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Effects of Monensin and Increasing Crude Protein in Early Lactation on Performance of Dairy Cows

[1]Behnam Ghorbani, [1]Taghi Ghoorchi, [2]Hamid Amanlou and [1]Saeed Zerehdaran
[1]Department of Animal Science,
Gorgan University of Agriculture Science and Natural Resources, Gorgan, Iran
[2]Department of Animal Science, Zanjan University, Iran

Abstract: Twenty-four Holstein dairy cows were used to evaluate the singular and combined effects of different level of crude protein and monensin treatments during the early lactation on digestion and milk yield of dairy cows. The experiment was designed as completely randomized 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). This experiment consist of three periods and each period was 3 week in length. Monensin did not affect DMI, milk yield, lactose and SNF but it reduced milk fat and protein percentage. Monensin premix significantly decreased rumen ammonia but rumen pH and microbial protein synthesis was not affected by monensin treatment. Although, Monensin treatment increased apparent digestibility of DM, NDF, ADF, CP, but they were not significantly. 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 diet CP from 19.5 to 21.4% did not significantly increase ruminal ammonia, but increasing to 23.4% have significant effect on it. There was a linear relationship between level of crud protein in the diet and urine volume excretion. Microbial protein synthesis was affected by increasing CP level; on this way maximum protein synthesis was achieved in 21.4% CP.

Key words: Monensin, crude protein, milk production, allantoin

INTRODUCTION

During early lactation, high yielding dairy cows cannot consume enough DM to meet nutrient requirements (National Research Council, 2001). Maltz and Silanikove (1996) determined that high yielding dairy cows had a negative nitrogen balance of 52 and 40 g day^{-1} at 2 and 7 week 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 the more efficient use of dietary protein in early lactation by dairy cows.

Monensin decreases methane losses and the ratio of acetate to propionate. Studies indicated that monensin could decrease amino acid deamination and ammonia accumulation and later work demonstrated that monensin could inhibit previously unrecognized ruminal bacteria that had very high rates of ammonia production (Ruiz *et al*., 2001). Phipps *et al*. (2000) reported that feeding monensin at 150, 300 and 450 mg day^{-1} improved milk yield.

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. Phipps *et al.* (2000) found a lower feed intake in cows treated with monensin. 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 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 yields on total lactation performance. Broderick (2003) reported that milk protein percentage of dairy cows that fed low protein diets was reduced slightly as were blood urea and albumin.

Corresponding Author: Behnam Ghorbani, Department of Animal Science,
Gorgan University of Agriculture Science and Natural Resources, Gorgan, Iran Tel: 0098-01233233426

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

MATERIALS AND METHODS

This experiment was conducted at the Animal husbandry of Tabriz (Azarnegin) during the summer of 2006. Twenty-four individually fed Holstein-Friesian cows were used to determine the effect of monensin and different levels of CP on feed intake, digestibility and milk production. There were two treatment groups receiving 0 and 350 mg day^{-1} of monensin and each group received three level of crude protein. Treatments were introduced at 3 days post calving and continued for 9 week. experiment consisted of 3 periods and each period was 21 days. For each cow, the mean value for each variable during the treatment period was calculated from the periodic data points and these values were analyzed using SAS. The study was done in a completely randomized design and differences between day of calving and entering to the experiment and lactation period were included in the statistical model as covariates. Cows were allocated to treatment according to lactation period, so each treatment had cows in first to fourth lactation period. The analysis of variance model used for each variable was:

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

Where:

Y_{ijkl} = Average value for cow L in treatments
μ = Overall mean
m_i = Effect of monensin treatment
p_j = Effect of different level of protein treatment
β_1 = Regression coefficient for difference between beginning of treatment
β_2 = Regression coefficient for difference between number of lactation
X = Average days of experiment
X_{ijl} = No. of days from beginning of experiment
$\bar{A}$ = Average lactation period
A_{ijk} = No. of lactation
t_m = Period of sampling
e_{ijkl} = Residual error

Data were analyzed as repeated measures using the MIXED procedure of SAS (1999-2000).

Feed Intake and BW: Diets were offered in equal amounts three times a day. Feed consumption was recorded daily by subtracting feed offered from feed refused by cows and data from day 16 to 21 third period were included in the statistical analysis. Samples of TMR, feed ingredients and orts were collected daily and kept frozen. Samples were composite by period, dried at 55°C for 48 h, ground through a 1 mm screen Wiley mill (standard model 4; Arthur M. Thomas, Philadelphia, PA) and analyzed for DM, OM, total N, NDF and ADF. Body weight was measured at the beginning and the end of each experimental period.

Apparent digestibility of diet composition: Feces and urine of each cow were collected separately from day 16 to 21 of third period of experimental. Feces were weighed and mixed daily and a sample (2%) was taken, stored at -20°C and subsequently thawed, dried at 55°C for 48 h and ground through a 1 mm screen (Wiley mill) for chemical analysis. Urine samples were collected from day 16 to 21 of third period (Table 1). Urine volume was calculated using creatinine as a marker assuming a creatinine excretion of 29 mg kg^{-1} of BW per day (Valadares *et al*., 1999). The ratio of allantoin:creatinine in spot samples of urine was used as an index of microbial protein synthesis (MPS) based on the methodology of Kolade (1994).

Approximately 100 mL of urine was collected daily using vulval stimulation from each cow between 12.00 h and 18.00 h from day 8 to 15 of each experimental period. These samples were acidified (pH 3) with the addition of 10 mL of 10% H_2SO_4 and then frozen at -20°C until analysed for creatinine using a Sigma Aldrich® creatinine determination kit and for allantoin using the method of Borchers (1977).

Ruminal fluid: Samples of ventral sac rumen fluid were obtained via hand vacuum pump just before feeding (0 h) and 2, 4, 8 h postfeeding on day 21 of each experimental period. The pH of Rumen fluid was recorded immediately (pH meter, model M90, Corning Inc., Corning, NY). Fifteen milliliter subsample preserved by adding 3 mL of metaphosphoric acid (25%) and freezed (-20°C) until analysis. Samples were later centrifuged (4000x) for 30 min at 4°C, repeated 3 times, to obtain clear supernatant. The supernatant was analyzed for rumen ammonia (N) using a phenolhypochlorite assay (Broderick and Kang, 1980).

Milk production and milk composition: Cows were milked three times a day and milk yield was recorded at each milking (Table 1). During the last week of each period, milk

samples were taken from each cow at each milking and stored at +4°C with a preservative (bronopol-B2). Milk samples were taken at three consecutive milkings once per three week and analyzed for milk fat, protein, lactose and SNF concentration by an infrared milk analyzer [Foss Electric (UK) Ltd.].

Chemical analyses: Analytical 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 the TMR, orts and feces was determined by incineration at 550°C overnight and the 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 was calculated as N × 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. Concentrations of NH_3-N in ruminal fluid were analyzed by colorimetry using the phenyl-hypochlorite reaction (Weatherburn, 1967). Protein, fat, lactose and Solids None Fat (SNF) in milk samples were analyzed by infrared spectrophotometer (System 4000 MilkoScan; Foss Electric, Hillerød, Denmark; AOAC, 1990).

RESULTS

Milk yield and its components: There were not any significant difference between milk yield, lactose and SNF, but there was a significant difference between milk fat and protein in monensin treated cows (Table 2). Increasing dietary crude protein from 19.5 to 21.4% had significant effect on milk yield and protein, but increasing it to 23.4% had no effect on mentioned treats. However, increasing crude protein had no effect on milk fat, lactose and SNF. Also, there was a significant difference between the lactation of cows and milk production and it's components. Milk yield in third and fourth lactation period cows were highest and cows in the first lactation have the highest milk fat, protein and SNF (Table 2).

Ruminal ammonia, BW changes and urine characteristic: Statistical analysis revealed that Monensin reduced significantly the amount of Ammonia in rumen liquid, but it did not have significant effect on rumen pH, body weight changes and urine volume excretion, Allantoin, Creatinine and ratio of them in urine (Table 3).

Table 1: Composition of diets

	Diets[1]		
Items	A	B	C
Legume Forage Hay, mature	30.95	31.13	31.25
Corn silage, normal	10.00	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, rendered	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	19.50	21.40	23.40
NDF	32.80	31.60	30.60
ADF	22.10	21.40	20.80
RDP[2]	12.40	12.50	13.20
RUP[2]	7.10	8.90	10.20
NE_L[2] (Mcal kg^{-1})	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 National Research Council (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)

The level of Ammonia in rumen liquid, Body weight changes and urine volume excretion were changed by dietary crude protein. But rumen liquid pH, Allantoin, Creatinin in the urine was not affected by crude protein treatment, but the ratio of (A) to (C) that is the index of protein synthesis, was significantly affected by increasing diet protein from 19.5 to 21.4%, but increasing it to 23.4 has 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 in 23% CP. Body weight change were not affected by increasing crude protein from 19.5 to 21.4% but increasing to 23.4% CP caused the minimum changes in body weight. Period of lactation of treated cows has a significant effect on these parameters. Thus, the third lactation cows have highest level of ammonia and body weight loses and the fourth lactation cows showed the highest urine volume excretion. There were no interaction between monensin and different level of protein on ruminal ammonia, Body weight loses and urine volume.

DMI and digestibility: Statistical analysis of the effects of monensin and different level of CP are there in Table 4. Monensin did not have any significant effect on dry matter intake and digestibility of DM, NDF, ADF or CP,

Table 2: Milk production and it's components in treated cows with monensin and different level of crude protein

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

Means within rows followed by different letter(s) are significantly different ($p<0.05$)

Table 3: Rumen pH, NH_3, urine volume, allantoin, creatinine and ratio of (A) to (C) in treated cows with monensin and different level of crude protein

	Monensin			Dietary CP (%DM)				Lactation				
Observation	0	350	SE	19.5	21.4	23.4	SE	1	2	3	4	SE
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
NH_3 (mg L^{-1})	247.29a	240.30b	2.21	232.56b	234.55b	265.71a	2.60	237.08b	239.67ab	251.34a	248.99ab	2.54
Urine (mg mL^{-1})												
Allantoin (A)	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 (C)	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	3.14	3.52	0.55	3.09b	3.52a	3.55a	0.56	2.98	3.18	3.19	3.22	0.61
Volume (L day^{-1})	24.51	24.62	0.34	23.52b	25.03ab	25.14a	0.40	22.31c	23.87bc	24.94b	27.15a	0.55

Means within rows followed by different letter(s) are significantly different ($p<0.05$)

Table 4: DMI and apparent digestibility in treated cows with monensin and different level of crude protein

	Monensin			Dietary CP (%DM)				Lactation				
Observation	0	350	SE	19.5	21.4	23.4	SE	1	2	3	4	SE
DMI(kg day^{-1})	17.92	17.69	0.09	18.15a	17.79ab	17.47b	0.11	16.79b	17.10b	18.86a	18.47a	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	61.20b	65.06a	62.72ab	0.89	66.33a	62.98ab	61.11b	61.55b	1.03
NDF	39.29	38.54	0.37	37.76b	39.68a	39.30ab	0.44	40.17	39.63	37.92	37.93	0.50
ADF	35.80	37.10	0.49	34.93b	37.52a	36.91ab	0.58	39.32a	37.85a	34.80b	33.85b	0.66

Means within rows followed by different letter(s) are significantly different ($p<0.05$)

but dietary crude protein had significant effects on DMI and digestibility of NDF, ADF and CP. 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 significant effect on DMI and digestibility of ADF and NDF, but it did not have an effect on digestibility of DM and NDF. There were no interaction between monensin and crude protein levels on DMI and digestibility of DM, NDF, ADF and CP.

DISCUSSION

Ionophores generally reduce DMI in feed lot 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 present study in which cows had either the same, or lower DMI dependent on level of DMI of cows. All cows lost BW during the 56 day postpartum period. This is consistent with other studies where monensin (Abe *et al*., 1994) were supplemented during early lactation.

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.

Monensin appeared to reduce NH_3 production by suppressing most if not all of the species of NH_3-hyperproducing bacteria that have been isolated from the rumen (Russell and Rychlik, 2000). However, at least one important species of NH_3-hyperproducing bacteria was not eliminated from the rumen at 350 mg day^{-1} of monensin in the diet (Russell and Rychlik, 2000).

Although, monensin decreased ruminal concentration of NH_3, but this diminution has no significant effect on ruminal microbial synthesis. As you see in our results (Table 3) monensin did not have significant affects on Allantoin excretion.

Monensin increased the apparent digestibility of NDF and ADF precalving, but not postcalving. 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.

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 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, increase propionate production, at the expense of acetate, butyrate and methane, will increase energy that is potentially available for milk synthesis (McGuffey, 1995).

Ipharraguerre and Clark (2005) increased the CP of diet from 11.3 to 23.1 (% of DM) and observed Maximum milk yield is achieved at 23% CP in the diet. These results are similar to results reported by National Research Council (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 AA 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 AA. 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) founded that, The 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.

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 which these results are agree with our findings (Table 4).

Estimated urine volume increased from 23.52 to 25.14 L day^{-1} in response to higher CP supplementation. Sannes *et al*. (2002) reported that urinary excretion increased from 22.2 to 25.6 L day^{-1} 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 N 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 as the index of microbial protein formation in the rumen (Stangassinger *et al*., 1995). There were no changes in urinary allantoin excretion, by 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 this increase can improve microbial protein synthesis.

However, 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 is agreed with suggestion of Olmos Colmenero and Brodrick (2006). Ruminal microbial protein synthesis depends on supply of adequate amounts and type of CHO as an energy source and NH_3 as a source of N (Bach *et al*., 2005). In early lactation CHO is a limiting factor that affecting microbial protein synthesis. This trial proved that in early lactation limiting in availability of CHO has a significant effect on microbial synthesis.

CONCLUSION

In the early lactation Supplementing 350 mg monensin per day per cow, has no effect on milk production and digestibility but increasing dietary crude protein increased milk yield and protein. Moreover, increasing crude protein and RUP, decreases lose weight in early lactation. Monensin did not have significant effect on body weight changes and urine volume excretion. However, observed that, increasing dietary

crude protein caused an increase in rumen ammonia and urine volume excretion which is the highest in level of 23% CP. Results from this study indicated that diets containing 19.5% CP supported maximal production in dairy cows in early lactation and improve performance of dairy cow.

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