

## Effects of replacing soybean meal with corn gluten meal on milk production and nitrogen efficiency in Holstein cows

M. Imran<sup>1</sup>, M.Q. Shahid<sup>2</sup>, T.N. Pasha<sup>1</sup> & M.N. Haque<sup>#1</sup>

<sup>1</sup> Department of Animal Nutrition, University of Veterinary & Animal Sciences, Lahore 54000, Pakistan

<sup>2</sup> Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore 54000, Pakistan

(Received 12 September 2017; Accepted 2 May 2018; First published online 5 July 2018)

Copyright resides with the authors in terms of the Creative Commons Attribution 4.0 South African License.

See: <http://creativecommons.org/licenses/by/4.0/za>

Condition of use: The user may copy, distribute, transmit and adapt the work, but must recognize the authors and the South African Journal of Animal Science.

### Abstract

The objective of the current study was to determine the effects of partial replacement of soybean meal with corn gluten meal on lactation performance and nitrogen (N) efficiency in lactating Holstein dairy cows. Nine multiparous lactating cows in mid lactation ( $109 \pm 19$  days in milk) received three treatments in a  $3 \times 3$  Latin square design for 21 days. The three treatments consisted of i) Ctrl: low protein diet with 15.2% crude protein (CP); ii) SBM: soybean meal-based diet with 18.4% CP; and iii) CGM: soybean meal partially replaced with corn gluten meal with 18.3% CP. Two pre-planned orthogonal contrasts were used to compare the treatments: i) Prot compared Ctrl and average of SBM and CGM to see the effect of increasing protein supplies; and ii) Rep compared SBM and CGM treatments. Increasing CP supplies did not affect dry matter intake, whereas it significantly increased milk protein and lactose yield by 3.6% and 3.3%, respectively. Increasing CP supplies decreased milk nitrogen efficiency (MNE) by 10.9%. The milk yield, milk component yield, milk composition and feed efficiency were similar in the SBM and CGM treatments. Similarly, no difference was observed on dry matter, N and net energy for lactation ( $NE_L$ ) intakes between SBM and CGM treatments. However, MNE significantly decreased by 4.8% in the CGM treatment compared with SBM. The results indicated that soybean meal could be partially replaced with corn gluten meal without negatively affecting productive performance.

**Keywords:** Dairy cows, feed efficiency, nitrogen utilization, production performance, protein sources

<sup>#</sup> Corresponding author: [muhammad.naveed@uvas.edu.pk](mailto:muhammad.naveed@uvas.edu.pk)

### Introduction

Protein is considered the most expensive nutrient in the diet of a dairy cow (St-Pierre, 2012). Dietary protein plays an important role in the nutrition of dairy cattle by providing amino acids (AA) and nitrogen (N) to the rumen microbes for microbial protein synthesis. The protein balance in terms of supply versus requirements, and source of proteins used in the diet, plays a key role in total feed cost. Pakistan ranks fourth for milk production in the world and is among the countries with highest milk-to-feed price ratio (Hemme & Otte, 2010). Soybean meal is among the most common sources of protein in the diet of lactating dairy cows. It contains high content of rumen degradable protein (RDP), good profile of AA (high level of lysine (Lys), and high cell wall digestibility (INRA, 1988). In dairy cows, increasing protein supplies with soybean meal increased the dry matter intake (DMI), milk yield and milk protein contents (Rego *et al.*, 2008). However, the low levels of rumen undegradable protein (RUP), low Met/Lys ratio and highly variable prices of soybean meal validate the need to continuously evaluate the potential usage of other protein sources as its replacement. There is also competition for soybean meal between poultry and dairy animals. The high N efficiency in poultry compared with dairy cows, however, makes the use of soybean meal more economical for poultry compared with the dairy industry.

Soybean meal has previously been replaced partially or completely by peas (Liponi *et al.*, 2007; Vander Pol *et al.*, 2008), field beans (Liponi *et al.*, 2007; Tufarelli *et al.*, 2012), cassava pulp mixed with urea (Paengkoum & Bunnakit, 2009), corn gluten meal (Voss *et al.*, 1988; Holter *et al.*, 1992) and canola meal (Broderick *et al.*, 2015). Among these sources, corn gluten meal has the highest value of RUP because of

high concentration of prolamins and glutelins, and a higher Met/Lys ratio (Clark *et al.*, 1987), making it more suitable as a source that can be fed in combination with soybean meal in the diets of lactating cows. However, the results of replacing soybean meal with corn gluten meal are inconsistent. Previous studies showed that it increased milk production in lactating dairy cows (Fox *et al.*, 2004), decreased it (Wohlt *et al.*, 1991; Polan *et al.*, 1997), or showed no effect (Klusmeyer *et al.*, 1990). The increase observed in most of the studies was a result of higher RUP supplies (Cozzi & Polan, 1994), whereas the decrease was related to deficient RDP supplies or imbalance in the essential amino acid (EAA) profile (McCormick *et al.*, 2001). Nevertheless, the objectives of these studies were mostly to investigate the effects of increasing RUP supplies through corn gluten meal. Limited literature is available in which soybean was partially replaced with corn gluten meal without modifying the RUP/RDP ratio in the diets and net energy for lactation.

The authors hypothesized that partial replacement of soybean meal with corn gluten meal would result in similar milk production and DMI at the same crude protein (CP), RUP, and RDP levels in both diets. The objective of the study was therefore to determine the production response of mid lactating Holstein cows when corn gluten meal partially replaced soybean meal in the diet without modifying the RDP/RUP ratio.

## Materials and Methods

The experiment was carried out at Sharif Dairy Farms, Chiniot-Pakistan, from 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 were used in this study. The body weights, milk yield, and days in milk of these cows were  $555 \pm 42$  kg,  $34.0 \pm 3.7$  kg and  $109 \pm 19$  days (mean  $\pm$  standard deviation), respectively. The three treatments consisted of i) Ctrl, a low protein diet with 15.2% CP; ii) SBM, a soybean meal-based diet with 18.4% CP; and iii) CGM, a diet with 18.3% CP with corn gluten meal partially replacing soybean meal. The diets were iso-energetic and thus designed to provide similar energy (7.13 MJ NE<sub>L</sub> per kg). In the SBM diet, soybean meal was the main source of protein for the animals and in the CGM diet corn gluten meal replaced 34% of the soybean meal. Urea was used in the CGM treatment to balance the resultant RUP and RDP contents in the two treatments (Table 1). The metabolizable protein supplies were similar in the two treatments, that is, 2509 g/d and 2527 g/d in SBM and CGM treatments, respectively (Table 1). The diets were supplied according to a 3  $\times$  3 Latin square design with a 21-day period. The total duration of the experiment was 63 days, following a week of adaptation. The diets were formulated with 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 (Fox *et al.*, 2004). The feed was offered five times a day as total mixed ration (TMR) at 01:00, 05:00, 09:00, 13:00 and 17:00 hours and adjusted to yield 10% orts. The first seven days in each treatment were taken as the dietary adaptation period. Cows were fed individually and milked three times daily at 01:00, 09:00 and 17:00 hours. All animals had free access to clean water.

The quantities of diet and orts were weighed daily. Samples of corn silage and refusal were collected twice a week to determine the DM and adjust the TMR for changes in moisture content. The samples of individual ingredients of TMR (corn silage, alfalfa hay and concentrates) were analysed twice during experiment for chemical composition. These samples were collected, dried immediately in a forced-air oven at 60 °C for 48 hours (AOAC, 1980), and sent to the Animal Nutrition Laboratory, UVAS, for proximate analysis. Samples were analysed for CP, crude ash, ether extract (AOAC, 1980), gross energy (using bomb calorimeter) and neutral detergent fibre (NDF) (Van Soest, 1965).

Milk yield was recorded daily at each milking, and samples were collected every third day at each milking and assayed with an ultrasonic milk analyser (Ekomilk instrument; Eon Trading Inc, USA) to determine protein, fat and lactose contents.

On the third last day of each period, blood samples were collected from the jugular vein 30 minutes after milking and 15 minutes before feed distribution, and were immediately centrifuged (2000  $\times$  g for 15 min) to separate plasma from whole blood. Plasma samples, separated from whole blood, were sent to the Quality Operations Laboratory, UVAS. They were further processed for plasma urea nitrogen (PUN), blood glucose and triglycerides (TG) using kits (HUMAN Max-Planck-Ring 21 D 65205, Wiesbaden, Germany). Plasma urea was hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia from this reaction combined with 2-oxoglutarate and NADH in the presence of glutamate dehydrogenase to yield glutamate and NAD<sup>+</sup>. The decrease in 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 analyser Micro Lab 300 (ELITech Group 13-15 bis rue Jean Jaurès 92800 Puteaux-France).

The gross efficiency of metabolizable protein (MP) was calculated by dividing milk protein yield by MP intake. The metabolic efficiency of MP was calculated using following equation:

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

**Table 1** Percentage composition of ingredients and chemical composition of nutrients in experimental diets

Items	Ctrl <sup>1</sup>	SBM	CGM
DM (%)	49.8	49.8	49.8
<b>Ingredients (% of DM)</b>			
Corn silage	45.4	45.4	45.4
Alfalfa hay	8.62	8.61	8.60
Soybean meal	7.45	13.1	8.71
Corn distiller grains soluble	13.0	13.5	13.3
Palm kernel cake	4.78	4.78	4.59
Corn grain	2.65	1.99	2.69
Corn gluten meal 60%		0.79	3.12
Rapeseed meal	2.72	3.62	2.76
Rice polish	6.77	0.45	2.11
Sugarcane molasses	7.45	6.71	6.93
Urea			0.56
Megalac <sup>2</sup>	0.20	0.15	0.20
Sodium bicarbonate	0.50	0.50	0.51
Minerals and vitamins mix <sup>3</sup>	0.48	0.48	0.51
<b>Nutrient composition (% of DM)</b>			
Organic matter	91.7	92.0	93.0
CP	15.2	18.4	18.3
NDF	39.2	39.3	39.3
EE	4.54	3.87	4.06
NFC <sup>4</sup>	32.8	28.2	31.2
<b>Predicted values</b>			
ME <sup>5</sup> (MJ/kg)	11.1	11.1	11.1
NE <sub>L</sub> <sup>6</sup> (MJ/kg)	7.12	7.23	7.07
RUP (g/d)	1075	1495	1508
MP (g/d)	2337	2509	2527

DM: dry matter; CP: crude protein; NDF: neutral detergent fibre; EE: ether extract; RUP: rumen undegradable protein; MP: metabolizable protein

<sup>1</sup> Ctrl: low protein diet, CGM: corn gluten meal based high protein diet, SBM: soybean meal based high protein diet.

<sup>2</sup> Church & Dwight Co., Princeton, NJ

<sup>3</sup> Minerals and vitamins premix contained (per kilogram): 22% Ca, 12% P, 2.5% Mg, 2% Na, 500000 IU of vitamin A, 80000 IU of vitamin D3, 300 IU of vitamin E, 1000 mg of Fe, 600 mg of Cu, 3000 mg of Zn, 2000 mg of Mn, 10 mg of Co, 20 mg of I and 3 mg of Se

<sup>4</sup> NFC: non-fibre carbohydrates (100- (CP+ NDF+ crude ash+ crude fat)) (DePeters *et al.*, 2000)

<sup>5</sup> ME: calculated from the ingredients gross energy by using energy conversion calculator of University of California, Davis

<sup>6</sup> NE<sub>L</sub>: calculated from the ingredients ME by using energy conversion calculator of University of California, Davis

Milk nitrogen efficiency was calculated by dividing milk N yield by total N intake. The data of second and third weeks of every period were analysed using mixed procedure of SAS (2001) according to this statistical model:

$$Y_{ijk} = \mu + \text{Cow}_i + \text{Period}_j + \text{AA}_k + \varepsilon_{ijk}$$

Where  $\mu$  is the grand mean,  $\varepsilon$  is the random error. Cow was taken as random effect. Two pre-planned orthogonal contrasts were used to compare the treatments: i) Prot: Ctrl vs. others (average of CGM and SBM treatments): to compare the effect of increasing protein supplies in SBM and CGM treatment vs. Ctrl; ii) Rep: SBM vs. CGM: to compare the effect of replacement of soybean meal with corn gluten meal. The significance level was set at  $P \leq 0.05$  and the tendency was set at  $0.05 < P \leq 0.10$ .

## Results

Increasing the protein supplies in SBM and CGM diets did not change the DMI and the intakes of ME and  $NE_L$  (Table 2) (contrast Prot,  $P > 0.10$ ). However, increased protein supply (Ctrl vs. SBM and CGM) increased CP intake by 21.5%, and MP intake by 8.14% ( $P < 0.01$ ). The MP balance was slightly negative in Ctrl diet (-4.4 g/d), whereas it was positive in high protein diets (SBM = 122 g/d and CGM = 146 g/d). The ME balance decreased with increased protein supply (Table 2;  $P = 0.04$ ).

**Table 2** Dry matter intake, protein and energy balance in experimental diets fed to Holstein cows

Items	Treatments <sup>1</sup>			SEM	p value <sup>2</sup>		
	Ctrl	SBM	CGM		Treat	Prot	Rep
DMI (kg/d)	22.6	22.5	22.8	0.27	0.64	0.95	0.35
Protein intake (g/d)							
CP	3435	4140	4172	52.2	<0.01	<0.01	0.24
Nitrogen	549	662	667	8.33	<0.01	<0.01	0.30
MP	2335	2501	2525	28.6	<0.01	<0.01	0.39
Energy intake (MJ/d)							
ME <sup>3</sup>	216	212	215	2.55	0.30	0.21	0.35
NE <sub>L</sub> <sup>4</sup>	138	136	138	1.63	0.30	0.21	0.35
Balance							
MP balance <sup>5</sup> (g/d)	-4.4	122	146	25.8	<0.01	<0.01	0.35
ME balance <sup>6</sup> (MJ/d)	16.2	9.58	12.5	4.89	0.08	0.04	0.35

SEM: standard error of mean; DMI: dry matter intake; CP: crude protein; MP: metabolizable protein

<sup>1</sup> Ctrl: low protein diet, CGM: corn gluten meal based high protein diet, SBM: soybean meal based high protein diet.

<sup>2</sup> p value, probability, corresponding to the null hypothesis with Prot and Rep contrasts.

<sup>3</sup> ME: calculated from the ingredients gross energy by using energy conversion calculator of University of California, Davis.

<sup>4</sup> NE<sub>L</sub>: calculated from the ingredients ME by using energy conversion calculator of University of California, Davis.

<sup>5</sup> MP balance = MP intake – MP requirement.

<sup>6</sup> ME balance = ME intake – ME requirement.

No effect was observed on DM and nutrient intake including protein (CP, N and MP), and energy (ME and NE<sub>L</sub>) when soybean meal was partially replaced with corn gluten meal (Table 2) (contrast Rep,  $P > 0.10$ ). Similarly, MP and ME balance were not modified (Table 2) (contrast Rep,  $P > 0.10$ ).

Increasing the dietary protein supplies in SBM and CGM treatments tended to increase milk yield by 3% compared with Ctrl treatment (Table 3) (contrast Prot  $P = 0.07$ ). Similarly, increasing the protein supply of the diet increased milk protein yield by 3.6% ( $P = 0.03$ ) and lactose yield by 3.3% ( $P = 0.04$ ) compared with the Ctrl, respectively. No effect was observed on milk fat yield (contrast Prot,  $P = 0.16$ ). Partially

replacing soybean meal with corn gluten meal had no effect on milk yield, milk component yield, and milk composition (contrast Rep,  $P > 0.10$ ).

**Table 3** Effects of replacement of soybean meal with corn gluten meal on milk production and composition in Holstein cows

Items	Treatments <sup>1</sup>			SEM	p value <sup>2</sup>		
	Ctrl	SBM	CGM		Treat	Prot	Rep
Milk yield (kg/d)	30.3	31.2	31.2	0.58	0.20	0.07	0.90
Milk components yield (g/d)							
Fat	1090	1143	1136	39.6	0.36	0.16	0.87
Protein	970	1003	1005	17.9	0.10	0.03	0.90
Lactose	1408	1453	1456	25.6	0.11	0.04	0.11
Milk composition (%)							
Fat	3.60	3.68	3.63	0.102	0.69	0.51	0.59
Protein	3.20	3.22	3.21	0.009	0.19	0.08	0.25
Lactose	4.65	4.67	4.66	0.016	0.53	0.29	0.69

SEM; standard error of mean

<sup>1</sup> Ctrl: low protein diet, CGM: corn gluten meal-based high protein diet, SBM: soybean meal-based high protein diet.

<sup>2</sup> p value, probability, corresponding to the null hypothesis with Prot and Rep contrasts

The results of feed and N efficiencies are presented in Table 4. Increasing the dietary protein supplies increased feed efficiency in SBM and CGM treatments compared with Ctrl (contrast Prot,  $P = 0.04$ ). However, the gross and metabolic efficiencies of MP and MNE decreased ( $P \leq 0.01$ ) by 4.8%, 9.8% and 10.9%, in high protein diets (SBM and CGM), respectively, compared with Ctrl. Feed efficiency, gross and metabolic efficiencies of MP were not affected by partial replacement of soybean meal with corn gluten meal (contrast Rep,  $P > 0.10$ ). However, MNE was decreased by 5% in CGM treatment compared with SBM (contrast Rep  $P = 0.03$ ).

**Table 4** Effects of replacement of soybean meal with corn gluten meal on feed and nitrogen efficiencies in Holstein cows

Items	Treatments <sup>1</sup>			SEM	p value <sup>2</sup>		
	Ctrl	SBM	CGM		Treat	Prot	Rep
Feed efficiency <sup>3</sup>	1.34	1.39	1.37	0.022	0.09	0.04	0.50
Gross efficiency MP <sup>4</sup>	0.42	0.40	0.40	0.006	0.03	0.01	0.50
Metabolic efficiency MP <sup>5</sup>	0.71	0.65	0.64	0.013	<0.01	<0.01	0.44
Milk N efficiency <sup>6</sup>	0.23	0.21	0.20	0.037	<0.01	<0.01	0.03

SEM; standard error of the mean

<sup>1</sup> Ctrl: low protein diet, CGM: corn gluten meal based high protein diet, SBM: soybean meal based high protein diet.

<sup>2</sup> p value, probability, corresponding to the null hypothesis with Prot and Rep contrasts

<sup>3</sup> Feed efficiency: milk yield/dry matter intake

<sup>4</sup> Gross efficiency MP: milk protein yield/MP intake

<sup>5</sup> Metabolic efficiency MP: milk protein yield/MP intake – (MP for growth + MP for maintenance + MP for pregnancy).

<sup>6</sup> Milk N efficiency: N in milk/N intake.

As shown in Table 5, neither increasing the protein content of the diet nor partially replacing soybean meal with corn gluten meal had any effect ( $P > 0.10$ ) on PUN, plasma glucose, and TG.

**Table 5** Effects of replacement of soybean meal with corn gluten meal on urea, triglycerides and glucose concentrations in serum (mg/dL)

Items	Treatments <sup>1</sup>			SEM	p value <sup>2</sup>		
	Ctrl	SBM	CGM		Treat	Prot	Rep
PUN	18.3	21.0	21.1	2.23	0.60	0.32	0.96
Glucose	61.7	67.1	60.7	4.26	0.31	0.56	0.16
TG	11.1	11.0	9.09	2.15	0.60	0.58	0.40

SEM: standard error of mean; PUN: plasma urea nitrogen; TG: triglycerides.

<sup>1</sup> Ctrl: low protein diet; CGM: corn gluten meal-based high protein diet; SBM: soybean meal-based high protein diet.

<sup>2</sup> p value, probability, corresponding to the null hypothesis with Prot and Rep contrasts.

## Discussion

The objective of the current study was to investigate the effects on milk yield, milk composition and MNE of partially replacing soybean meal with corn gluten meal. The dietary treatments were designed to provide similar energy supplies.

In the present study, the lack of DMI response to the increasing dietary protein supply was similar to other studies, in which protein supplies were increased through diets or infusions in the intestine (Cyriac *et al.*, 2008). Contrary to the current findings, studies conducted by Faverdin *et al.* (2003) and Metcalf *et al.* (2008) showed an increase in DMI by increasing protein supplies. The duration of the experimental period (21 days) in the current study might not be sufficient to observe any clear response on DMI (Cyriac *et al.*, 2008). Responses to altered protein supply require time to manifest owing to the buffering effect of labile protein reserves, particularly when the CP of the diets is relatively high ( $\geq 15\%$ ) and they have small differences between them. Usually more than four weeks is adequate to test any potential deficiency (labile Histidine reserves of intramuscular carnosine and anserine, dipeptides and circulating haemoglobin) of protein in dairy cows (Kröber *et al.*, 2000; Hristov & Giallongo, 2014). Moreover, the small increase in MP supplies in the current study (Ctrl vs. SBM and CGM treatments: 7.62%) compared with higher increases (37% and 57%) in studies of Faverdin *et al.* (2003) and Metcalf *et al.* (2008), respectively, might explain the lack of response in DMI.

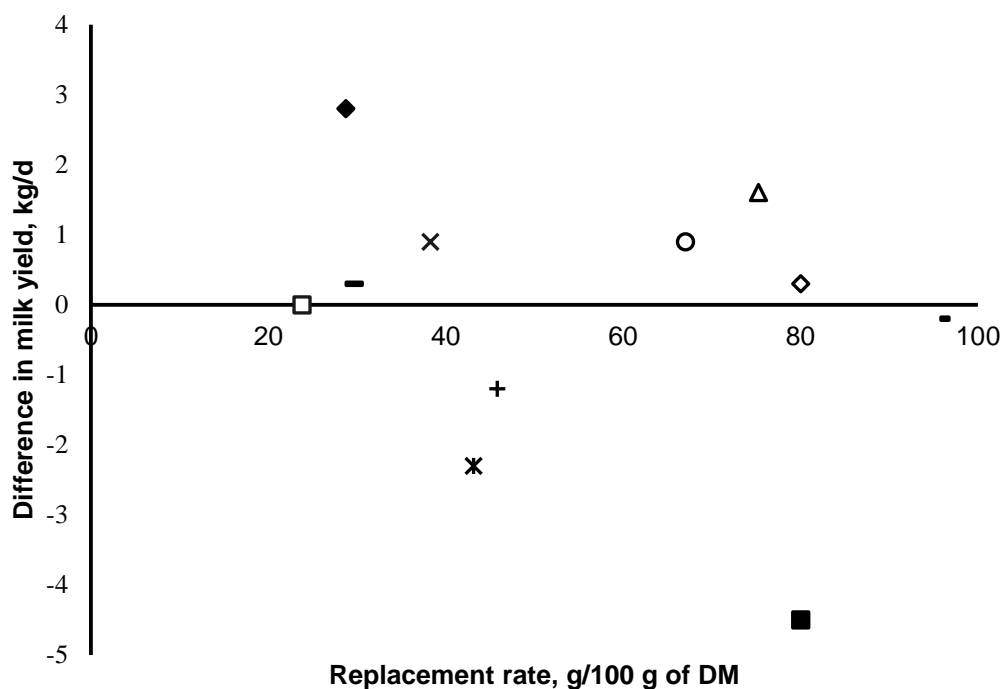
Dry matter intake remained unaffected when soybean meal was partially replaced with corn gluten meal, which was in agreement with the literature, in which soybean meal was partially or completely replaced with corn gluten meal (Robinson *et al.*, 1998; McCormick *et al.*, 2001). Contrary to this, studies indicated a decrease (Polan *et al.*, 1997) and an increase (Cozzi & Polan, 1994) in DMI. The decrease in DMI in the study of Polan *et al.* (1997) could be owing to the use of a blend of CGM with poultry by-product blood and feather meal. The increased DMI in the study of Cozzi & Polan (1994) could be related to the change in the site of protein digestion (rumen vs. small intestine) owing to increased RUP supplies, as described by Faverdin *et al.* (2003). However, the current authors observed no such response, mainly because RUP and RDP supplies were similar in the two diets (CGM and SBM). All experimental diets were formulated to supply a similar amount of energy (as shown in Table 1). However, ME balance was lower in the high protein diets compared with Ctrl. The MP/NE<sub>L</sub> ratio in the current diets was increased in the SBM and CGM treatments (see Table 2), resulting in higher utilization of energy for milk protein synthesis by the mammary glands.

Increased protein supply in the CGM and SBM diets tended to increase milk yield compared with Ctrl, which was in agreement with Imran *et al.* (2017). Increased protein and lactose yields observed in CGM and SBM diets compared with Ctrl were in agreement with the findings of Imran *et al.* (2017).

In the present study, the authors observed no effect of partially replacing soybean meal with corn gluten meal on milk yield. Previous studies showed that it increased milk production in lactating dairy cows (Cozzi & Polan, 1994; Fox *et al.*, 2004;), decreased it (McCormick *et al.*, 2001), or showed no effect (Klusmeyer *et al.*, 1990).

The increased RUP (CGM and SBM treatments) had RUP 42.3% vs. 34.3% of CP, respectively) and MP supplies might explain the increased milk yield in the study of Cozzi & Polan (1994). Moreover, the treatments were not iso-nitrogenous (Cozzi & Polan, 1994). On the other hand, the possible explanation for a decrease was insufficient energy supplies required for efficient protein utilization (McCormick *et al.*, 2001). The variation in milk yield response might also be the effect of variations in DMI in these studies. The changes in milk production were related more to variations in DMI (or, in other words, the supply of MP). DMI was unaffected in the studies of Wohlt *et al.* (1991) and Klusmeyer *et al.* (1990), whereas it increased in the

study of Cozzi & Polan (1994) and decreased in the studies of Polan *et al.* (1991) and McCormick *et al.* (2001). To further research the effect of replacement rate on milk yield, the authors regrouped the studies. The basal diets in those studies were composed principally of corn silage (Cozzi & Polan, 1994; McCormick *et al.*, 2001), oat pasture (McCormick *et al.*, 2001) and alfalfa silage (Robinson *et al.*, 1991). Figure 1 shows no relationship between replacement rate and the change in milk production. It was reported that high soybean meal and high corn gluten meal diets had imbalanced Lys/Met ratio compared with the ideal (2.9:1) (Rulquin *et al.*, 1993). This could explain the lack of response on milk in studies replacing soybean meal with corn gluten meal at higher rates. In the current study, SBM treatment was even more imbalanced in the Lys/Met ratio compared with Ctrl and CGM. Hence the authors observed no difference in milk yield between the CGM and SBM treatments. This also indicates that strategy of balancing the EAA profile of diets through rumen protected AA could be used for improving milk response. Nevertheless, the objective of this study was to quantify only the effects of replacement of protein sources on an iso-nitrogenous basis without modifying the EAA profile.



Symbols correspond to the data from following studies:  $\diamond$  Klusmeyer *et al.* (1990);  $\blacksquare$  Polan *et al.* (1991);  $\triangle$  Wohlt *et al.* (1991);  $\times$  Cozzi & Polan (1994);  $\star$  Keery & Amos (1993);  $\circ$  Korhonen *et al.* (2002);  $+$  McCormick *et al.* (2001);  $\blacksquare$  Robinson *et al.* (1991);  $-$  Seymour *et al.* (1990);  $\blacklozenge$  Wheeler *et al.* (1995);  $\square$  Current study

**Figure 1** Change in milk production, kg/d (y-axis) in response to the replacement (x-axis) of soybean meal with corn gluten meal (g/100g DM) in the literature

In the present study, no effect was observed on milk component yield. Not only did milk volume remain unchanged, but the milk composition remained unaffected owing to the similar supplies of RDP and RUP in the two diets. As described earlier, when soybean meal was replaced with corn gluten meal, milk protein yield increased (Cozzi and Polan, 1994), decreased (Polan *et al.*, 1991; McCormick *et al.*, 2001), or remained unaffected (Seymour *et al.*, 1990; Robinson *et al.*, 1998). However, only a few studies reported a change in milk protein concentration owing to the addition of corn gluten meal. The studies of Wohlt *et al.* (1991) and Klusmeyer *et al.* (1990) showed a negative effect on milk protein contents when corn gluten meal replaced 75% or more soybean meal. No effect on milk protein contents was observed in diets in which corn gluten meal replaced less than 70% of the soybean meal (Seymour *et al.*, 1990; Keery & Amos, 1993). Again, if levels of corn gluten meal are too high, this may decrease Lys concentration, which may consequently decrease milk protein concentration (Polan *et al.*, 1991).

The increased protein supplies decreased the efficiency of conversion of dietary N into milk by 10.9% in the current study (see Table 4). Previous studies reported a decrease in MNE when dietary CP was increased from 12% to 16% (Cozzi & Polan, 1994), from 14.6% to 18.3% (Broderick & Reynal, 2009) and from 15.6% to 18% (Broderick *et al.*, 2008). The efficiency of utilization of dietary N for milk protein synthesis depends on the protein to energy ratio (MP/NE<sub>L</sub>) and declines with an increasing MP/NE<sub>L</sub> (Hof *et al.*, 1994). The diets in the current study were not balanced for EAA. It could be possible that the absorbed AA that were not utilized for the synthesis of milk proteins were de-aminated by the liver to urea, which ultimately reduced the MNE in high protein diets (Wohlt *et al.*, 1991). The numerically high PUN observed in the SBM and CGM diets compared with the Ctrl diet could explain this mechanism. Milk urea nitrogen (MUN) is correlated with the PUN and can be estimated through the equation (milk urea nitrogen = 0.620 × PUN + 4.75) derived by Broderick & Clayton (1997). The average MUN value calculated from the PUN value in the current experiment was 17.2 mg/dL (Ctrl = 16.1, SBM = 17.8, and CGM = 17.8 mg/dL), which was above the range of 10 to 16 mg/dL that is recommended by some researchers (Jonker *et al.*, 1999; Wattiaux *et al.*, 2005). The recommended range of MUN (10 to 16 mg/dL) was particularly associated with ration CP less than 17%. The researchers from University of Wisconsin (Wattiaux *et al.*, 2005) suggested that for each 1% increase in CP there was a 2 mg/dL increase in MUN when the diet contained 15% to 18.5% CP. In the current experiment, the average CP of the diets was 17.3%, which corresponded with the MUN value above the recommended range. In the current study, partial replacement of soybean meal with corn gluten meal decreased MNE, which was in agreement with the study conducted by Klusmeyer *et al.* (1990), and contrary to the findings of Wohlt *et al.* (1991) in which they reported no change. This change in MNE was due mainly to a slightly higher N supply in the CGM as opposed to the SBM treatment. However, because the authors designed the diets to provide similar MP supplies, the gross and metabolic MP efficiencies remained unaffected between the CGM and SBM treatments. Partial replacement of soybean meal with corn gluten meal did not change feed efficiency, which was in agreement with the findings of Robinson *et al.* (1998) in which soybean meal was partially or completely replaced with corn gluten meal. Contrary to this, Keery & Amos (1993) found increased feed efficiency from 1.8 to 1.9 owing to decreased DMI when soybean meal was completely replaced with heat-treated soybean meal plus corn gluten meal.

## Conclusions

In conclusion, milk yield and milk component yield (except milk fat) increased as the dietary protein increased from 15.4% to 18.3% of DM. Corn gluten meal could partially replace soybean meal without negatively affecting milk yield and composition under iso-nitrogenous, iso-caloric, and balanced RUP and RDP diets.

## Acknowledgements

This project was funded by Sharif Dairy Farms, Chiniot and UVAS, Lahore. The authors are grateful to the management of Sharif Dairy Farms for providing animals and for support at the farm in conducting the research trial. They also thank to the University Diagnostic Laboratory, Quality Operations Laboratory and Animal Nutrition Laboratory, UVAS, for giving financial and technical support for blood and feed analyses.

## Authors' Contributions

MI and MNH conceived, designed and performed the experiment. MI, TNP and MNH supervised the experiment including laboratory analysis related to feed and blood parameters. MI and MNH analysed the data and contributed to the writing of the manuscript. MQS carried out critical reading and drafting of the manuscript. All the authors agreed with the final version to be submitted.

## Conflict of Interest Declaration

The authors certify that they have no affiliations with any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this manuscript.

## References

- AOAC, 1980. Official methods of analysis. 13th edition. AOAC. Benjamin Franklin Station, Washington DC.
- Broderick, G.A. & Clayton, M.K., 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. *J. Dairy Sci.* 80, 2964-2971.
- Broderick, G.A. & Reynal, S.M., 2009. Effect of source of rumen degraded protein on production and ruminal metabolism in lactating dairy cows. *J. Dairy Sci.* 92, 2822-2834.
- Broderick, G.A., Faciola A.P. & Armentano L.E., 2015. Replacing dietary soybean meal with canola meal improves production and efficiency of lactating dairy cows. *J. Dairy Sci.* 98, 5672-5687.
- Broderick, G.A., Stevenson, M.J., Patton, R.A., Lobos, N.E. & Olmos Colmenero, J.J., 2008. Effect of supplementing rumen-protected methionine on production and nitrogen excretion in lactating dairy cows. *J. Dairy Sci.* 91, 1092-1102.



- Clark, J., Murphy, M. & Crooker, B., 1987. Supplying the protein needs of dairy cattle from by-product feeds. *J. Dairy Sci.* 70, 1092-1109.
- Cozzi, G. & Polan, C., 1994. Corn gluten meal or dried brewers grains as partial replacement for soybean meal in the diet of Holstein cows. *J. Dairy Sci.* 77, 825-834.
- Cyriac, J., Rius, A.G., McGilliard, M.L., Pearson, R.E., Bequette, B.J. & Hanigan, M.D., 2008. Lactation performance of mid-lactation dairy cows fed ruminally degradable protein at concentrations lower than National Research Council recommendations. *J. Dairy Sci.* 91, 4704-4713.
- DePeters, L.E., Fadel, J.G., Arana, M.J., Ohanesian, N., Etchebarne, M.A., Hamilton, C.A., Hinders, R.G., Maloney, M.D., Old, C.A., Riordan, T.J., Perez-Monti, H. & Pareas, J.W., 2000. Variability in the chemical composition of 17 selected byproduct feedstuffs used by the California dairy industry. *Profess. Anim. Sci.* 19, 3.
- Faverdin, P., M'hamed, D. & Vérité, R., 2003. Effects of metabolizable protein on intake and milk production of dairy cows independent of effects on ruminal digestion. *Anim. Sci.* 76, 137-146.
- Fox, D.G., Tedeschi, L.O., Tylutki, T.P., Russell, J., Van Amburgh, M.E., Chase, L.E., Pell, A.N. & Overton, T.R., 2004. The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. *Anim. Feed Sci. Technol.* 112, 29-78.
- Hemme, T. & Otte, J., 2010. Status of and prospects for smallholder milk production—A global perspective, Rome: FAO.
- Hof, G., Tamminga, S. & Lenaers, P.J., 1994. Efficiency of protein utilization in dairy cows. *Livest. Prod. Sci.* 38, 169-178.
- Holter, J., Hayes, H., Urban, W., Ramsey, S. & Rideout, H., 1992. Response of Holstein cows to corn gluten meal used to increase undegradable protein in early or later lactation. *J. Dairy Sci.* 75, 1495-1506.
- Hristov, A.N. & Giallongo, F., 2014. Feeding protein to dairy cows – What should be our target? *Proc. Tri-State Dairy Nutr. Conference.* Fort Wayne, IN, pp. 75-84.
- Imran, M., Pasha, T.N., Shahid, M.Q., Babar, I. & Naveed Ul Haque, M., 2017. Effect of increasing dietary metabolizable protein on nitrogen efficiency in Holstein dairy cows. *Asian-Australas. J. Anim. Sci.* 30, 660-665.
- INRA, 1988. *Alimentation des bovins, ovins et caprins*, INRA, Paris.
- Jonker, J.S., Kohn, R.A. & Erdman, R.A., 1999. Milk urea nitrogen target concentrations for lactating dairy cows fed according to National Research Council recommendations. *J. Dairy Sci.* 82, 1261-1273.
- John, M., 2005. *Tropical dairy farming: Feeding management for smallholder dairy farmers in the humid tropics.* Landlinks Press, Australia. 312 pp.
- Keery, C. & Amos, H., 1993. Effects of source and level of undegraded intake protein on nutrient use and performance of early lactation cows. *J. Dairy Sci.* 76, 499-513.
- Klusmeyer, T., McCarthy, R., Clark, J. & Nelson, D., 1990. Effects of source and amount of protein on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. *J. Dairy Sci.* 73, 3526-3537.
- Korhonen, M., Vanhatalo, A. & Huhtanen, P., 2002. Evaluation of isoleucine, leucine, and valine as a second-limiting amino acid for milk production in dairy cows fed grass silage diet. *J. Dairy Sci.* 85, 1533-1545.
- Kröber, T.F., Külling, D.R., Menzi, H., Sutter, F. & Kreuzer, M., 2000. Quantitative effects of feed protein reduction and methionine on nitrogen use by cows and nitrogen emission from slurry. *J. Dairy Sci.* 83, 2941-2951.
- Liponi, G., Casini, L., Martini, M. & Gatta, D., 2007. Faba bean (*Vicia faba minor*) and pea seeds (*Pisum sativum*) as protein sources in lactating ewes' diets. *Ital. J. Anim. Sci.* 6, 309-311.
- McCormick, M., Ward, J., Redfearn, D., French, D., Blouin, D., Chapa, A. & Fernandez, J., 2001. Supplemental dietary protein for grazing dairy cows: Effect on pasture intake and lactation performance. *J. Dairy Sci.* 84, 896-907.
- Metcalf, J.A., Mansbridge, R.J., Blake, J.S., Oldham, J.D. & Newbold, J.R., 2008. The efficiency of conversion of metabolisable protein into milk true protein over a range of metabolisable protein intakes. *Animal* 2, 1193-1202.
- Paengkoum, P. & Bunnakit, K., 2009. Replacement of soybean meal with cassava pulp mixed with urea gelatinizes (caspurea) in concentrate diets of beef cattle. *Agric. J.* 4, 242-249.
- Polan, C., Cozzi, G., Berzaghi, P. & Andrighetto, I., 1997. A blend of animal and cereal protein or fish meal as partial replacement for soybean meal in the diets of lactating Holstein cows. *J. Dairy Sci.* 80, 160-166.
- Polan, C.E., Cummins, K., Sniffen, C., Muscato, T., Vicini, J., Crooker, B., Clark, J., Johnson, D., Otterby, D. & Guillaume, B., 1991. Responses of dairy cows to supplemental rumen-protected forms of methionine and lysine. *J. Dairy Sci.* 74, 2997-3013.
- Rego, O.A.D., Regalo, S., Rosa, H.J.D., Alves, S., Borba, A.E.S.d., Bessa, R.J.B., Cabrita, A.R. & Fonseca, A., 2008. Effects of grass silage and soybean meal supplementation on milk production and milk fatty acid profiles of grazing dairy cows. *J. Dairy Sci.* 91, 2736-2743.
- Robinson, P., McQueen, R. & Burgess, P., 1991. Influence of rumen undegradable protein levels on feed intake and milk production of dairy cows. *J. Dairy Sci.* 74, 1623-1631.
- Robinson, P.H., Chalupa, W., Sniffen, C.J., Julien, W.E., Sato, H., Watanabe, K., Fujieda, T. & Suzuki, H., 1998. Ruminally protected lysine or lysine and methionine for lactating dairy cows fed a ration designed to meet requirements for microbial and post-ruminal protein. *J. Dairy Sci.* 81, 1364-1373.
- Rulquin, H., Pisulewski, P.M., Vérité, R. & Guinard, J., 1993. Milk production and composition as a function of post-ruminal lysine and methionine supply: a nutrient-response approach. *Livest. Prod. Sci.* 37, 69-90.
- SAS, 2001. *SAS System for Windows Release 8.2 (TS2M0)*, SAS Institute Inc, Cary, NC, USA.
- Seymour, W.M., Polan, C.E. & Herbein, J.H., 1990. Effects of dietary protein degradability and casein or amino acid infusions on production and plasma amino acids in dairy cows. *J. Dairy Sci.* 73, 735-748.
- St-Pierre, N.R., 2012. The cost of nutrients, comparison of feedstuffs prices and the current dairy situation. *Buckeye Dairy News* 14.

- Tufarelli, V., Khan, R.U. & Laudadio, V., 2012. Evaluating the suitability of field beans as a substitute for soybean meal in early lactating dairy cow: Production and metabolic responses. *Anim. Sci. J.* 83(2), 136-140.
- Vander Pol, M., Hristov, A., Zaman, S. & Delano, N., 2008. Peas can replace soybean meal and corn grain in dairy cow diets. *J. Dairy Sci.* 91, 698-703.
- Van Soest, P.J., 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: voluntary intake in relation to chemical composition and digestibility. *J. Anim. Sci.* 24, 834-843.
- Voss, V., Stehr, D., Satter, L. & Broderick, G., 1988. Feeding lactating dairy cows proteins resistant to ruminal degradation. *J. Dairy Sci.* 71, 2428-2439.
- Wattiaux, M.A., Nordheim, E.V. & Crump, P., 2005. Statistical evaluation of factors and interactions affecting dairy herd improvement milk urea nitrogen in commercial midwest dairy herds. *J. Dairy Sci.* 88, 3020-3035.
- Wheeler, J.G., Amos, H.E., Froetschel, M.A., Coomer, J.C., Maddox, T. & Fernandez, J.M., 1995. Responses of early lactation cows fed winter and summer annual forages and undegradable intake protein. *J. Dairy Sci.* 78, 2767-2781.
- Wohlt, J., Chmiel, S., Zajac, P., Backer, L., Blethen, D. & Evans, J., 1991. Dry matter intake, milk yield and composition, and nitrogen use in Holstein cows fed soybean, fish, or corn gluten meals. *J. Dairy Sci.* 74, 1609-1622.