



ABSTRACT

This experiment was performed to investigate whether feeding xylose-treated soybean meal (XSBM) or corn gluten meal (CGM) is economically better under field conditions. Ninety-four multiparous early lactating Holstein cows (55±5 days in milk and 2.82±0.41 body condition score) were used in a completely randomized design. Cows were randomly assigned to one of two treatments: XSBM or CGM as the main rumen undegradable protein source in the diet. Dry matter intake (DMI), milk yield and fat corrected milk (FCM) were not affected by treatments. A tendency was detected for higher FCM: DMI (1.57 *vs.* 1.47) and income:cost (1.97 *vs.* 1.83) in CGM treatment. Milk urea nitrogen was greater (15.89 *vs.* 15.16 mg/dL) in the XSBM group. Plasma glucose, non-esterified fatty acid, beta-hydroxy butyric acid and albumin were not different between treatments. The results showed that under field conditions and feed ingredients used in this experiment, using CGM was economically beneficial during early lactation, which is in contrast with traditional beliefs.

KEY WORDS benefit, corn gluten meal, early lactation, performance, treated soybean meal.

INTRODUCTION

Feeding high crude protein (CP) diets to ensure a sufficient supply of the metabolizabale protein (MP) required for maximum milk and protein production of dairy cows is a common procedure throughout the world. However, overfeeding CP reduces profit margins because of the relatively high cost of protein supplements and the poor efficiency of N use by dairy cows fed with high protein diets (Broderick, 2003; Ghelich Khan *et al.* 2010). It seems that rumen degradable protein (RDP) requirement of dairy cattle has been overestimated by NRC (Huhtanen and Hirstov, 2009), and despite the fact that increasing diet RDP can linearly increase microbial protein synthesis (Broderick *et al.* 2010), milk protein secretion occurs at less than maximum microbial protein synthesis (Reynal and Broderick, 2005). Hence, microbial protein synthesis should be "optimized" rather than "maximized" and this may imply more rumen undegradable protein (RUP) inclusion in the diets. Early lactation cows are in negative N balance (Lapierre *et al.* 2007) and our research studies also have favored of feeding high protein diets immediately postpartum (both RDP and RUP; Ghelich Khan *et al.* 2010; Amir-Abadi, 2010). Regarding to environmental pollution and N efficiency, however, it appears that RUP use would be more beneficial (Jahani-Moghadam *et al.* 2009). In a recent study, we showed that with decreasing RDP:RUP from 11.6:5.2 to 8.5:8.1, milk yield and protein content increased, but high RUP diets were not economical. We also showed that a RDP:RUP of 10.6:6.1 and of 9.5:7.1 are economically efficient. In addition, Olmos Colmenero and Broderick (2006) showed that diets containing 16.5% CP support maximal production in dairy cows with minimal N excretion to the environment compared with diets with higher CP content.

Thus, we decided to conduct an experiment with a relatively moderate RUP level, similar to our previous trial, and under farm conditions. Soy proteins are highly valued in animal nutrition because they are rich in lysine (Lys; NRC, 2001). Soybean meal (SBM) has the highest intestinal digestibility among the feedstuffs (NRC, 2001), but it is poor in methionine (Met) content, the most limiting amino acid (AA) in the dairy cows rations. Soy bean meal processing with xylose can increase its RUP content (Cleale *et al.* 1987; Nakamura *et al.* 1992; Ipharraguerre *et al.* 2005). Soy products have been suggested to have the AA profile that best resembles the milk protein (Santos *et al.* 1998; NRC, 2001). Corn gluten meal is also a good source of RUP (Met) and energy (NRC, 2001).

It has been shown that ruminal outflow of nonammonia, nonmicrobial N (NANMN) is higher in corn RUP diets than soy RUP diets (Ipharraguerre and Clark, 2005), although Reynal *et al.* (2007) showed that NANMN was higher for XSMB compared to CGM. What is more, Ipharraguerre and Clark (2005) found ruminal essential AA and Met outflow to the small intestine were greater in corn RUP diets. Milk production responses to soy RUP supplementation have been greater compared to corn RUP diets (Ipharraguerre and Clark, 2005). We hypothesized that part of this difference would be explained by the source of CP in the control diets and conditions in which the experiment was conducted.

Each of these RUP sources has its own special features that others do not have. Also, Tempelman (2009) noted that we should move towards conducting experiments at commercial farms to attain more applicable results. To our knowledge, there is no experiment that has compared between soy and corn RUP under commercial and practical circumstances. Thus, the objective of this trial was to compare between 2 commonly used RUP sources in Iran and at the best economically level obtained in our previous experiment on productive performance of lactating dairy cows.

MATERIALS AND METHODS

Animal and experimental design

This experiment lasted 50 d and was done in a commercial farm (Talise Asl-e-Jahan) with 1000 lactating dairy cows with an average milk yield of 33 kg/cow. Ninety-four multiparous early lactating Holstein cows (55 \pm 5 days in milk (DIM) and 2.82 \pm 0.41 body condition score (BCS)) were used in a completely randomized design.

The cows were randomly assigned to one of 2 treatments: xylose-treated soy bean meal (XSBM, Yasna Mehr Co. Shams Abad Industrial City, Hasan Abad, Qom Highway, Tehran, Iran), or corn gluten meal (CGM) as the main sources of RUP in the diets. Cows were housed in a free-stall barn with a slatted floor and were group fed *ad libitum* as a total mixed ration (TMR), twice daily at 0700 and 1500 h to achieve 5% orts. Each barn had a separate feed bunk and trough. The amount of offered and refused diet was measured weekly (a total of 7 times) to calculate the group DMI. The chemical composition of dietary ingredients of the two diets is presented in Table 1.

Ingredients (% of DM)	XSBM	CGM
Legume forage	21.4	21.4
Corn silage	23.3	23.3
Barley grain	11.1	11.1
Corn grain	10.7	10.7
Wheat grain	3.8	3.8
Cottonseed whole	5.5	5.5
Canola meal	3.7	3.7
Soybean meal	8.7	10.1
Yasmino (max soy) ¹	3.9	0
Corn gluten meal	0	2.5
Fish meal	0.7	0.7
Calcium soaps of fat ²	1.8	1.8
Salt	0.3	0.3
Sodium bicarbonate	1.1	1.1
Calcium carbonate	1	1
Bentonite	2.2	2.2
Vitamin and mineral supplement ³	0.4	0.4
Di-calcium phosphate	0.4	0.4
Chemical composition		
DM (%)	44.2	44.5
NE_{L} (Mcal/kg) ⁴	1.59	1.59
CP (%)	17	17.2
RDP $(\%)^4$	10.2	10.6
RUP (%) ³	6.8	6.6
Lys:Met ratio ⁴	3.61	3.29
NDF (%)	31	30.3
ADF (%)	19.7	19.6
NFC (%)	39.3	38.8
$EE(\%)^{3}$	5.1	5.1
DCAD (mEq/kg)	270	248

¹ Manufactured by: Yasna Mehr Co. Shams Abad Industrial City, Hasan Abad, Qom Highway, Tehran, Iran.

² Magnapac contains 1.5% myristic acid, 44% palmitic acid, 5% stearic acid, 40% oleic acid and 9.5% linoleic acid and was provided by Attaka Industrial Zone-Suez gulf. Suez. Egypt.

³ Provided (per kg of DM): vitamin A: 500000 IU; vitamin D: 100000 IU; vitamin E: 1000 mg; P: 9000 mg; Ca: 195000 mg; Mn: 2000 mg; Na: 55000 mg; Zn: 2000 mg; Fe: 2000 mg; Cu: 280 mg; Co: 100 mg; Br: 100 mg and Se: 1 mg; Antioxidant: 3000 mg.

Calculated using the NRC (2001) model.

DM: dry matter, CP: crude protein; NEL: net energy for lactation; RDP: rumendegradable protein; RUP: rumen-undegradable protein; NDF: neutral detergent fiber; ADF: acid detergent fiber; NFC: nonfiber carbohydrates; EE: ether extracts and DCAD: dietary cation-anion difference. XSBM: xylose-treated soybean meal and CGM: corn gluten meal.

Measurements and sampling

The general sampling framework in the current experiment was similar to our previous experiment (Jahani-Moghadam *et al.* 2009).

Samples of TMR and diet ingredients were analyzed for dry matter (DM; oven-dried at 105°C for 48 h), CP (AOAC, 2000; ID 984.13), ether extract (AOAC, 2000; ID 920.39), ash (AOAC, 2000; ID 942.05), NDF and ADF (Van Soest et al. 1991). Corn silage DM was measured weekly to correct its amount in the diet. Cows were milked with Westfalia Metatron 21 equipment thrice a day at 0600, 1400 and 2200 h. The amount of milk yield was recorded weekly (a total of 5 times). On Mondays of weeks 1, 3 and 5, milk samples were collected weekly from consecutive milkings, pooled and analyzed by CombiFoss 5000 (Foss Electric, Hillerød, Denmark) for fat, protein, lactose, MUN and total solid. Blood samples (10 mL) were collected 3 weeks after (20 per treatments; a total of 40 samples) the beginning of the experiment from the coccygeal vein, 4 h after the a.m. feeding. Blood was centrifuged at $3000 \times g$ for 15min at 5 °C and plasma was harvested and frozen at -20 °C until analysis. Samples of plasma were analyzed for non-esterified fatty acids (NEFA) and beta hydroxyl butyric acid concentrations (BHBA; Randox Laboratories Ltd., UK). For total protein (Biuret method), albumin (Bromcresol Green method), glucose (GOD-PAR enzymatic method) and blood urea nitrogen content (BUN; Berthelot enzymatic method) Pars Azmun kits (Pars Azmun Laboratory, Tehran, Iran) were used. Absorbance was read using a spectrophotometer (PerkinElmer, Coleman Instruments Division, Oak Brook, IL, USA). At the beginning and the end of the experiment, the BCS was recorded (five point scale where 1=emaciated and 5=obese; Wildman et al. 1982) by three skilled individuals and the average of three people was used in data analysis. Feed intake was determined daily through the study by offered and refused diet for TMR and was averaged per week. NRC (2001) requirements were used for diet formulation. Also, the tabular amounts of AA in NRC (2001) were used to evaluate the AA composition of the diets.

Statistical analysis

Milk yield, milk composition and DMI data were analyzed as repeated measures using the PROC MIXED of SAS (SAS, 2003). Cow was considered as a random effect and was nested within treatment. Blood metabolites and BCS data were analyzed using PROC MIXED. The main source of variation in the model was the treatment and the cow was considered as a random effect. Milk yield before the experiment was used as a covariate adjustment and because it was not significant, it was removed from the final model. Income:cost ratio was calculated as individual milk sold per cow divided by the average DMI (as fed) cost. In this study, differences among treatments were considered significant if P < 0.05, whereas when $0.05 < P \le 0.20$, differences were considered to indicate a trend towards significance.

RESULTS AND DISCUSSION

DMI, milk yield and milk composition

All data are presented in Table 2. Dry matter intake was not affected by treatments (P<0.22), however, was slightly numerically greater for XSBM group, which is important under farm conditions and for farmers. Similarly, the milk yield showed no differences between treatments, as long as the yield of 3.5% FCM. However, FCM:DMI (P<0.13) and the income:cost (P<0.06) ratios tended to be higher for CGM compared with XSBM. Fat content was higher for CGM (P<0.03), while fat yield tended to be higher for CGM (P<0.13). Protein content, but not yield, was greater for XSBM (P<0.02, Table 2). Milk lactose content tended to be higher in CGM group (P<0.16) but total solid and solid non-fat were not affected by treatments. Milk urea nitrogen tended (P<0.14) to be higher for XSBM group.

Blood metabolites and BCS

Results on blood parameters for two treatments are presented in Table 3. None of the blood parameters evaluated (glucose, NEFA, BHBA, albumin, total protein and BUN) was affected by treatments. A change in BCS tended to be more negative for CGM fed cows (-0.05 *vs.* -0.12; P=0.10).

To our knowledge, there is no experiment in which CGM and XSBM feeding have been compared under commercial and practical conditions. In our previous study (Jahani-Moghadam *et al.* 2009), we showed that replacing SBM with XSBM and, consequently changing RDP:RUP ratio had no effect on DMI in early lactation cows. In contrast to the current study, Ipharraguerre and Clark (2005) showed that corn RUP diets resulted in higher DMI compared with SBM RUP diets.

Because in early lactation, the level of milk yield, and hence, energy demands are main controllers of DMI (Aghaziarati et al. 2011; Ingvartsen and Andersen, 2000), this lack of difference was expected. In contrast, Reynal et al. (2007) showed a 4.5 kg higher DMI for XSBM than for CGM group; this might be due to a lower number of cows used or to different experimental conditions. Although milk yield was not affected by RUP source, the difference of as much as 0.6 kg/cow/d is still economically important under farm conditions. We must also consider the amount of DMI/kg milk produced and especially the milk component or FCM. Greater FCM:DMI and income:cost ratios for CGM treatment are very significant for the farmers. With high feed cost and low milk price, the selection of more economically beneficial feed would be important. Recently, we showed that during early lactation decreasing dietary RDP:RUP from 11.6:5.2 up to 10.6:6.1 and 9.5:7.1 led to, respectively, 18% and 16% gains in profits (Jahani-Moghadam et al. 2009).

Table 2 Effects of xylose-treated soybean meal or corn gluten meal as rumen-undegradable protein (RUP) source on DMI, milk production and composition

Item	Treatments		- SE	P-value
	XSBM	CGM	- 5E	r-value
DMI, (kg/d)	27.3	26.8	1.26	0.22
Milk yield, (kg/d)	40.65	40.05	1.37	0.66
FCM 3.5%, (kg/d)	40.27	41.82	1.71	0.36
FCM 3.5%:DMI	1.47	1.57	0.07	0.13
Income:cost (FCM 3.5%:DMI)	1.83	1.97	0.08	0.06
Fat, (g/kg)	34.6	37.3	0.13	0.03
Fat, (kg/d)	1.40	1.50	0.07	0.17
Protein, (g/kg)	31.7	30.9	0.03	0.02
Protein, (kg/d)	1.29	1.25	0.05	0.40
Lactose, (g/kg)	44.6	45.2	0.05	0.16
Total solid, (g/kg)	119.8	121.1	2.3	0.57
SNF, (g/kg)	83.9	83.6	0.8	0.67
MUN, (mg/dL)	15.89	15.16	0.50	0.14

DMI: dry matter intake; FCM: fat corrected milk; SNF: solid non fat and MUN: milk urea nitrogen.

XSBM: xylose-treated soybean meal and CGM: corn gluten meal.

 Table 3
 Effects of xylose-treated soybean meal or corn gluten meal as rumen-undegradable protein (RUP) source on plasma metabolites and body conditions score

Item	Trea	Treatments		D 1
	XSBM	CGM	SE	P-value
Glucose, (mg/dL)	63.66	65.66	2.94	0.63
NEFA, (mmol/L)	0.261	0.244	0.018	0.39
BHBA, (mmol/L)	0.85	0.73	0.095	0.39
Total protein, (g/dL)	6.94	6.86	0.09	0.57
Albumin, (g/dL)	2.78	2.83	0.04	0.48
BUN, (mg/dL)	19.70	18.64	1.11	0.51
BCS change	-0.05	-0.12	0.03	0.10

NEFA: non esterified fatty acids; BHBA: beta hydroxyl butyric acid concentrations; BUN: blood urea nitrogen and BCS: body condition score. XSBM: xylose-treated soybean meal and CGM: corn gluten meal.

SE: standard error.

When the ratio drop to 8.5:8.1, there were no net profits, suggesting that the 8.5:8.1 was not the economically optimum RDP:RUP ratio. In the current study we demonstrated that with a RDP:RUP of approximately 10.4:6.7, the source of RUP has also a determinant effect on profits.

From a nutritional point of view and regarding the insignificant and variable production responses to RUP sources (Santos et al. 1998; Ipharraguerre and Clark, 2005), their profitability may be questioned. But this must not result in ignoring the important role of RUP in the diet, and thus, overemphasizing RDP. There is evidence of linear increases in microbial protein synthesis in response to increasing dietary RDP (Broderick et al. 2010), while maximum milk protein yield occurs at less than maximum microbial protein synthesis (Reynal and Broderick, 2005; Olmos Colmenero and Broderick, 2006). This implies that we should not emphasize RDP at the expense of RUP, especially when we know that NRC (2001) over-predicts the RDP requirement (Huhtanen and Hristove, 2009). Except for the work performed by Reynal et al. (2007), to our knowledge, there is no experiment in which CGM has been compared with XSMB and this makes it difficult to interpret and discuss the results.

It is well known that supplements of Met often increase the yield and concentration of milk fat (Chamberlain and Yeo, 2003). Ipharraguerre and Clark (2005) showed that corn by-products and fish meal were more effective sources of RUP to enhance the ruminal outflow of Met to the small intestine than soy RUP sources. It has been suggested that the effects of Met on the secretion of milk fat might be related to a stimulation of the synthesis of lipoproteins, and hence, increased transport of triglycerides to the udder (Pullen *et al.* 1989). Since there was no difference in DMI, it appears that cows fed CGM partitioned more energy precursors to the mammary gland.

Our findings provide evidence that CGM compared with XSBM can support more milk production and fat yield (which is more favorable in Iran's conditions) and in spite of a lower DMI it improved feed efficiency and economics. Our data is in contrast with Santos *et al.* (1998), who speculated that CGM usually has a negative effect on the cow performance. One possible reason can be the limited number of cows used in those experiments.

In corn-based diets (forage and grain), duodenal Lys rather than Met is more likely to be the first AA limiting milk protein synthesis (Schwab *et al.* 1992).

SE: standard error.

The flow of Lys and Met to the duodenum was not significantly increased by high RUP supplementation (Santos *et al.* 1998). However, Ipharraguerre and Clark (2005) showed that Lys flow for soy RUP was greater than for corn RUP, and milk protein yield was slightly higher for soy RUP. Given its high Lys and RUP (72-79%, Jahani-Moghadam *et al.* 2009), feeding XSBM can increase intestinal Lys flow (Mabjeesh *et al.* 1996) and milk protein synthesis (Ipharraguerre *et al.* 2005).

It seems that there is a discrepancy between our results and the literature recommendations on Lys:Met ratio. NRC (2001) recommended that for maximum content of milk protein, the Lys:Met ratio should be 3:1 and, recently, Schwab and Foster (2009) suggested that for NRC model, this ratio must be 2.97 for the highest milk protein content and 2.82 for the highest milk protein yield. Schwab and Foster (2009) also suggested that balancing the diet as close as possible to obtain the model determined optimal concentrations of Lys and Met in MP, will result in more milk components, especially milk protein. Because NRC (2001) predictions of intestinal flow of essential AA are reliable under field conditions (Pacheco et al. 2012), it was expected that milk protein was higher for CGM group (Lys:Met ratio=3.29) compared with XSBM (Lys:Met ratio=3.61). Also, in agreement with our data, Chase et al. (2009) showed that in 14 high yielding farms, the Lys:Met ratio ranged from 2.38:1 to 3.4:1 without any relationship between this ratio and milk components. This discrepancy between field and experimental results may warrant further investigations on the role and magnitude effect of this ratio on milk protein content and yield. It appears that this ratio should be adjusted based on diet ingredients as well.

The MUN values between 10 to 16 mg/dL indicate an appropriate diet formulation (Ouellet *et al.* 2007).

In addition, lower or higher values are indicative of the inadequacy of N use. In practical conditions with dairy cows, approximately one half or two thirds of synthesized urea-N is derived from ammonia, while the remainder is from the catabolism of amino acid, either as a part of maintenance functions or surplus to the needs to support anabolic processes such as milk protein output (Ouellet et al. 2007). Regardless that the MUN values are in that range, higher values for XSBM perhaps implies that digesta AA profile or synchronization between N and energy in the rumen was better for CGM group. An undetectable difference in concentrations of NEFA and BHBA between groups, which are energy balance indicators in dairy cows (Ingvartsen, 2006), shows that cows were in relatively similar conditions of energy balance. However, BUN was at dangerous levels for XSBM group. Blood urea nitrogen is the major end product of N metabolism in ruminants, and its high concentrations are indicative of an inefficient utilization of dietary N.

Although it is believed that ureagenesis needs energy (Tyrrell *et al.* 1970), Reynolds (2006) showed that on an ATP basis, the synthesis of urea from ammonia requires 4 moles of ATP and produces 6 moles of ATP equivalents through NADH generation and thus produces 2 moles of net ATP.

Oba and Allen (2003) also reported similar results about positive effects of urea synthesis on ATP production; in this context, the lower BCS loss in XSBM group (-0.05 *vs.* - 0.12, Table 3) may be partially justified. Because early lactation Holstein cows do not use AA for gluconeogenesis (Doepel *et al.* 2009), comparable blood glucose (Table 3) may indicate that hepatic inflow of propionate and lactate, the two major glucogenic substances in fed cows, remained unaffected by treatments. Therefore, it is possible that the increased AA supply was likely used towards mammary protein synthesis at the expense of gluconeogenesis (Doepel *et al.* 2009).

CONCLUSION

Feeding corn gluten meal compared to XSBM resulted in higher fat content and yield. As a result, FCM:DMI (1.57 *vs.* 1.47) and income:cost (1.97 *vs.* 1.83) were better for the CGM RUP group and this is very important under field conditions. While the ratio of Lys:Met improved with CGM feeding, and it was expected that milk protein production was greater for this group, the protein content was higher in the soy RUP group, implying more experiments to determine the exact role of Lys:Met ratio under field conditions. Also, MUN was higher for the soy RUP group. None of the plasma metabolites were influenced by treatments. These results show that under field conditions and feed ingredients used here, using CGM can support similar milk yield compared to XSBM, in contrast to conventional beliefs.

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