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Effects of Partial Replacement of Soybean Meal with Corn Gluten Meal, Fish Meal, or Their Combination on Dairy Calves' Performance and Insulin Concentration

Research Article

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ABSTRACT

The partial replacement of soybean meal (SBM) with different protein sources (corn gluten meal; CGM, and fish meal; FM) was evaluated on the performance, blood metabolites, microbial protein synthesis, and insulin concentration in Holstein dairy calves. Forty-four Holstein female calves with average body weight (BW) 42 ± 0.81 kg and 3-d age were allocated in a completely randomized design with four treatments: 1) control diet with soybean meal as a sole protein source (SBM); 2) partial replacement (5%) of SBM with corn gluten meal (CGM); 3) partial replacement (5%) of SBM with fish meal (FM) and 4) replacement of SBM with a combination (2.5% each) of CGM and FM meal (CGM-FM). The study lasted 11 weeks with the weaning date on d-66, though the study lasted until d-80. The statistical analysis was considered as three separate periods including pre-weaning (3-66 d), post-weaning (66-80 d), and the entire period (3-80 d). The results revealed that the starter intake and average daily gain were not affected among experimental treatments (P>0.05). Replacing SBM with a combination of fish meal and corn gluten meal (CGM-FM) caused improved feed efficiency (P<0.05). Among the growth indices, wither height was enhanced in the CGM-FM treatment at weaning time (P<0.05), and in the final stage of recording, a tendency was significant for this trait (P=0.05). The results suggested that the protein digestibility slightly improved in the CGM-FM treatment compared to other treatments (P=0.06); however, it did not affect others. The microbial protein yield did not differ among experimental treatments (P>0.05). The greatest insulin concentration was found to be for CGM-FM among experimental treatments (P<0.05). In conclusion, the results showed that the partial replacement of SBM with the combination of CGM and FM in dairy calves' starter diet improved the growth performance and contributed to a higher insulin concentration.

KEY WORDS dairy calves, growth parameters, insulin, performance, undegradable protein.

INTRODUCTION

Starter protein content and quality in dairy calves have an important role in the growth and performance as well as in improving the immune system. The starter used for calves less than 2 months normally contain 18% crude protein (NRC, 2001); however, greater protein content in the starter diet could improve the growth performance of dairy calves (Kazemi-Bonchenari *et al.* 2018). The consumption of starters with a lower level of crude protein reduces the daily growth and nutritional efficiency of these calves. The most common source of protein in ruminant rations is SBM (Baker, 2000) and this feedstuff provides more balanced amino acids for animal requirements rather than other protein sources (NRC, 2001). The protein requirement of ruminant nutrition is commonly divided into ruminal degradable (RDP) and ruminal undegradable protein (RUP) that its optimum ratio is found to be an important factor influencing dairy calves' growth performance (NRC, 2001). Some studies have been shown that higher starter dietary RUP content improved structural growth indices in dairy calves (Kazemi-Bonchenari *et al.* 2020). The undegradable protein can lower the turnover of microbial protein synthesis and the flow of microbial amino acids to the small intestine (Siddons *et al.* 1985; Cecava *et al.* 1991). In the alimentation of calves, like dairy cattle, along with the crude protein diet, special attention should be paid to the replaced protein sources and amino acids especially lysine and methionine. The RUP fraction of the protein enters amino acid to the small intestine. The RUP could be provided through different sources of feedstuffs such as meat meal, FM, and roasted SBM (NRC, 2001). However, some RUP-based rations seem to transfer some of the specific amino acids to a small amount in the intestine. For instance, corn has a small amount of lysine amino acids. High production of bovine products is limited by lysine unless much of the RUP is made from corn grits or CGM. The FM has more lysine and methionine than SBM and CGM. Methionine and lysine are two determinative amino acids in the protein to be metabolized and synthesized by the protein of milk (DePeters and Cant, 1992; Koenig *et al.* 2002). Methionine has an exclusive role as an amino acid primer in protein production (Brosnan and Brosnan, 2006). Weekes *et al.* (2006) found that equipoising the diet in terms of amino acids has a beneficial effect on the dry matter of lactating cows, where diet supplements with upside quality proteins or protected amino acids, in particular methionine and lysine, redound to an increase in DMI and digestion in cattle and sheep (Ali *et al.* 2009). The CGM has more leucine in its combination compared to SBM and fishmeal. As with other essential amino acids, the first role of leucine is in the body's protein structure. One of the major hormones regulating glucose uptake is insulin. Insulin stimulates glycogenesis, protein synthesis, and lipogenesis (Dimitriadis *et al.* 2011). Insulin also supports protein synthesis and, thereupon, has a significant effect on the growth hormone (GH) (Guyton and Hall, 2000; Hadley, 2000). Insulin sensibility in infant's calves is noticeably reduced during the final period of breeding (Doppenberg and Palmquist, 1991; Hugi *et al.* 1997). Some previous results have suggested that in growing ruminants, a greater insulin concentration can cause improved growth performance (Wester *et al.* 2000). The level and type of amino acids reaching the small intestine from the replaced protein sources can also affect insulin density (Wester *et al.* 2000). The major amino acids absorbed from the small intestine can stimulate anabolism via increased insulin secretion (Davis, 1972). We hypothesized that replacing some RUP sources such as CGM and FM may provide more leucine and some other amino acids contributed to insulin concentration regulation that consequently is influencing the animal growth performance. Based on the abovementioned status the current study addressed the effect of partial replacement of SBM by CGM, fish meal, and their combination in dairy calves' nutrition on performance, structural growth, microbial protein production as well as insulin concentration.

MATERIALS AND METHODS

Calves, housing, and diets

The present study was conducted at the Zarrin-Khoosheh Commercial Dairy Farm, Arak, Iran. A total of 44 Holstein female calves with a mean weight of 42 ± 0.81 kg with 3-d age were allocated in a completely randomized design with four treatments (11 animals per treatment) for 11 weeks. The calves were separated from their dams, weighed, and moved to individual pens (1.2×2.5 m), bedded with straw which was cleaned every 24 h. The ingredients and chemical composition of the experimental diets are presented in Table 1. The calves were fed twice daily at 0800 and 1600. The calves had free also access to water throughout the experimental period. The treatments were: 1) control diet (SBM) containing soybean meal; 2) 5% replacement of soybean meal with corn gluten meal (CGM); 3) 5% replacement of soybean meal with fish meal (FM), and 4) 5% replacement of soybean meal with CGM with fish meal (CGM-FM) in combination (2.5% each). The calves were fed individually and the feed was provided as free access throughout the experiment. The starter feed was offered as mash form for all four treatments.

Sampling times and chemical analyses

BW was measured weekly during the preweaning period (d 3 to 66) and post-weaning period (d 66-80). The amounts of the starter feed offered and refused were recorded daily throughout the study for each calf to calculate the feed intake. The average daily gains and feed efficiency (kg of BW gain/kg of total DMI) were also calculated based on previous works (Kazemi-Bonchenari *et al.* 2020). In addition, daily fecal scores (Khan *et al.* 2007) were recorded. Body measurements (cm), including heart girth (circumference of the chest), hip-width (distance between the points of hook bones), body length (distance between the points of shoulder and rump), body barrel (circumference of the belly before feeding), wither height (distance from the base of the front feet to wither), and hip height (distance from the base of the rear feet to the hook bones) were measured at the start of the experiment (d 3), with a 10-day interval.

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Table 1 Ingredients and chemical composition (% of DM, unless otherwise noted) of the experimental diets

Item	Treatments			
	SBM (T1)	CGM (T2)	FM (T3)	CGM-FM (T4)
Ingredients				
Barley grain, ground	10	10	10	10
Corn grain, ground	51.5	51.5	51.5	51.5
Soybean meal	26	20	20	20
Corn gluten meal	0	5	0	2.5
Fish meal	0	0	5	2.5
Soybean seed	3	3	3	3
Beet pulp	5	6	6	6
Vitamin-mineral premix[1]	2	2	2	2
Salt	0.5	0.5	0.5	0.5
Sodium bicarbonate	1	1	1	1
Calcium carbonate	0.5	0.5	0.5	0.5
Di-calcium phosphate	0.5	0.5	0.5	0.5
Chemical composition				
Metabolisable energy, Mcal/kg	2.73	2.69	2.74	2.72
Crude protein, %	20.6	20.7	20.8	20.8
Rumen undegradable protein, %	7.63	8.69	8.81	8.75
Ether extract, %	3.07	3.0	3.30	3.15
Ca, %	0.9	0.9	0.9	0.9
P, %	0.5	0.5	0.5	0.5

[1] Contained per kg of supplement: vitamin A: 500000 IU; vitamin D: 100000 IU; vitamin E: 1800 IU; Mn: 2.25 g; Ca: 130 g; Zn: 7.7 g; P: 15 G; Mg: 20 g; Na: 170 g; Fe: 1.25 g; S: 3 g; Co: 12 g; Cu: 1.20 g; I: 58 mg and Se: 15 mg.
T1: starter diet with soybean meal as control (SBM); T2: starter diet with partial replacement of SBM with corn gluten meal (CGM); T3: starter diet with partial replacement of SBM with fish meal (FM) and T4: starter diet with partial replacement of SBM with the combination of corn gluten meal and fish meal (CGM-FM).

The digestibility iatrial and the microbial protein synthesis iatrial in dairy calves were conducted in the current study as extensively explained in previous works (Kazemi-Bonchenari *et al.* 2016; Kazemi-Bonchenari *et al.* 2020). Blood samples were collected 4 h after the morning feeding from the jugular vein into 10-mL tubes on d-3, d-35, and d-65 of the study. The blood samples were placed on ice immediately after collection and within 20 min of drawing, the samples were centrifuged at 3,000 × *g* for 15 min at 4 ˚C to harvest serum. The serum was preserved at –20 ˚C until analysis for metabolites. Later, the serum metabolites including glucose, beta-hydroxy butyric acid (BHBA), total protein (TP), albumin, blood urea nitrogen (BUN), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and insulin were measured using Auto Analyzer (Hitachi 717, Japan).

Statistical analyses were performed using PROC MIXED of SAS (2004) with the individual calf as an experimental unit. Starter intake, ADG, and FE was statistically analyzed as a repeated measures with the period (each period was 10 d) as repeated variable using the following model:

$$Y_{ijk}= \mu + CP_i + T_j + (CP\times T)_{ij} + \beta(Xi-\bar{X}) + \varepsilon_{ijk}$$

Where:
Y_{ijk}: dependent variable.
μ: overall mean.
CP_i: effect of protein source i.
T_j: effect of sampling time j.
$(CP\times T)_{ij}$: interaction between protein source and time.
$\beta(Xi-\bar{X})$: covariate variable.
ε_{ijk}: overall error term.

The data was analyzed in three separate intervals (3-66; 66-80; 3-80) and divided in to 3 periods in result section as pre-weaning (d 3 to 66), post-weaning (d 66 to 80) and the entire period (d 3 to 80). Initial BW was used as a covariate for weaning weight and final weight. The differences among treatment means were determined using Tukey's multiple range tests.

Effects were considered to be significant when $P \leq 0.05$, and a tendency was considered when $0.05 < P \leq 0.10$. All reported values are least-square means.

RESULTS AND DISCUSSION

Calf performance

Regardless of the source of the protein, elevated levels of the inclusion protein did not affect the amount of feed consumed or on the weight gain of the experimental animals. The mean daily weight (P<0.05) and food conversion ratio (P<0.05) showed a relative improvement upon increasing the inclusion protein level (Table 2).

Skeletal growth

Body growth measurements data are shown in Table 3. According to the results, the heart girth at any stage of measurement was not affected by replaced the protein sources (P>0.05). The body length was not affected by protein sources (P>0.05) either in any of the experimental stages. Again, the wither height in the initial stage of the record keeping was not influenced by the protein sources (P> 0.05) and no significant effect was observed. However, at weaning and at the end of the experiment, this measurement was affected by RUP resources (P<0.05). The height of the hip at weaning (P=0.65) and at the end of the experiment of recording growth records tended to be significant.

Blood metabolites

The blood metabolites data are reported in Table 4. Blood metabolite was analyzed at three intervals between the start of the experiment, its end, and the mean. Blood glucose was not affected by the replaced protein sources (P>0.05) during any of the time intervals, and no significant effect was observed. Blood beta-hydroxy butyrate was not affected by the replaced protein sources (P>0.05) either during the experiment. However, total protein (TP) was influenced by transient protein sources in both time intervals (before and after weaning) (P<0.05) and significant effects were observed. The AST and ALT liver enzymes were not affected by replaced protein sources (P>0.05) during any of the four-time intervals, and no significant effect was observed. The blood insulin concertation was increased by replacing RUP sources instead of SBM (P=0.035) during pre-weaning and it was tended to be significant during post-weaning period.

Digestibility and microbial protein synthesis

According to the reported data (Table 5), the purine derivatives excretion as well as the synthesis of microbial protein was not affected by different treatments (P>0.05). The results of the nutrients' digestibility indicated that CP digestibility slightly improved in the CGM-FM treatment compared with other treatments (P=0.06).

Our results suggested that replacing SBM with a combination of FM and CGM caused improved feed efficiency.

Some researchers found that methionine plays a major role in controlling muscle protein in calving growth, so a higher protein content has usually higher methionine levels, though methionine supplementation may still be required. The jam of passing protein can be useful for growing calves. Reduction of calf starter usage decreases the beneficial effects of calves (Yari *et al.* 2018). The addition of methionine and lysine protected to the diet resulted in increased dry matter intake (Broderick and Craig, 2009), which could be due to the safety of limiting amino acids (Ali *et al.* 2009), balancing the diet in terms of amino acids (Weekes *et al.* 2006). Some studies have suggested that the density of crude starter protein does not affect the performance and calves' feed intake (Sekine *et al.* 2004; Labussiere *et al.* 2008; Ozkaya and Toker, 2012). Nevertheless, Drackley *et al.* (2002) stated that starter-fed calves showed a 22% better crude protein compared to the use of crude protein 18%. Ozkaya and Toker (2012) reported that feeding 22% CP to 18% CP improves the calves' performance in the pre-lactation period, but there is no difference between protein levels at 56 days of age.

Our results indicated that the synthesis of microbial protein was not affected, but the results of the nutrients digestibility showed that CP digestibility slightly improved in CGM-FM treatment compared with other treatments. This may have related partly to amino acid composition of the protein sources (NRC, 2001). Bruckental *et al.* (2002) found that the effect of partial replacement of SBM with different protein sources (CGM and FM) increases the digestibility of the CP and non-structural carbohydrate.

Bunnakit and Khampa (2011) stated that DMI digestibility in Thai Native × Brahman cattle rose linearly upon elevation of the RUP levels. Felisberto *et al.* (2011) clarified that the use of RUP source promoted an increase in the flow of nutrients and changes in the digestive contents of the omasum, but it compromised the production of microbial efficiency in dairy goats.

Widyobroto *et al.* (2018) reported that supplementation with different RUP levels on early lactating dairy cattle could not increase feed consumption, nutrient digestion, BW, and body condition score. Some studies also reported interactive effects of RUP content of the diet and nutrient digestibility in ruminants (Pattanaik *et al.* 2003; Flis and Wattiaux, 2005).

Regarding microbial protein synthesis, we found no changes among experimental treatments. This is probably because of adequate RDP content of the starter diet among experimental treatments.

The adequate ruminal available nitrogen content which is supplied through adequate RDP content of the diet can guarantee the optimum microbial protein synthesis in ruminants (NRC, 2001).

Table 2 Effect of partial replacement of soybean meal with corn gluten meal, fish meal, and their combination on the starter intake, gain, and feed efficiency in Holstein dairy calves (n=11 per treatment)

Item	Treatments				SEM	P-value
	SBM (T1)	CGM (T2)	FM (T3)	CGM-FM (T4)		
Starter feed intake, g/day						
Pre-weaning (d 3-66)	736	662	688	728	31.20	0.286
Post-weaning (d 67-80)	2167	2273	2328	2124	98.04	0.435
Entire period (d 3-80)	996	955	986	982	31.14	0.805
Milk intake (DM), g/day	643	646	647	641	3.12	0.763
Average daily gain, g/day						
Pre-weaning (d 3-66)	562	514	590	621	30.13	0.079
Post-weaning (d 67-80)	742	723	787	823	60.88	0.649
Entire period (d 3-80)	595	552	626	658	27.02	0.039
Bodyweight, kg						
Initial (d 3)	42.8	42.7	42.5	43.1	0.87	0.963
Weaning (d 66)	77.6	75.2	77.0	80.8	2.37	0.424
Final (d 80)	87.8	85.3	89.8	93.2	3.04	0.319
Feed efficiency						
Pre-weaning (d 3-66)	0.40	0.40	0.45	0.49	0.027	0.045
Post-weaning (d 67-80)	0.35	0.32	0.36	0.39	0.032	0.528
Entire period (d 3-80)	0.39	0.39	0.43	0.47	0.029	0.023
Fecal scoring	1.33	1.34	1.37	1.25	0.038	0.202

T1: starter diet with soybean meal as control (SBM); T2: starter diet with partial replacement of SBM with corn gluten meal (CGM); T3: starter diet with partial replacement of SBM with fish meal (FM) and T4: starter diet with partial replacement of SBM with the combination of corn gluten meal and fish meal (CGM-FM).
SEM: standard error of the means.

Table 3 Effect of partial replacement of soybean meal with corn gluten meal, fish meal, and their combination on growth parameters (cm) (n=11 per treatment)

Item	Treatments				SEM	P-value
	SBM (T1)	CGM (T2)	FM (T3)	CGM-FM (T4)		
Heart girth						
Initial (d 3)	72.5	73.0	73.1	73.0	0.95	0.973
Weaning (d 66)	86.0	87.2	89.4	89.2	1.41	0.265
Final (d 80)	96.9	97.1	96.0	98.2	1.14	0.609
Hip width						
Initial (d 3)	20.9	21.3	21.5	21.4	0.48	0.835
Weaning (d 66)	28.1	28.3	29.0	29.1	0.39	0.198
Final (d 80)	32.0	31.5	33.1	32.2	0.61	0.296
Body length						
Initial (d 3)	51.3	51.0	51.2	52.8	0.77	0.713
Weaning (d 66)	60.2	60.7	62.0	63.1	1.18	0.362
Final (d 80)	68.7	67.9	71.2	70.4	1.15	0.168
Body barrel						
Initial (d 3)	75.2	75.4	74.3	76.2	1.09	0.677
Weaning (d 66)	92.0	92.1	95.3	95.9	2.43	0.549
Final (d 80)	111.2	109.4	112.2	115.0	1.66	0.130
Wither height						
Initial (d 3)	74.3	73.7	74.8	74.9	0.99	0.826
Weaning (d 66)	85.8	85.2	87.6	90.2	1.27	0.035
Final (d 80)	92.4	91.5	91.0	94.3	0.89	0.058
Hip height						
Initial (d 3)	77.7	79.3	77.0	77.1	1.09	0.406
Weaning (d 66)	89.0	88.1	90.6	92.1	1.13	0.068
Final (d 80)	93.2	95.0	93.9	96.0	0.79	0.095

T1: starter diet with soybean meal as control (SBM); T2: starter diet with partial replacement of SBM with corn gluten meal (CGM); T3: starter diet with partial replacement of SBM with fish meal (FM) and T4: starter diet with partial replacement of SBM with the combination of corn gluten meal and fish meal (CGM-FM).
SEM: standard error of the means.

Table 4 Effect of partial replacement of soybean meal with corn gluten meal, fish meal and their combination on blood metabolites, liver enzymes and insulin (n=11 per treatment)

Item	Treatments				SEM	P-value
	SBM (T1)	CGM (T2)	FM (T3)	CGM-FM (T4)		
Glucose, mg/dL						
Initial (d 3)	120.5	109.3	108.4	115.0	13.65	0.915
Pre-weaning (d 38)	101.8	97.0	96.1	102.8	5.62	0.775
Post-weaning (d 76)	74.3	78.3	90.2	86.8	5.17	0.139
Overall	98.88	94.87	98.22	101.56	5.21	0.842
BHB, mmol/L						
Initial (d 3)	0.07	0.06	0.05	0.06	0.007	0.585
Pre-weaning (d 38)	0.14	0.13	0.15	0.16	0.01	0.397
Post-weaning (d 76)	0.23	0.24	0.28	0.30	0.03	0.478
Overall	0.148	0.146	0.165	0.173	0.012	0.349
Total protein, g/dL						
Initial (d 3)	7.11	8.13	7.61	7.78	0.33	0.228
Pre-weaning (d 38)	6.81	7.03	6.95	7.93	0.26	0.029
Post-weaning (d 76)	6.58	6.91	6.73	7.48	0.23	0.061
Overall	6.83	7.36	7.10	7.73	0.162	0.002
Albumin, g/dL						
Initial (d 3)	3.35	3.26	3.41	3.20	0.09	0.379
Pre-weaning (d 38)	3.56	3.81	3.70	3.56	0.08	0.120
Post-weaning (d 76)	3.45	3.55	3.56	3.50	0.11	0.889
Overall	3.45	3.54	3.56	3.43	0.05	0.318
Blood urea nitrogen, mg/dL						
Initial (d 3)	26.66	23.0	25.50	24.16	3.89	0.917
Pre-weaning (d 38)	28.33	24.32	22.66	22.10	2.17	0.240
Post-weaning (d 76)	18.16	17.33	15.16	14.50	1.08	0.081
Overall	24.38	21.55	21.11	20.44	1.53	0.286
AST, IU/mL						
Initial (d 3)	29.50	37.0	45.0	52.10	7.79	0.229
Pre-weaning (d 38)	45.16	38.17	45.18	41.83	2.98	0.318
Post-weaning (d 76)	66.0	53.66	64.50	64.16	10.12	0.815
Overall	46.88	42.94	51.55	52.66	4.37	0.378
ALT, IU/mL						
Initial (d 3)	10.83	14.50	14.0	12.51	2.10	0.612
Pre-weaning (d 38)	10.16	9.17	8.83	10.33	1.06	0.699
Post-weaning (d 76)	23.66	20.67	20.16	24.84	3.58	0.789
Overall	14.88	14.77	14.50	15.88	1.43	0.910
Insulin, μIU/mL						
Initial (d 3)	18.55	19.91	17.79	20.29	1.66	0.693
Pre-weaning (d 38)	12.61	13.60	16.56	17.84	1.32	0.035
Post-weaning (d 76)	13.12	13.28	15.08	18.97	1.68	0.081
Overall	13.59	14.43	15.32	17.87	0.90	0.009

T1: starter diet with soybean meal as control (SBM); T2: starter diet with partial replacement of SBM with corn gluten meal (CGM); T3: starter diet with partial replacement of SBM with fish meal (FM) and T4: starter diet with partial replacement of SBM with the combination of corn gluten meal and fish meal (CGM-FM).
BHB: beta-hydroxy-butyrate; AST: aspartate aminotransferase and ALT: alanine aminotransferase.
SEM: standard error of the means.

In addition, the constant starch content of the starter diets among experimental treatments caused similar microbial protein synthesis observed in the current study.

Our results indicated that replacing SBM with CGM and FM and their combination improved the growth performance of dairy calves. The reports of Richardel (2004) suggested that a diet of more than 16% crude protein improves performance in dairy calves. Therefore, protein supplements in the form of RUP in the diet may be a better alternative. Adding protein to the diet will keep the tissues healthy during the weaning period (Stamey *et al.* 2012).

Therefore, research has been conducted to show that the use of soybean protein as a protein source in comparison with milk protein improves the growth performance. We found that replacing RUP source instead of SBM in the current study improved wither and hip weights in dairy calves. This is mostly because of the increased entrance of amino acid into small intestine in RUP enriched diets (NRC, 2001; Kazemi-Bonchenari *et al.* 2020).

The higher amino acids can contribute to improved structural growth in dairy calves that were found in the present study.

Table 5 Effect of partial replacement of soybean meal with corn gluten meal, fish meal, and their combination on purine derivatives, microbial protein synthesis, and nutrients digestibility (n=11 per treatment)

Item	Treatments				SEM	P-value
	SBM (T1)	CGM (T2)	FM (T3)	CGM-FM (T4)		
Purine derivatives and microbial crude protein (MCP)						
Allantoin, mmol/d	16.18	15.65	14.60	15.16	1.60	0.91
Uric acid, mmol/d	0.82	0.59	0.74	0.73	0.11	0.59
Purine derivatives (PD), mmol/d	17.01	16.25	15.34	15.89	1.65	0.92
Microbial protein, g/d	90.93	86.88	82.03	84.99	8.86	0.92
Digestibility, %						
Dry matter (DM)	56.8	52.0	53.62	52.47	1.97	0.33
Crude protein (CP)	59.9	63.1	63.8	67.1	1.72	0.06
Neutral detergent fiber (NDF)	46.51	52.12	50.68	48.34	4.47	0.81
Ether extracts (EE)	75.86	78.85	72.70	75.20	2.20	0.30

T1: starter diet with soybean meal as control (SBM); T2: starter diet with partial replacement of SBM with corn gluten meal (CGM); T3: starter diet with partial replacement of SBM with fish meal (FM) and T4: starter diet with partial replacement of SBM with the combination of corn gluten meal and fish meal (CGM-FM).
SEM: standard error of the means.

In this study, two sources of rumen undegradable protein (CGM and FM) have been used to replace SBM, which has a different amino acid balance than the feed. Note that no specific processing has been carried out on them, feed, or performance intake.

Our results indicated that blood insulin concentration was greater in CGM-FM treatment which was followed by improved growth performance of animals in this treatment. This was due to that this treatment may provide greater amino acids in the small intestine and promote increased insulin secretion and subsequently improved growth performance. A previous work suggested that some amino acids have a potential to increase insulin concentration in animals (Docherty and Clark, 1994). In the current study, blood glucose levels were within the normal range. Lately, the appropriateness of serum beta-hydroxy-butyrate (BHB) as an indicator for rumen growth and feed intake in calves has been confirmed (Deelen *et al.* 2016). Butyric acid is the basic stimulant for the growth of rumen epithelia (Mentschel *et al.* 2001). Indeed, nonesterified fatty acids (NEFA) and plasma β hydroxybutyric acid (BHBA) levels decreased in transition cows fed either with rumen protected methionine (Sun *et al.* 2016) or higher levels of rumen undegradable protein (Amanlou *et al.* 2017). Further, a postpartum reduction in BHBA levels was reported in dairy cows in response to prepartum feeding of the above levels of metabolizable protein (Farahani *et al.* 2017). Albumin levels and total protein levels in the blood can be used as immune status and long-term markers of amino acids (NRC, 2001; Kohn *et al.* 2005; Jahani-Moghadam *et al.* 2009). Furthermore, branched amino acids constitute an important part of skeletal muscle, where there is a direct correlation between increased protein synthesis and leucine (Shimomura *et al.* 2004). It seems that leucine plays an inhibitory role in the protein degradation of the body (Hargrove *et al.* 1985).

The observation of leucine inhibition on protein degradation was demonstrated by studying experimental rats under circumstances of starvation. Under these conditions, tissue decomposition, the presence of leucine in the diet of rats minimized the amount of tissue protein degradation and increased protein synthesis. According to Elsabagh *et al.* (2018) studies on sheep, threonine amino acid improves insulin concentrations. Immunoglobulins and total plasma protein concentrations constitute a significant amount of immunoglobulins since the plasma level is critical for passive passivation of immunoglobulin (Weaver *et al.* 2000; Furman-Fratczak *et al.* 2011). Blood urea nitrates may show short-term effects on ammonia production in the nitrogen circulation and rumen (NRC, 2001; Kohn *et al.* 2005; Jahani-Moghadam *et al.* 2009). In the report of Kazemi-Bonchenari *et al.* (2018), plasma BUN density was reported in the post-lactation period. Elevation of RUP levels also led to a tendency to reduced nitrogen excretion through urine, when the supplementation was transferred from the rumen to the reticulum (Batista *et al.* 2016). The elevated levels of liver enzymes, ALT and AST, demonstrate impaired liver functioning, which may be related to decreased appetite or excessive fat accumulation in the liver (Cebra *et al.* 1997). It is indeed a marker of abnormal conditions for the metabolism of the liver. It seems that the livestock did not encounter any of the mentioned problems in the current test, and there was no increase in the concentration of liver enzymes. Insulin promotes the aggregation of DNA and protein synthesis by controlling the absorption of amino acids (Hadley, 2000). Increased protein intake decreases insulin resistance (Gerrits and Blum, 1998) which leads to increased gluconeogenesis (Barthel and Schmoll, 2003). One of the patterns proposed for leucine is the activation of insulin as a promotor for protein synthesis in the muscle, especially when enough amino acids and energy are available (Garlick, 2004).

In goats, energy utilization affects the local and systemic expression of IGF-I and rumen growth (Shen *et al.* 2004), while in steers, IGF-I regulates the plasma via energy consumption and protein (Elsasser *et al.* 1989). Postpartum somatotropic maturity depends on the nutrition beginning during the post-natal period, indicating the insulin activity and state of glucose (Breier *et al.* 1988; Brameld *et al.* 1996; Butler *et al.* 2003). In rats, after intensive nourishment in the early life, high levels of insulin were observed (Srinivasan *et al.* 2003). It has been determined that incremented insulin levels can improve the growth of ruminant muscles (Wester *et al.* 2000). It seems that replacing the SBM with RUP sources such as CGM and FM in growing calves is likely to influence the incidence of higher levels of amino acids to the small intestine as well as insulin concentrations and ultimately improve the growth of growing calves.

CONCLUSION

In conclusion, the results suggested that partial replacing SBM with CGM and FM and their combination improved the growth performance of dairy calves. Replacing SBM with a combination of CGM and FM (CGM-FM treatment) resulted in the best growth performance among treatments which could be attributed to higher blood insulin concentration. It seems that the use of sources of protein instead of SBM in growing calves would affect the growth of growing calves through entering the higher level of amino acids to the small intestine and improving metabolizable protein levels. In summary, it can be concluded that regarding the performance of dairy calves, partial replacement of the SBM with FM and CGM can be recommended due to improvement in structural improvement which is supposed to be obtained through higher blood insulin concentration.

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