ISSN: 1023-1072 (Print), ISSN: 2663-7863 (Online)

Pak. J. Agri., Agril. Engg., Vet. Sci., 2019, 35 (1): 57-62

DEGRADABILITY OF DIFFERENT PROTEIN SOURCES AND ITS IMPACT ON MILK COMPOSITION

A. Shah[2], S. Sultan[1], Z. Shah[2], S. Ahmad[2] and H. Khan[2]

[1]Department of Animal Nutrition, [2]Department of Animal Health,
Faculty of Animal Husbandry and Veterinary Sciences,
University of Agriculture Peshawar, Pakistan

ABSTRACT

The study was planned to determine the effect of degradability of different protein sources and its impact on milk composition and milk urea nitrogen in Holstein Friesen cows at Peshawar Khyber, Pakhtunkhwa, Pakistan. For this purpose nine healthy early lactating cows were selected. All the chosen animals were assigned into three different groups on the basis of feeding group A, B and C, respectively. Total mixed ration (TMR) was formulated as per availability of different degradable protein sources (cotton seed cake and mustard seed cake) and named as ration 1, 2 and 3, respectively. One group was kept as control and fed on only TMR at libitum and the other two groups B and C were supplemented with TMR added with differentials degradable proteins for one month. The milk production and composition (milk fat, Solid not fat (SNF), milk protein) and milk urea nitrogen were significantly *($P<0.05$)* increased in group B (ration 2). Hence, it may be concluded that ration 2 (group 2) of different sources of protein have improved the milk production, milk composition and milk urea nitrogen.

Keyword: cattle, feed, milk composition, protein

INTRODUCTION

Livestock plays an important role in the economy of the country and constituting more than 8.5% of the total exports. It mainly raised more than 8.5 million small and landless families in the rural as well as in the urban areas as their livelihood source. Livestock is a form of social security for poor people and can be cashed at the time of need. Farmers leaving in Barani area utilize livestock as an alternate source of income during drought and crop failure. Dairy sector is an important component of the country's economy. The value of milk alone exceeds the combined value of wheat, rice, maize and sugarcane in country largest for the milk production (Afzal, 2016).

Pakistan produces 46.4 million tons milk per year, about 70% of the total milk is produced in the rural areas. Pakistan imports 25000 tons dry milk every year (Economic Survey of Pakistan, 2010-11). The market value of milk is based on fat content; therefore high milk-fat percentage has good sale price. In Pakistan only 3-3.5% milk is processed, 30% is marketed and most of the milk approximately 70% is consumed at home (Economic Survey, 2011-2012).

Protein is the limiting nutrient in the dairy animal's diet. Protein plays a vital role in lactating animals, because there's low dry matter intake (DMI) (Oba *et al.,* 2004). In the new era scientists have developed new procedure for different feeding crude protein (Dhali, 2006). The ammonia produced in the rumen is due to the increase level of protein degradation that ultimate results in the increase of blood urea (B4 N) concentration and bloat (Eastridge, 2006). In feed of ruminants, the rumen degradable protein (RDP) and un-degradable protein could endanger the microbial production of protein; disturb digestion and unavailability of protein in animal milk if RDP is not sufficient to meet the requirement of microbes (Broderick, 2003). Therefore crude protein (CP) should provide in-sufficient quantity of the RDP to produce rumen microbes that are good for production of milk and rumen amino acids. Milk urea nitrogen (MUN) was increased linearly with increasing RDP (Santos *et al.*, 1998). The MUN arises primarily from passive

Corresponding author: drzahir@aup.edu.pk

© 2019, Sindh Agriculture University, Tandojam (http://pjaaevs.sau.edu.pk/index.php/ojs)

transfer of urea from blood similarly, high correlation between MUN and blood urea nitrogen (Broderick and Clayton, 1997).

Increasing the CP content in the feed of dairy cow may result not only in greater milk production (Jonker *et al.,* 1998), but also increase in the concentration of ruminal ammonia and BUN and consequently greater urinary nitrogen losses (Jonker *et al.,* 1998; Castillo *et al.,* 2001). Limited of studies investigating the effect of CP level, diets supplied different amount of metopilzable (MP), RDP or RUP. Thus the individual contributors of RDP, RUP, or MP to urinary or overall N losses cannot be readily distinguished. Dietary RDP can be used for Microbial Protein Synthesis (MPS) if enough energy is provided (Christensen *et al.,* 1993). Here in our study the main objective was to produce economic ration and its effects on production and composition of milk.

MATERIALS AND METHODS

Holstein Friesian cows 2 ± 0.29 years in age and weighing 350 ± 10.09 kg were used to examine the effect of different protein sources in Total Mixed Ration (TMR) on milk production, composition and MUN at Peshawar Khyber Pakhtunkhwa. The study was conducted under the guideline approved by the Animal Care Center, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan.

Total mixed ration

Total mixed ration was formulated as per availability of different degradable protein sources (cotton-seed cake and mustard seed cake).

Experimental design

Completely randomized study design was used for this purpose. Nine animals in total were selected for the experiment. Experimental animals were selected on the basis of same milking stage. All the experimental dairy cows were fed with Isocaloric and Isonitrogenous diet as per protocol approved by National Research Council (NRC). The animals were treated for infestation with Nilzan^{R} (ICI Pakistan) two weeks before the start of the experiment. The animals were divided into three main groups A, B and C (three animals in each group) and experimental ration 1, 2 and 3 were fed to the animals. Total mixed ration was prepared. Animals in group A were fed on only TMR at libitum. Animals of group B and C were fed (5 kg each animal) on TMR added with differential degradable proteins, respectively. Different degradable protein sources were added to TMR, and cows were free accessed to fresh drinking water throughout the day. Economic analysis was carried out in terms of cost of products and increase in milk production. Trail lasted for 30 days. Fourteen days were used as adaptation period and further 16 days for data recording regarding milk yield and the concentration of the milk components.

Feed formulation and composition

The concentrate feed ingredients (cotton seed cake, maize oil cake, maize gluten, mustard seed cake, maize grain, wheat bran and degradable crude proteins (DCP)) were formulated in three different parts. The formulation changes in feed were made in concentrates at ingredients level with different degradability of protein sources. While the level of crude protein was kept invariable for all ration parts (16%). Five kg formulated ration was fed to each cow twice a day according to the protocol approved by the Animal Care Center, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agricultural Peshawar. Fresh forages (Berseem) were fed at libitum as well 5 kg wheat straw per animal was fed as a basal feed (Table 1, 2).

Table 1. Ingredients of formulated ration (kg)

Ingredients	A	B	C
Cotton seed cake	15	20	00
Maize oil cake	10	10	15
Wheat bran	45	48	48
Maize gluten	10	10	10
Maize grain	10	10	15
Mustard seed cake	08	00	10
Di-Calcium phosphate	02	02	02
Total	100 kg	100 kg	100 kg

Table 2. Proximate composition of formulated ration (%)

Components	Concentrates			Basal diet	
	A controlled	B	C	Berseem	Straw
Dry matter	90.25	90.35	91.35	21.00	91.28
Crude protein	15.00	16.00	16.00	18.89	02.21
Ether extract	06.25	05.92	05.23	01.90	01.09
Crude fiber	09.53	13.67	10.28	22.98	40.98
Nitrogen free extract	50.00	45.00	47.20	40.98	42.21
Total digestible nutrients	73.00	68.29	70.00	61.48	42.00
Ash	04.98	05.13	05.00	14.18	12.00

Proximate analysis of dietary samples

Formulated ration and fresh forages were collected for proximal analysis. According to Association of Official Analytical Chemist (16) proximate analysis of feed sample was performed in a Laboratory of the Department of Animal Nutrition, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agricultural Peshawar.

Feed intake

Feed intake was measured by subtracting the refused feed from the offered feed to calculate the exact feed intake.

Feed efficiency

For the economic analysis of feed addition, cost of feed per kg was taken. Price of milk increase was compared with cost of feed consumed.

Milk production and composition

To examine the increase in milk production, milk of each cow was weighed after milking and was recorded in a chart. Fats, protein and SNF were analyzed by Elko Milk (EON Trading INC, Korea) in Dairy Technology Center Lab. at Veterinary Research Institute (VRI), Peshawar, Pakistan. Milk sample of 20 ml from each cow was taken at 7, 14, 21 and 28 days to carry out analysis of milk composition.

Milk urea nitrogen

Milk urea nitrogen was determined by a procedure described by Dhali *et al*. (2005). The Colorimetric dimethylaminobenzaldehyde (DMAB) was used in the procedure. About 10 ml of milk sample was taken to warm at room temperature 30^{o}C and mixed thoroughly. By using (10 ml) cold trichloroacetic acid (pH1) solution 12% to deproteinised the milk and allowed to stand for 1 hour, centrifuged at 3,000×g for 30 minutes and then filtered. Supernatant (2 ml) was taken to mix with 2 ml DMAB reagent (1.6 g DMAB+90 ml ethanol+10 ml concentrated HCl) and reading was recorded.

Table 3. Milk (ml) fat and lactose in holstein fresien cows supplemented with different levels of degradable protein (Mean± SE, %)

Group	Week 1	Week 2	Week 3	Week 4
Fat %				
A	2.51±0.04^{c}	2.15±0.17^{c}	2.10±0.31^{c}	2.25±0.14^{c}
B	4.40±0.06^{a}	4.43±0.18^{a}	4.52±0.13^{a}	4.62±0.04^{a}
C	3.80±0.02^{b}	3.85±0.05^{b}	3.68±0.14^{b}	3.82±0.01^{b}
Lactose %				
A	3.30±0.66^{c}	3.32±0.81^{b}	3.34±0.05^{b}	3.30±0.03^{b}
B	4.01±0.02^{a}	3.92±0.08^{a}	4.00±0.33^{a}	3.95±0.77^{a}
C	3.90±0.01^{b}	3.90±0.77^{a}	3.92±0.80^{a}	3.90±0.77^{a}

^{a,b,c} Mean values having different superscripts in a column differ significantly *(P<0.05)*

Statistical analysis

All data were expressed as means ± standard Error. Data analysis was performed with a commercially available software program (SPSS Version 18, SPSS Inc, Chicago, IL, USA). The data were analyzed using analysis of a variance (ANOVA) for repeated measurements of changes in time and comparisons between treatments, followed by Bonferroni test for differences between treatment groups at each time point. The $P< 0.05$ value was considered to be significant.

RESULTS

Effect of feed on fat and lactose content of milk

The fat content of the milk was linearly increased ($P< 0.05$) in group B throughout the experimental period, compared to group C and control. Overall the group B represented significantly higher ($P< 0.05$) fat composition; while lactose content in the milk was significantly ($P< 0.05$) higher for the first week in group B as compared to group C and control. In the 2nd, 3rd and 4th week both groups B and C showed linear increase ($P< 0.05$) in fat content compared to control group as it is shown in the Table 3.

Effect of feed on milk yield, protein, solid not fat and milk urea nitrogen

Milk yield was significantly ($P< 0.05$) increased due to the supplementation of different levels of degradable protein in the ration. Milk production was higher ($P< 0.05$) in group B, from 1st to 4th week in comparison to control (A) and treatment group C. The milk protein was significantly ($P< 0.05$) improved by the supplementation of different levels of degradable protein. Group B showed significant result into the experiment as compared to control and group. By the addition of different levels of degradable protein in TMR, supplemented to the experimental groups showed significant ($P< 0.05$) improvement in the milk SNF in both group B and C, respectively compared to the control group (A). There was also significant increase ($P< 0.05$) in MUN concentration by the supplementation of degradable protein sources in TMR in group B from 1-4 week as compared to group C and control (Table 4).

Economic importance of experimental ration

Economic analysis was carried out in terms of cost of production and increase in milk production (Table 5).

Table 4. Milk productions, protein, solid not fat, milk urea nitrogen in Holstein Fresien cows supplemented with different levels of degradable protein at different weeks (Mean ± SE, %)

Groups	Week 1	Week 2	Week 3	Week 4
Milk production				
A	7.16±0.44[c]	6.66±0.33[c]	7.16±0.44[c]	7.00±0.28[c]
B	8.50±0.28[a]	8.66±0.60[a]	8.70±0.28[a]	8.77±0.28[a]
C	7.66±0.33[b]	7.66±0.16[b]	7.33±0.16[b]	7.50±0.50[b]
Protein %				
A	2.32±0.05[c]	2.33±0.08[c]	2.34±0.03[c]	2.34±0.02[b]
B	2.83±0.01[a]	2.80±0.01[a]	2.81±0.01[a]	2.74±0.33[a]
C	2.76±0.00[b]	2.75±0.00[b]	2.52±0.24[b]	2.62±0.16[ab]
SNF				
A	6.24±0.01[c]	6.28±0.01[b]	6.30±0.08[b]	6.29±0.05[b]
B	7.61±0.03[a]	7.43±0.15[a]	7.55±0.03[a]	7.49±0.01[a]
C	7.42±0.06[b]	7.38±0.06[a]	7.48±0.08[a]	7.38±0.04[a]
Milk urea nitrogen				
A	15.33±0.33[b]	15.00±0.57[b]	14.33±0.3[b]	14.67±0.33[b]
B	16.33±0.33[a]	16.77±0.33[a]	17.53±0.33[a]	17.83±0.16[a]
C	14.83±0.16[c]	14.33±0.33[c]	12.33±0.33[c]	11.67±0.33[c]

[a,b,c] Mean values having different superscripts in a column differ significantly *(P<0.05)*

Table 5. Economic importance of experimental ration (price in Rupees)

Ingredients	A	B	C
Cotton seed cake	420	560	0
Maize oil cake	450	450	675
Wheat bran	900	960	960
Maize gluten	260	260	260
Maize grain	240	240	360
Mustard seed cake	240	0	300
Di-Calcium phosphate	140	140	140
Total	2650	2610	2695

Group B is economically low in price and available in market

DISCUSSION

The current study was conducted to determine the influence of degradability of various protein sources and its impact on milk yield and its composition. Several studies have reported that increasing the dietary protein level in ration increase the fat content in milk (Dhali. 2005). In the present study, similar results were obtained, where increase in the dietary proteins concentration in feed, simultaneously increased the fat content of milk. Inconsistence with our report several studies indicated no obvious change in milk fat content (Kalscheur *et al.,* 2006). Our finding is disagree to the result of Choung and Chamberlain (1992) who indicated that imbalance in amino acids profile in feed leads to increase in fat content (Kung and Huber, 1983). Studies have reported that increased concentration of fat precursors in the blood stream resulted into milk produce with high fat content. This increase in fat precursor in the blood stream enhances *denovo* fatty acid synthesis (Choung and Clamberlain, 1992). While by increasing dietary protein level in the diet made enhance the acetic acid production in the rumen due to which cellulolytic work increase and because of this reason milk fat content increased (Varvikko *et al.,* 1999).

Milk lactose increased in our study, which are inconsistence with other findings (Colmenero and Broderick, 2006) who observed, when degradable protein source increased in feed have no effect on milk lactose content (Varvikko *et al.,* 1999). However, another study reported that lactose helps in the regulation of milk osmolality and hence the volume of water passing into the mammary alveolus which resolves the volume of milk secreted (Spain *et al.,* 1990). While another study indicated that milk lactose concentration remains constant despite changes in the diet composition (Vanhatalo *et al.,* 1999).

In consistence to our findings Clark (1975) reported that high protein supplemented to the lactating cows, enhanced in protein streamed at the duodenum which results in high milk production (Kim *et al.,* 2001). Gulati *et al*. (2005) also reported that high protein feed improves milk production in animals. In contrast to our findings, another studies determined that dietary protein have no effect on milk production in dairy cows, when it increased from 16.5 to 18.5% and from 16.1 to 18.9%, respectively (AOAC, 1990; Kalscheur *et al.,* 2006). However, this increase in milk production may be due to high feed intake or may be the availability of high amount of the metabolizable protein and amino acids (Westwood *et al.,* 2000). However, degradable protein increased in milk production (Kim *et al.,* 2001). We do not know the precise mechanism, but can speculate that increase in the milk production in the present study might be due to high supply of amino acids to the mammary glands, or by increasing the glucogenesis in liver resulting in increased synthesis of lactose. While the indirect role may be reconciled during the change in the hormonal type, particularly better concentration of growth hormone which keeps foundation for division of nutrients in favor of milk production and growth and from fat deposition (Gulati *et al*., 2005). In this study we noticed that the high level of degradable protein in TMR may have increase the protein level in milk. These findings, are in agreement to another findings where they observed that the concentration of milk protein were obviously increase by the supplementation of protein in feed (Derrig *et al.,* 1974).

The protein supplements increased milk protein concentration (Orskov and Kay, 1977).

While this increased supply of dietary protein ultimately resulted in the high level of amino acids for synthesis of milk protein (Korhonen *et al.,* 2002). Inconsistence to our findings, another report indicated no obvious effect on milk protein concentration by increasing protein content in the diet (Varvikko *et al.,* 1999). However, Cunningham *et al*. (1996) reported that increase in the dietary protein significantly increased the SNF in milk. Rafieei (2011) reported that increase in ration protein resulted in the increase SNF in milk composition. Similar to our finding Schepers and Meijer (1998) reported that high level of MUN under the negative energy increase body protein metabolism. Saville and Rajala (2003) said that high level of different dietary protein source in the diet increases MUN because this caused lowering of rumen bacteria activity to utilize free ammonia. According to Sommer (1995) protein has a great relationship with MUN. The passive transfer of urea from blood may cause the MUN production. In the same way another study reported that there is high relation between MUN and Blood Urea Nitrogen (BUN) (Broderick and Clayton, 1997). The level of MUN and BUN may by affected by various factors. Which consist intake of crude protein, crude protein and energy disturbance between rumen un-degradable protein and RDP, imbalance between nonstructural carbohydrates RDP and diseases (Broderick and Clayton, 1997).

CONCLUSION

The present experiment was conducted to assess the relationship of degradability of different protein sources and its impact on milk composition and MUN. Hence, here we conclude that there was improvement in milk production, milk composition like SNF, milk lactose, milk protein and milk fat and also improvement in MUN. The following recommendations are made on the present work: The TMR along with different sources of degradable protein can be used in mid lactation period. Combination of different sources of concentrates may be used with different degradable protein sources. In future experiments may be designed to link the different degradable protein sources with reproductive parameters in dairy cattle.

REFERENCES

Annual Report the State of Pakistan Economy. Pakistan Economy Survey. 2010-2011. Government of Pakistan.

Afzal, M. 2016. Re-designing small holder dairy production in Pakistan. Pakistan Veterinary Journal, 30: 187-190.

Association of Official Analytical Chemist (AOAC). 1990. Official methods of analysis. (15^{th} Ed.) AOAC Arlingon, VA, 1990.

Broderick, G. A. and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentration of milk urea nitrogen. Journal of Dairy Science, 80 (11): 2964-2971.

Broderick, G. A. 2003. Effect of vary dietary protein and energy levels on the production of lactating dairy cows. Journal of Dairy Science, 86 (4): 1370-1381.

Castillo, A. R., E. Kebreab, D. E. Beever, J. H. Barbi, J. D. Sutton, H. C. Kirby and J. France. 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. Journal of Animal Science, 79 (1): 247-253.

Choung, J. J. and D. G. Clamberlain. 1992. Protein nutrition of dairy cows receiving grass silage diets. Effect on silage intake and milk production of postruminal supplement of casein or soya-protein isolate and the effects of intervenous infusions of a mixture of methionine, phenylalanine and tytophon. Journal of the Science of Food and Agriculture, 58: 307-314.

Christensen, R. L., G. L. Lynch, J. H. Clark and Y. Yu. 1993. Influence of amount and degradability of protein on production of milk and milk components by lactating Holstein cows. Journal of Dairy Science, 76 (11): 3490-3496.

Clark, J. H. 1975. Effect of amount and composition of feed given over three lactations on the performance of the dairy cows. Journal of Dairy Science, 4: 1170-1178.

Colmenero, C. J. J. and G. A. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. Journal of Dairy Science, 89 (5): 1704-1712.

Cunningham, K. D., M. J. Cecava, T. R. Johnon and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. Journal of Dairy Science, 79 (4): 620-630.

Derrig, R. G. J. H. Clark and C. L. Davis. 1974. Effect of degradability of crude protein and energy levels on the production of lactating cows. Journal of Nutrition, 2: 104-151.

Dhali, A., R. K. Mehla and S. K. Sirohi. 2005. Effect of urea supplemented and urea treated straw based diet on milk urea concentration in crossbred Karan Fries cows. Italian Journal of Animal Science, 4 (1): 25-34.

Dhali, A., D. P. Mishra, R. K. Mehla and S. K. Sirohi. 2006. Usefulness of milk urea concentration to monitor the herd reproduction performance in close breed Karan Fries cows. Asian-Aust. Journal of Animal Science, 1: 1926-1930.

Eastridge, M. L. 2006. Major advances in applied dairy cattle nutrition. Journal of Dairy Science, 89 (4): 1311-1323.

Gulati, K., M. R. Garg and T. W. Scott. 2005. Rumen protected protein and fat produced from oil seeds and meals by formaldehyde treatment their role in ruminant production and product quality. Australian Journal of Experimental Agriculture, 45 (10): 1189-1203.

Jonker, J. S., R. A. Kohn and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. Journal of Dairy Science, 81 (10): 2681-2692.

Kalscheur, K. F., R. L. Baldwin, B. P. Glenn and R. A. Kohn. 2006. Milk production of dairy cows fed different concentrations of rumen degradable protein. Journal of Dairy Science, 89 (1): 249-259.

Kim, C. H., J. H. Chonug and P. G. Chemberlain. 2001. Estimate of the efficiency of transfer of L. histidine from blood to milk when in the first limiting amino acid secretion of milk protein in dairy cows. Journal of the Science of Food and Agriculture, 81 (12): 1150-1155.

Korhonen, M., Vanhatalo, A. and Huhtanen, P., 2002. Effect of protein source on amino acid supply, milk production, and metabolism of plasma nutrients in dairy cows fed grass silage. Journal of Dairy Science, 85 (12): 3336-3351.

Kung, L. and J. T. Huber. 1983. Performance of high producing cows in early lactation fed protein of varying amount, source and degradability. Journal of Dairy Science, 66 (2): 227-234.

Oba, M., R. L. Baldwin, S. L. Owens and B. J. Bequette. 2004. Urea synthesis by ruminal epithelial and duodenal mucosal cell from growing sheep. Journal of Dairy Science, 87 (6): 1803-1805.

Orskov, R. E. and R. N. B. Kay. 1977. Effect of level of degradable protein during the second lactation period in Holstein Fresien dairy cows, feed intake and milk production. British Journal of Nutrition, 21: 381-397.

Rafieei, H. 2011. Responses of milk urea nitrogen content to dietary rumen degradable protein level in lactating Holstein dairy cows. Iranian Journal of Applied Animal Science, 1 (2): 111-116.

Santos, F. A., P. J. E. P. Santos, C. D. Theurer and J. T. Hubber. 1998. Effects of rumen degradable protein on dairy review. Journal of Dairy Science, 81 (12): 3182-3213.

Saville, W. J. A., P. J. Rajala-Schultz. 2003. Sources of variation in milk urea nitrogen in Ohio dairy herds. Journal of Dairy Science, 86 (5): 1653-1661.

Schepers, A. J., R. G. Meijer. 1998. Evaluation of the utilization of dietary nitrogen by dairy cows based on urea concentration in milk. Journal of Dairy Science, 81 (2): 579-584.

Spain, J. N., M. D. Alvarado, C. E. Polan, C. N. Miller and M. L. McCillard. 1990. Variation in milk output and milk protein in response to the level of degradable protein. Journal Dairy Science, 73: 445-448.

Varvikko, T., A. Vanhatalo, T. Jalava and P. Huhtanen. 1999. Lactation and metabolic responses to graded abomasal doses of methionine and lysine in dairy cows fed grass silage diets. Journal of Dairy Science, 82 (12): 2659-2673.

Westwood, C. T., I. T. Lean, J. K. Garvin and P. C. Wynn. 2000. Effect of genetic and varying dietary protein degradability on lactation in dairy cows. Journal of Dairy Science, 83 (12): 2926-2940.

(Accepted: March 03, 2019)