Open Access
n-Australas J Anim Sci
Vol. 30, No. 5:660-665 May 2017
https://doi.org/10.5713/ajas.16.0564
pISSN 1011-2367 eISSN 1976-5517

AJAS
Asian-Australasian Journal of Animal Sciences

Effect of increasing dietary metabolizable protein on nitrogen efficiency in Holstein dairy cows

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Submitted Jul 24, 2016; Revised Oct 24, 2016; Accepted Nov 18, 2016

Objective: The objective of the study was to determine the effects of increasing levels of metabolizable protein (MP) on lactation performance and nitrogen (N) efficiencies in lactating dairy cows.
Methods: Nine multiparous cows in mid lactation [113±25 days in milk] received three treatments in a 3×3 Latin square design with a period length of 21 days. The treatments were three diets, designed to provide similar energy and increasing supply of MP (g/d) (2,371 [low], 2,561 [medium], and 2,711 [high] with corresponding crude protein levels [%]) 15.2, 18.4, and 20.9, respectively.
Results: Increasing MP supplies did not modify dry matter intake, however, it increased milk protein, fat, and lactose yield linearly. Similarly, fat corrected milk increased linearly (9.3%) due to an increase in both milk yield (5.2%) and milk fat content (7.8%). No effects were observed on milk protein and lactose contents across the treatments. Milk nitrogen efficiency (MNE) decreased from 0.26 to 0.20; whereas, the metabolic efficiency of MP decreased from 0.70 to 0.60 in low to high MP supplies, respectively. The concentration of blood urea nitrogen (BUN) increased linearly in response to increasing MP supplies.
Conclusion: Increasing MP supplies resulted in increased milk protein yield; however, a higher BUN and low MNE indicated an efficient utilization of dietary protein at low MP supplies.

Keywords: Dairy Cows; Metabolizable Protein; Milk Production; Milk Nitrogen Efficiency

INTRODUCTION

Dietary protein represents 42% to 50% of the total cost of dairy rations [1,2] and plays an important role in farm profitability as it affects dairy cow performance as well as environment [3]. There are several factors which affect milk nitrogen efficiency (MNE) (milk N yield/dietary N intake) including diet, cow, production system, environment, and models that predict nutrient requirements of cows. Increasing the dietary protein supplies below the requirement has been shown to increase milk protein yield, however, MNE decreased [4,5]. High producing cows are more efficient in nutrient partitioning compared to low producing cows. Similarly, a variation in MNE exists due to difference in stage of lactation [6]. In intensive production system, MNE varies from 25% to 35% [7] and from 21% to 27% in pasture based extensive production system [8]. The environmental temperature also affects MNE and it decreases with increasing temperature [9]. Different nutrient requirement models use different metabolizable protein (MP) efficiencies for prediction of protein requirements in lactating dairy cows e.g., NRC [10] model proposes an MP efficiency of 0.67, which is higher than INRA [11] and Cornell Net Carbohydrate and Protein System (CNCPS) 5.0 [12] that propose 0.64 and 0.65, respectively. Under such scenario, a continuous evaluation of MNE based on cow, production system, and environment is needed

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so that strategies can be designed for high MNE.

One of the strategies to increase MNE is to feed protein that closely matches to its requirements in dairy cows. The protein requirements for milk production in lactating dairy cows are calculated as milk protein yield divided by the MP efficiency. For this purpose, a careful assessment of protein supply vs. requirement is a pre-requisite because of the negative effects on milk protein yield with the low protein supplies in dairy cows [4,13]. We hypothesized that increasing dietary MP supplies will increase the milk production to a level where cows' requirements for protein will be met while decreasing the MNE. The objective of this study was to determine optimal protein supply level and MNE in mid lactating Australian Holstein cows by increasing dietary MP supplies.

MATERIALS AND METHODS

Animals, diets and experimental design

The experiment was carried out at Sharif Dairy Farms, Chiniot-Pakistan during the period of February to May, 2015 (outdoor temperature 23°C to 38°C). All the procedures were followed in accordance with the guidelines set out by the ethical committee of University of Veterinary and Animal Sciences (UVAS), Lahore-Pakistan.

Nine multiparous (parity = 2) cows in mid-lactation (body weight = 554 kg [standard deviation {SD} 27]; days in milk = 113 [SD 25], milk yield = 32.0 kg/d [SD 4.07]) were used in this study. Treatments were diets formulated to provide three levels of MP; i) Low MP = diet supplied 2,371 g/d of MP; ii) Medium MP = 2,561 g/d of MP, and iii) High MP = 2,711 g/d of MP. The crude protein (CP) contents of diets were 15.2%, 18.4%, and 20.9% in low, medium, and high MP diets, respectively. The diets were supplied according to a 3×3 Latin square design with a 21 days' period. The total duration of experiment was 63 days, following a week of adaptation period.

The ingredients and nutrient composition of diets are presented in Table 1. Dietary dry matter (DM) contained approximately 54% forage in each treatment. Soybean meal and rapeseed meal were increased from low to high MP diets in the concentrates. The diets were formulated using CPM-Dairy 3.0.10 from Cornell University (Ithaca, NY, USA), University of Pennsylvania (Philadelphia, PA, USA), and Miner Institute (Chazy, NY, USA), based on CNCPS 5.0 [12]. The diets were formulated to provide similar amount of energy i.e. 1.70 Mcal net energy for lactation (NE_L) per kg on DM basis. Low MP diet was designed to meet MP requirements for the cows producing 32 kg of milk; whereas, medium and high MP diets were formulated to provide MP supplies 7.9% and 14.2% above the predicted requirements, respectively. The feed was offered five times a day at 0100, 0500, 0900, 1300, and 1700 h and adjusted to yield about 10% orts. The first seven days in each treatment were taken as dietary adaptation period. Cows were fed individually and milked at 0100, 0900, and 1700 h. All animals had free access to clean water.

Table 1. Ingredients and nutrient composition of diets

Items	Low MP[1]	Medium MP	High MP
DM (%)	49.8	49.9	49.8
Ingredients (% of DM)			
Corn silage	45.4	45.4	45.4
Alfalfa hay	8.62	8.60	8.61
Soybean meal	7.45	13.1	18.6
Corn distillers' grains soluble	13.0	13.5	4.95
Palm kernel cake	4.78	4.78	0.48
Corn grain	2.65	1.98	0.89
Corn gluten meal 60%	0.00	0.79	4.67
Rice polish	6.77	0.45	0.45
Rapeseed meal	2.72	3.62	9.54
Sugarcane molasses	7.45	6.70	4.49
Megalac[2]	0.20	0.15	0.98
Sodium bicarbonate	0.50	0.50	0.50
Minerals and vitamins premix[3]	0.48	0.48	0.48
Nutrient composition (% of DM)			
Organic matter	91.7	92.0	92.1
CP	15.2	18.4	20.9
NDF	39.2	39.3	39.3
EE	4.54	3.87	3.71
NFC[4]	32.8	30.5	28.2
Predicted values			
ME (Mcal/kg)	2.66	2.66	2.65
NE_L (Mcal/kg)	1.70	1.70	1.70
RUP (g/d)	1,261	1,531	1,799
MP (g/d)	2,371	2,561	2,711

MP, metabolizable protein; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; EE, ether extract; NFC, non-fiber carbohydrates; ME, metabolizable energy; NE_L, net energy for lactation; RUP, rumen undegradable protein.

[1] Low MP, diet with 2,371 g/d supply of MP; medium MP, diet with 2,561 g/d supply of MP; high MP, diet with 2,711 g/d supply of MP.

[2] Church & Dwight Co., Princeton, NJ.

[3] Minerals and vitamins premix contained (per kilogram): 22% Ca; 12% P; 2.5% Mg; 2% Na; 500,000 IU of Vitamin A; 80,000 IU of Vitamin D_3; 300 IU of Vitamin E; 1,000 mg of Fe; 600 mg of Cu; 3,000 mg of Zn; 2,000 mg of Mn; 10 mg of Co; 20 mg of I; 3 mg of Se.

[4] NFC = 100–(CP+NDF+crude ash+crude fat).

Sampling, measurement, and analyses

The quantity of the diet offered and orts were weighed daily. Samples of corn silage and refusal were collected twice a week to determine the DM and adjust the total mixed ration (TMR) for changes in moisture content. The samples of individual ingredients of TMR (corn silage, alfalfa hay and concentrates) were analyzed twice during the experiment for chemical composition. These samples were collected, immediately dried in a forced-air oven at 60°C for 48 h [14], and sent to the Animal Nutrition Laboratory, UVAS, for proximate analysis. Samples were analyzed for crude protein (CP), crude ash, ether extract [14], and neutral detergent fiber (NDF) [15].

Milk yield was recorded daily at each milking, and samples were collected every 3rd day at each milking and assayed by infrared analysis using an Ekomilk instrument (Eon Trading Inc, Stara Zagora, Bulgaria) to determine protein, fat, and lactose contents.

On the 3rd last day of each period, blood samples were collected from jugular vein 30 minutes after milking and 15 minutes before feed distribution, and were immediately centrifuged (2,000 ×g for 15 min) to separate plasma from the whole blood. Plasma samples, separated from whole blood, were sent to the Quality Operations Laboratory, UVAS. Plasma samples were further processed for blood urea nitrogen (BUN), blood glucose, and triglycerides (TG) by using kits (HUMAN Max-Planck-Ring 21 D 65205 Wiesbaden, Germany). Urea was hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia from this reaction combined with 2-oxoglutarate and nicotinamide adenine dinucleotide- hydrogen in the presence of glutamate dehydrogenase to yield glutamate and nicotinamide adenine dinucleotide. The decrease in the absorbance was proportional to the urea concentration within the given time intervals. Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. Concentration of TG was determined after enzymatic hydrolysis with lipases. Indicator was quinoneimine, formed from hydrogen peroxide 4-aminoantipyrine and 4-chlorophenol under the catalytic influence of peroxidase. Tests were performed on chemistry analyzer Micro Lab 300 (ELITech Group 13-15 bis rue Jean Jaurès 92800 Puteaux, France).

Calculations and statistics

The feed efficiency was calculated by dividing milk yield by dry matter intake (DMI). The gross efficiency of MP was calculated by dividing milk protein yield by MP intake. The metabolic efficiency of MP was calculated using following equation [11]:

Metabolic efficiency MP

$$= \frac{\text{Milk protein yield (g/d)}}{\text{MP intake (g/d) - MP requirement for maintenance and gestation (g/d)}}$$

The MNE was calculated by dividing milk N yield by total N intake. The data of 2nd and 3rd week were analyzed using Mixed Procedures of SAS [16] with cow treated as random variable and periods and treatments were taken as main effects. Treatments were compared in linear and quadratic effects by using polynomial contrasts (orthogonal polynomial contrasts). The significance level was set at $p \le 0.05$ and the tendency was set at $0.05 < p \le 0.10$.

RESULTS

Dry matter, protein, and energy intakes

Increasing the MP supply did not change DMI and consequently the intake of NE_L was not effected (Table 2; p>0.10). As expected, the CP, N, and, MP intakes increased linearly by increasing the dietary MP supplies from low to high MP (p<0.01). The MP balance was slightly negative in low MP diet (–2.85 g/d); whereas, it was positive in medium and high MP diets (medium MP = 155 g/d and high MP = 255 g/d; p<0.01). The metabolizable energy balance was 13.9, 12.9, and 11.1 Mcal/d for low, medium, and high MP diets, respectively (p<0.01).

Milk yield and composition

Milk yield and composition results are presented in Table 3. Milk yield and fat corrected milk (FCM) increased linearly by increasing the MP supplies, averaging 29, 29.6, and 30.5 kg/d for milk yield (p = 0.01) and 26.9, 27.9, and 29.4 kg/d for FCM (p<0.01) in low, medium, and high MP, respectively. Similarly, milk fat, protein, and lactose yield increased linearly by 12.5%, 5.3%, and 5.11% with increasing MP supplies from low to high, respectively.

Table 2. Dry matter intake, protein and energy balance

Items	Treatments[1]			SEM	p value[2]		
	Low MP	Medium MP	High MP		Treat	Linear	Quadratic
DMI (kg/d)	23	23	22.9	0.29	0.91	0.76	0.76
Protein intake (g/d)							
CP	3,486	4,221	4,787	135.3	<0.01	<0.01	0.09
Nitrogen	556	675	766	8.83	<0.01	<0.01	0.09
MP	2,371	2,561	2,711	32.1	<0.01	<0.01	0.52
Energy intake (Mcal/d)							
ME	61	61.1	60.8	0.78	0.90	0.75	0.76
NE_L	39.1	39.1	38.9	0.50	0.91	0.75	0.76
Balance							
MP balance[3] (g/d)	–2.85	155	255	36.9	<0.01	<0.01	0.40
ME balance[4] (Mcal/d)	13.9	12.9	11.1	0.86	0.01	<0.01	0.64

MP, metabolizable protein; SEM, standard error of the mean; DMI, dry matter intake; CP, crude protein; ME, metabolizable energy; NE_L, net energy for lactation.
[1] Low MP = diet with 2,371 g/d supply of MP; medium MP, diet with 2,561 g/d supply of MP; high MP, diet with 2,711 g/d supply of MP.
[2] p value, probability, corresponding to the null hypothesis with linear and quadratic contrasts.
[3] MP balance = MP intake – MP requirement.
[4] ME balance = ME intake – ME requirement.

Table 3. Effects of metabolizable protein levels on milk yield and composition

Items	Treatments[1)]			SEM	p value[2)]		
	Low MP	Medium MP	High MP		Treat	Linear	Quadratic
Milk yield (kg/d)							
Milk	29.0	29.6	30.5	0.54	0.03	0.01	0.73
4% FCM[3)]	26.9	27.9	29.4	0.65	0.01	<0.01	0.78
Milk components yield (g/d)							
Fat	1,017	1,075	1,144	36.1	<0.01	<0.01	0.85
Protein	943	968	993	18.0	0.03	0.01	1.00
Lactose	1,370	1,400	1,440	26.3	0.04	0.01	0.97
Milk composition (%)							
Fat	3.57	3.70	3.85	0.122	0.09	0.03	0.93
Protein	3.27	3.28	3.27	0.012	0.51	0.95	0.25
Lactose	4.76	4.78	4.76	0.020	0.51	0.93	0.25

MP, metabolizable protein; SEM, standard error of the mean; FCM, fat corrected milk.
1) Low MP, diet with 2,371 g/d supply of MP; medium MP, diet with 2,561 g/d supply of MP; high MP, diet with 2,711 g/d supply of MP.
2) p value, probability, corresponding to the null hypothesis with linear and quadratic contrasts.
3) 4% FCM = $(0.4\times MY)+[15\times(fat/100)\times MY]$.

Milk fat contents increased linearly from 3.57% to 3.85% by increasing MP supplies from low to high levels (p = 0.03). Milk protein and lactose contents remained unaffected across the dietary treatments.

Efficiency of utilization of feed and nitrogen

The feed efficiency increased linearly by increasing the MP supplies (Table 4; p = 0.01), averaging 1.25, 1.28, and 1.32 in low, medium, and high MP, respectively. Similarly, FCM per unit of DMI increased linearly (p<0.01). The gross and metabolic efficiency decreased linearly by increasing the MP supplies (p<0.01) and averaged 0.40, 0.38, and 0.37 for gross and 0.70, 0.62, and 0.60 for metabolic efficiency of MP in low, medium, and high MP, respectively. The MNE decreased in high MP compared with low and medium MP (linear: p<0.01 and quadratic: p = 0.04) and averaged 0.26, 0.22, and 0.20 in low, medium, and high MP, respectively.

Plasma metabolites

Effects of different dietary MP supplies on plasma metabolites

Table 4. Effects of metabolizable protein levels on feed efficiencies

Items	Treatments[1)]			SEM	p value[2)]		
	Low MP	Medium MP	High MP		Treat	Linear	Quadratic
Feed efficiency[3)]	1.25	1.28	1.32	0.026	0.03	0.01	0.62
FCM:DMI	1.16	1.21	1.28	0.030	<0.01	<0.01	0.70
Gross efficiency MP[4)]	0.40	0.38	0.37	0.008	<0.01	<0.01	0.40
Metabolic efficiency MP[5)]	0.70	0.62	0.60	0.023	<0.01	<0.01	0.13
Milk N efficiency[6)]	0.26	0.22	0.20	0.005	<0.01	<0.01	0.04

MP, metabolizable protein; SEM, standard error of the mean; FCM, fat corrected milk; DMI, dry matter intake.
1) Low MP, diet with 2,371 g/d supply of MP; medium MP, diet with 2,561 g/d supply of MP; high MP, diet with 2,711 g/d supply of MP.
2) p value, probability, corresponding to the null hypothesis with linear and quadratic contrasts.
3) Feed efficiency = milk yield/dry matter intake.
4) Gross efficiency MP = milk protein yield/MP intake.
5) Metabolic efficiency MP = milk protein yield/MP intake – (MP for growth+MP for maintenance+MP for pregnancy).
6) Milk N efficiency = N in milk/N intake.

Table 5. Effect of metabolizable protein levels on plasma metabolites (mg/dL)

Items	Treatments[1)]			SEM	p value[2)]		
	Low MP	Medium MP	High MP		Treat	Linear	Quadratic
BUN	15.5	16.1	21.7	2.55	0.07	0.03	0.32
Glucose	67.7	64	67.8	6.23	0.79	0.98	0.51
TG	8.00	10.3	10.0	1.81	0.42	0.30	0.42

MP, metabolizable protein; SEM, standard error of the mean; BUN, blood urea nitrogen; TG, triglycerides.
1) Low MP, diet with 2,371 g/d supply of MP; medium MP, diet with 2,561 g/d supply of MP; high MP, diet with 2,711 g/d supply of MP.
2) p value, probability, corresponding to the null hypothesis with linear and quadratic contrasts.

are presented in Table 5. Increasing the MP supplies increased the BUN linearly (p = 0.03) and averaged 15.5, 16.1, and 21.7 (mg/dL) in low, medium, and high MP, respectively. Plasma glucose and TG remained unaffected across the treatments (p>0.10).

DISCUSSION

The objective of the current study was to investigate the effects of increasing MP supplies on MNE, milk yield, and milk composition in mid lactating Australian Holstein cows in Pakistan. There was no change in DMI with increasing MP supply from low to high MP levels in the present study. Similar results have been observed by Groff and Wu [17] who reported no change of MP supply on DMI at similar levels of dietary CP (16.2% to 19.8%). Previous studies reported that the effect of protein supplies on DMI was related to the duration of treatment [18]. Minimum four weeks are required in order to observe a protein deficiency in dairy cow due to the buffering effect of labile protein reserves [19]. The short duration of treatment in the current study (21 days) could explain no effect of MP on DMI. Hence, it may be suggested that the duration of treatment in the current study may not be long enough to observe the effect of varying MP supplies on DMI.

The increased milk protein yield with increasing dietary MP supply was in agreement with previous findings [20,21]. However, the increase of 5.3% protein yield from low to high MP diet (Table 3) was smaller compared to the increase of 8.54%, 38.8%, and 9.0% observed by Wang et al [20], Metcalf et al [4], and Arriola Apelo et al [21], respectively. In fact, the low MP level (2,371 g/d) in our study was higher compared to other studies i.e. 1,751 g/d in Wang et al [20], 1,573 g/d in Metcalf et al [4], and 2,005 g/d in Arriola Apelo et al [21]. Moreover, the increase in MP supply from low to high MP diets was less i.e. 14% in our study compared to 38% on an average in other studies [4,20,21]. The increase in milk protein yield in response to increasing MP supplies was related to increase in milk volume in agreement with the literature [4,22]. Increasing the MP (or total amino acid) supplies to the mammary gland increased milk protein synthesis [4,5] mainly through increasing milk volume by providing α-lactalbumin for lactose synthesis [23].

The rsult of the current study indicated no effect of increasing MP supplies on milk protein concentration. Earlier studies have shown that milk protein concentration increased [5,13], decreased [24] or remained unaffected [25] in response to increasing protein supplies. Such variations could be related to the interaction between protein and energy supplies. The high protein supplies increase DMI and consequently the energy intake that has been shown to increase the milk protein concentration [13]. The energy intakes (Table 2) in our study remained unaffacted due to similar DMI across the treatments explaining no effect of MP supplies on milk protein concentration.

The MNE averaged 23% in the present study was comparable to the values of 20.5% and 24.7% observed in the studies of Brito et al [26] and Arndt et al [27], respectively. The decreased MNE with increasing MP supplies were in accordance with previous finding [28]. The MNE depends on protein to energy ratio (MP/NE_L) and declines with an increasing MP/NE_L [29]. The demand for energy increases with the increasing protein supplies and energy could be the first limiting factor at high protein levels for decreased MNE [5]. The fixed energy supplies across the treatments could be the one reason of decreased MNE with increasing MP in our study. Secondly, the diets in the current study were not balanced for essential amino acids that have been reported to affect the MNE [30]. The absorbed amino acids that are not utilized for the synthesis of milk or body protein are de-aminated by the liver to produce urea which ultimately decreases the MNE at higher protein diets. This assumption could further be supported by the increased BUN from 15.5 to 21.7 mg/dL (Table 5) in current study. The current findings of linear increase in BUN by increasing MP supplies were in agreement with previous studies [22]. The average metabolic MP efficiency value (0.64) in the current study was in agreement with the values reported by INRA [11] and Metcalf et al [4] and lower than NRC [10] and CNCPS [12] system. The higher MP efficiency observed in low MP treatment (0.70) could be an opportunity to reduce wastage of N and its negative impact on environment.

Increasing dietary MP supplies increased milk fat yield and content in this study. Previous studies have reported an increase [18,24] on milk fat content with increasing MP supplies. It could be possible that increased oxidation rate of amino acids to CO_2 in high MP diets resulted in increased *de novo* synthesis of short and medium chain fatty acids in the mammary gland. It could be possible that high MP supplies increased the production of chylomicrons and lipoproteins in the blood that may indirectly increase the fatty acid supplies to the udder [10].

CONCLUSION

The current study demonstrated that MNE decreased when MP supplies were increased from 2,371 to 2,711 g/d in low to high MP treatments, respectively. The increased concentration of BUN in high MP diet indicated an increase in the wastage of N. However, a careful assessment on return over investment is important before implementation of a low protein dietary strategy to improve MNE because of the increased milk yield and fat percentage observed in high MP treatment in the current study. Further research is needed to investigate the MNE at other stages of lactation and at herd level under subtropical conditions.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

ACKNOWLEDGMENTS

The authors acknowledge the financial support provided by Sharif Dairy Farm. The authors are grateful to Mr Hanif and Mr Sagheer for their help in laboratory analysis of feed stuffs as well as the staff of University Diagnostic Laboratory and Quality Operations Laboratory for blood analysis.

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