of DM, NDF, ADF and CP.

DISCUSSION

The present study has made several important findings with respect to the influence of monensin on milk yield and its components in Holstein dairy cows during early lactation. Phipps et al. (2000) reported that monensin treatment significantly reduced milk protein content in a study with a much larger sample size than the current study. However, in other studies (Van der Werf et al., 1998; Duffield et al., 1999; Green et al., 1999; Ruiz et al., 2001), monensin had no effect on milk protein content.

As in our experiment, other studies did not detect any effects of monensin on milk lactose content or yield (Green et al., 1999; Phipps et al., 2000; Vallimont et al., 2001).

Ionophore effects on ruminal fermentation, which may influence lactation performance, may explain some of these results. For example, increased propionate production, at the expense of acetate, butyrate and methane, will increase energy that is potentially available for milk synthesis (McGuffey, 1995).

Monensin decreases rumen ammonia concentration because gram-positive bacteria that are sensitive to monensin have a higher specific activity for ammonia production than do gram-negative bacteria that are resistant to monensin (Duffield et al., 2002). Experiments with sheep (Poos et al., 1979) and dairy cows (Haimoud et al., 1995) have observed lower rumen ammonia concentrations (63 and 53%, respectively) when monensin was administered than when a control treatment was administered. A similar trend appeared in the current experiment; a decrease in rumen ammonia was observed for cows treated with monensin.

Although, monensin decreased ruminal concentration of ammonia, this diminution had no significant effect on ruminal microbial synthesis. As shown in our results (Table 3) monensin did not have significant effects on allantoin excretion.

Low rumen pH can affect fiber digestibility (Calsamiglia et al., 1999). Green et al. (1999) also found that monensin increased rumen pH in dairy cows. Hence, monensin could have affected fiber digestibility through its effect on rumen pH.

Glucose concentrations were not significantly affected by monensin in the current study. However, a numerical trend supports previous studies (Duffield et al., 1998a; Abe et al., 1994). The lack of effect of monensin on blood glucose in the current study might be explained by the relatively small number of experimental cows and insufficient statistical power of the experiment.

Stephenson et al. (1997) reported that monensin-treated cows had significantly lower blood glucose in the immediate pre-calving period. Other studies reported a significantly higher blood glucose concentration in monensin-treated cows post-calving (Abe et al., 1994; Duffield et al., 1998a).

Monensin-treated cows had better energy status in the early weeks of lactation, as indicated by significantly lower BHBA and higher cholesterol. The significantly higher concentrations of cholesterol in monensin-treated cows post-calving are supportive of improved energy metabolism in these cows (Gerloff et al., 1986; Kaneene et al., 1997). The tendency for lower post-partum BHBA concentrations in monensin-treated cows was consistent with several studies demonstrating lower ketone concentrations in cows receiving monensin in the post-calving period (Sauer et al., 1989; Duffield et al., 1998a; Green et al., 1999). Monensin may also provide a glucogenic effect that is independent of rumen fermentation to improve glucose production and decrease BHBA concentrations (Abe et al., 1994). Haimoud et al. (1995) found intestinal digestion of undegraded rumen starch was higher when monensin was administered and found a higher concentration of glucose (3.6 vs. 3.1 mM for control animals). Therefore, the shift in site of starch

digestion would allow more carbon to be absorbed as glucose and not as volatile fatty acids, a more efficient use of potential energy by the cow (Blaxter, 1989).

Ionophores generally reduce DMI in feedlot cattle, but BW gain is increased, or unaffected, and feed efficiency is improved. In pasture-fed cattle, ionophores do not reduce DMI, but BW gain is increased, thereby resulting in improved feed efficiency (Nagaraja et al., 1997). Thus, in dairy cattle diets, which are intermediate between feedlot and pasture diets, a moderate depression in DMI might be expected. Reported effects of monensin on DMI have been variable, with either no effect or a decrease in DMI (Phipps et al., 2000), which is similar to results of the present study in which cows had either the same, or lower DMI dependent on level of DMI of cows.

Ipharraguerre and Clark (2005) increased CP of the diet from 11.3 to 23.1 (% of DM) and observed maximum milk yield at 23% CP. These results are similar to results reported by NRC (2001). Considerable variation in the relationship between the percentage of dietary CP and milk yield might be accounted for by the source of CP.

In the present experiment, delivery of RUP amino acids to the small intestine increased as dietary RUP increased, which resulted in greater milk protein production. The RUP fraction in the digesta delivered to the small intestine increased milk and protein production by supplying essential amino acids. Milk fat production declined numerically when a greater concentration of the RUP supplement was fed (Table 2). The decline in milk fat production when unsaturated fat is fed (as is commonly found in meat and bone meal) has been described by Sutton (1989).

Lactose production tended to increase as the concentration of RUP supplement in the diet increased (Table 2). This result corresponded to the linear increase in protein in this experiment and agrees with the summary by Sutton (1989), who indicated that the lactose content in milk was relatively constant. The milk and lactose production responses to increased concentrations of RUP supplement complemented each other.

If RDP supply is lower than the minimum required for microbial growth, intake may be restricted because of depressed ruminal digestion, especially of fiber. Olmos Colmenero and Broderick (2006), who found a linear increase in acetate concentration in the rumen with increasing dietary CP, also suggested that cellulolytic activity was increased and may be related to the linear increase in milk fat content.

Estimated urine volume increased from 23.52 to 25.14 L/d in response to higher CP supplementation. Sannes et al. (2002) reported that urinary excretion increased from 22.2 to 25.6 L/d when dietary CP was increased from 17.2 to 19.1%. These data indicated that greater urine volume was required for excreting the excess of nitrogen consumed by the cows (Holter et al., 1982).

Urinary excretion of purine derivatives, of which allantoin is the major component, reflects microbial nucleic acid absorption from the small intestine and the ratio of allantoin to creatinine is taken as the index of microbial protein formation in the rumen (Stangassinger et al., 1995). There were no changes in urinary allantoin excretion with increasing dietary crude protein (Table 3), but the ratio of allantoin to creatinine was increased by increasing CP from 19.5 to 21.4%. This increase suggests that increased CP content can improve microbial protein synthesis.

However, the ratio of allantoin to creatinine did not increase statistically above 21.4% dietary CP, suggesting no elevation in bacterial CP formation in the rumen beyond this point. This finding agreed with the suggestion of Olmos Colmenero and Brodrick (2006). Ruminal microbial protein synthesis depends on supply of adequate amounts and type of carbohydrate as an energy source and ammonia as a source of nitrogen (Bach et al., 2005). In early lactation, carbohydrate is a limiting factor that affects microbial protein synthesis. This trial proved that, in early lactation, limiting availability of carbohydrate has a significant effect on microbial synthesis.

In the current study, increasing dietary crude protein from 19.5 to 23.4% had no significant effect on glucose concentration. Blauwiekel and Kincaid (1986) reported that increasing dietary crude protein from 14 to 19% had no significant effect on glucose concentration, but weeks of lactation had a linear relationship with glucose concentration. Park et al. (2002) reported that increasing dietary crude protein from 9.7 to 16.2% had no significant effect on blood glucose of treated cows.

Schwalm and Schultz (1975) reported that increasing protein intake had a stimulating effect on insulin secretion, but this latter increase had no effects on blood cholesterol of treated cows. However, Zimmerman et al. (1992) observed increases in blood glucose concentrations when cows received a normal fiber, high protein diet supplemented with 12 g/d of niacin. On the other hand, weeks of lactation had a significant effect on blood cholesterol levels; at 60 days after calving, blood cholesterol increased from 161 to 244 mg/dl.

Blauwiekel and Kincaid (1986) reported no effect of dietary protein solubility on BUN when dietary CP was held constant, but a significant difference in BUN between dietary CP levels was observed. Absorbable protein that is not converted to milk protein is catabolized for energy, and this nitrogen contributes to the urea pool, some of which appears as BUN and MUN. Gabler and Heinrich (2003) stated that dietary crude protein influences BUN

concentration; hence in their study increasing dietary CP from 11.9 to 20.9 increased BUN from 10.12 to 16.68 mg/dl. However, in the present study increasing dietary CP from 19.5 to 23.4% increased BUN from 13.96 to 17.72 mg/dl.

Christensen et al. (1993) did not detect improvement in intake or apparent ruminal digestibility of OM, NDF, and ADF when increasing the CP content of the diet from 16.4 to 19.6% of DM, but these results do not agree with our findings (Table 5).

CONCLUSION

In early lactation, supplementing 350 mg monensin per day per cow had no effect on milk production, digestibility, blood glucose and triglycerides but increasing dietary crude protein increased milk yield and protein and decreased BHBA. Moreover, monensin did not have a significant effect on urine volume excretion. However, we observed that increasing dietary crude protein caused an increase in rumen ammonia and urine volume excretion which was highest at a level of 23% CP. Results from this study indicated that diets containing 21.4% CP supported maximal production in early lactation and improved performance of dairy cows.

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