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Effects of Different Carbohydrate Sources on the Performance, Ruminal and Blood Metabolites and Nutrients Digestibility of Fattening Male-Lambs Fed Corn Steep Liquor

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Abstract

The current experiment was conducted to evaluate the effects replacing dietary corn/barley mixture with molasses at levels of 0, 50 and 100 g/kg dietary Dry Matter (DM) as a ruminal fermentable carbohydrate source, in the ration containing Corn Steep Liquor (CSL, 130 g/kg DM), as a ruminal degradable protein source. Twenty four male Lori lambs were randomly assigned into three groups of eight lambs each in a balanced completely randomised design. Nutrients digestibility, ruminal parameters, blood metabolites, feed intake and growth performance were determined. Results indicated that supplementing dietary CSL with increasing levels of molasses up to 100 g/kg of DM linearly increased organic matter (L, $p=0.05$) and ash-free neutral detergent fibre (L, $p<0.05$) digestibility, while DM, crude protein and ash-free acid detergent fibre digestibility remained unchanged ($p>0.05$). Increasing the level of molasses in the CSL containing diets had no effect on ruminal pH ($p>0.05$), but linearly decreased rumen concentration of NH3-N (L, $p<0.05$). Except for total Volatile Fatty Acids (VFAs) and molar proportion of butyrate which were increased linearly (L, $p<0.05$) with increasing dietary molasses level, other individual VFAs were similar ($p>0.05$) among the experimental rations. Increasing the level of molasses in the diet up to 100 g/kg of DM linearly increased (L, $p=0.05$) plasma total protein concentration, while linearly reduced blood urea nitrogen concentration (L, $p<0.05$). Total weight gain and average daily gain were improved (L, $p<0.05$), while feed conversion ratio was decreased linearly (L, $p<0.05$) with increasing dietary level of molasses. However, final body weight and feed intake remained unchanged ($p>0.05$) by feeding the experimental diets. In conclusion, results of present study indicated that supplementing CSL with molasses at levels of 100 g/kg dietary DM increased nutrients digestibility and production performance of fattening lambs.

Keywords

Carbohydrate • Blood metabolites • Microbial protein • Nutrients • Metabolizable energy

Introduction

Dry climatic conditions and shortage of good quality forage have considerably increased animal feed costs in several developing countries including Iran. Therefore, in these situations, appropriate incorporation of cheap agricultural by-products or co-products will enhance animal production and decrease environmental pollutions [1]. Corn Steep Liquor (CSL), a main co-product of corn starch processing, is one such a co-product. The high Crude Protein (CP) [420 g/kg Dry Matter (DM)] and Metabolizable Energy (ME) [12.6 MJ/kg DM] as well as its good mineral and B-vitamin contents has made CSL a valuable co-product for being incorporated in ruminants' rations [2,3]. Annual production of CSL in Iran is about 4.000 t [4]. The acidic pH, viscous slurry nature with light to dark brown colour and ensiled odour are the other CSL characteristics [5]. The acidic pH of CSL is due to its high lactic acid content [200-250 g/kg DM] which might be a hurdle for its inclusion in the rations of ruminants [6]. In some studies, CSL also was used as a pellet binder [7].

Several studies have been done on the use of CSL on performance of ruminants, but the results were conflicting. It has been shown that Feed Intake (FI) and Average Daily Gain (ADG) were not affected when CSL was used in the diet of steers at the inclusion levels up to 100 g/kg DM [8]. In another study, incorporation of CSL in finishing lambs' diet up to 50 g/kg DM increased ADG and Feed Conversion Ratio (FCR), but at the levels upper than 100 g/kg DM it decreased animal performance [9]. Recently, in a study supplementation of fattening lambs' diet with CSL up to 100 g/kg decreased activities of rumen fibrolytic microbial enzymes, digestibility of nutrients and consequently animal performance, while increased the activity of rumen proteases.

More than 90% of CP content in CSL is in the form of amino acids and peptides, which can be rapidly degraded by rumen microbes. Researchers also reported that CSL protein was fully degraded in the rumen [10]. The high protein solubility and degradability of CSL in the rumen may lead to an asynchrony of protein and energy utilization. These problems can be alleviated by adding a readily available source of carbohydrate such as cereal grains or molasses, which may improve Microbial Protein Synthesis (MPS) in the rumen and consequently animal performance [11]. Molasses is a cost-effective energy source and has a higher ruminal degradation rate than cereal grains. Therefore, its incorporation in the ruminants' rations when CSL is used as a protein source may lead to a better synchrony of protein and energy utilisation in the rumen compared to those rations in which cereal grains such as barley and corn are being used as the sources of readily available carbohydrates. In Iran, annual production of molasses is around 480,000 t [12]. Therefore, the present study was aimed to evaluate effects of different dietary carbohydrate sources on the production performance, some ruminal and blood metabolites and nutrients digestibility in fattening male-lambs fed diets containing CSL.

Materials and Methods

Corn steep liquor

The CSL was purchased from a commercial supplier (Glucozan Company, Qazvin, Iran) and its chemical composition is shown in Table 1.

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Animals and experimental diets

Animal care and use were approved by Ethical Committee of Lorestan University and conducted according to guidelines outlined in the Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching [13]. Twenty four fat-tailed male Lori lambs with an average of 120 ± 7.5 days of age and Initial Body Weight (IBW) of 27.7 ± 3.41 kg were randomly allocated to three experimental diets in a balanced completely randomized design. Lambs were housed in individual pens (1.3 × 1.2 m). The feeding trial lasted 89 days with two weeks for acclimatizing to the pens and diets, and 75 days for fattening period. During the adaptation period, lambs were vaccinated against enterotoxaemia (3 mL per lamb; Razi Vaccine and Serum Research Institute, Iran), and treated with anthelmintic for external (1 mL of Azantole 10% per 7 l of water, as spraying method; Bayer, Germany) and internal (Triclabendazole+levamisole, 12 mL per lamb; Darou-Pakhsh Co., Iran) parasites.

All experimental diets were formulated to be iso-energetic and iso-nitrogenous experimental diets according to NRC recommendations [14]. The diet ingredients were similar, except that corn/barley was replaced by sugar beet molasses at levels 0 (control), 50 (M5) or 100 (M10) g/kg DM. The diets were provided ad libitum as a total mixed ration in tree equal meals daily at 08:00, 14:00 and 20:00 h for 75 days with a 5% of daily refusal. Feed offered and orts per lamb were collected and recorded daily during the experimental period. All animals had free access to drinking water. Lambs were individually weighed on days 0, 30, 45, 60 and 75 at 8:00 h after 16 h of feed deprivation. In each period, the ADG for individual lambs was calculated using Total Gain (TG) divided by number of days, and the FCR was calculated by dividing daily DM intake (DMI) to ADG (Table 2).

On day 50 of the experiment, faecal samples (50 g) were collected for 5 consecutive days for all lambs, and pooled (5 samples) to determine total-tract apparent digestibility of nutrients using acid-insoluble ash as an internal marker [15].

Rumen fluid samples (RF, 40-50 mL) were collected from on day 55 of trial 3 h after the morning feeding using a stomach tube. To avoid saliva contamination, the first 20 mL of RF form each animal was discarded [16]. The RF was strained through 4 layers of cheese cloth and pH was immediately determined using a pH meter (Sentron, model A102-003). For ammonia (NH3-N) determination, 5 mL of strained RF (SRF) was acidified with 1 mL of 0.2 N HCl to stop fermentation and frozen (-20°C). For Volatile Fatty Acids (VFAs) analysis, 1 mL of SRF was mixed with 0.25 mL of an acid solution containing 200 mL/L of orthophosphoric acid and 20 mM 2-ethyl-butyric acid and frozen at -20°C.

On day 57, blood samples were collected from all lambs using jugular venipuncture containing lithium heparinate 3 hrs after the morning feeding. Plasma was harvested by centrifugation at 3000 × g for 15 min and kept at -20°C pending further analyses.

Analytical procedures

Experimental rations, CSL and obtained orts were for DM, ash and CP, NDFom and ADFom (without sodium sulphite and with amylase treatment and Lignin (sa) [16-19]. Soluble Protein (SP) content in CSL was determined as described by and Metabolisable Energy (ME) conntent of CSL was estimated by gas production technique [20,21].

Rumen concentration of NH3-N was determined according to the procedure described by Broderick and Kang [22]. VFA concentrations, in centrifuged samples, were determined by gas liquid chromatography with ethyl-butyric acid as the internal standard [23]. Plasma samples were analysed by colorimetric methods for Total Protein (TP), albumin, blood urea-N (BUN), creatinine, triglycerides and glucose using Pars Azmun Diagnostic kits (Tehran, Iran).

Statistical analysis

Data on the digestibility of nutrients, ruminal and blood parameters were analysed using MIXED procedures of SAS. The model used was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is dependent variable assessed, μ is the population mean for the variable, T_i is the treatment effect on the assessed parameters (i.e., nutrient digestibility, blood and ruminal parameters) and eij is the random error associated with the observation ij.

Data on growth performance were analysed using the following model:

$$Y_{ij} = T_i + \beta_i (X_{ij} - \bar{X}) + eij$$

where, Y_{ij} is observation parameters, Ti is the fixed effect of treatment on the assessed parameters, β_i is the regression coefficient, X_{ij} is the initial body weight with mean $\bar{X}$ (covariate) and eij is the standard error of term. The IBW was used as covariate for analysing differences in body weight gain.

Orthogonal contrasts were used to test Linear (L) or Quadratic (Q) effects of molasses levels on assessed parameters.

Results

Nutrient digestibility

Chemical composition of the CSL is shown in Table 1. As shown in, supplementing dietary CSL with increasing levels of molasses up to 10% of DM linearly increased OM (L, p=0.05) and NDFom (L, p<0.05) digestibility, while DM, CP and ADFom digestibility were similar among the dietary treatments (p>0.05) (Table 3).

Table 1. Chemical composition of Corn Steep Liquor (CSL, % of dry matter or as stated).

Chemical composition	Amount (%)
Dry matter	52.5
Organic matter	92.5
Crude protein	42.5
Soluble protein	36.5
Neutral detergent fibre	0
Acid detergent fibre	0
Lactic acid	15.5
Metabolizable energy (MJ/kg DM)	12.6
Each value is average of 4 replicates	7.9

Table 2. Feed ingredients, chemical composition and metabolizable energy of experimental diets.

	Different energy sources		
	Control	M5	M10
Ingredients (% of DM)			
Wheat straw	10.0	10.0	10.0
Alfalfa hay (dried)	20.0	20.0	20.0
Wheat bran	10	9.0	9.0

Soybean meal	3.0	4.0	4.0
Corn Steep Liquor (CSL)	13.0	13.0	13.0
Barley grain, ground	19.75	17.25	14.75
Corn grain, ground	19.75	17.25	14.75
Molasses	0	5.0	10
Vitamin-mineral premix	2.0	2.0	2.0
Salt	0.5	0.5	0.5
Sodium bicarbonate	2.0	2.0	2.0
Chemical composition (% of DM or as stated)			
Dry matter (% of fresh weight)	86.8	85.7	85.5
Organic matter	89.5	89.1	88.6
Crude protein	15.8	15.7	15.6
Neutral detergent fibre	28.9	27.8	26.6
Acid detergent fibre	15.8	15.4	15.1
Metabolizable energy (MJ/kg DM)	10.7	10.7	10.6

M5, diet containing 5% molasses; M10, diet containing 10% molasses. 1Contained (per kg): 99.2 mg Mn, 50 mg Fe, 84.7 mg Zn, 1 mg Cu, 1 mg I, 0.2 mg Se, 9000 IU vitamin A, 2000 IU vitamin D and 18 IU vitamin E (Roshd-Daneh, Karaj, Iran).

Table 3. *In vivo* nutrients digestibility (% of DM) of Lori-male lambs fed experimental diets containing Corn Steep Liquor (CSL).

Item	Experimental diets (energy sources)			SEM	Contrast	
	Control	M5	M10		L	Q
Dry matter	0.777	0.790	0.797	0.013	0.35	0.81
Organic matter	0.790	0.802	0.828	0.011	0.05	0.81
Crude protein	0.782	0.792	0.795	0.021	0.68	0.88
Neutral detergent fibre	0.575	0.603	0.610	0.008	0.02	0.36
Acid detergent fibre	0.522	0.513	0.538	0.014	0.47	0.34

M5, diet containing 5% molasses; M10, diet containing 10% molasses; SEM, standard error of means; L, linear; Q, quadratic.

Ruminal parameters and blood metabolites

The effects of experimental diets on rumen fermentation parameters and biochemical blood metabolites are presented in Table 3. Increasing the level of molasses in the CSL containing diets had no effect on ruminal pH ($p>0.05$), but linearly decreased rumen concentration of NH3-N (L, $p<0.05$). Except for total VFA and molar proportion of butyrate were increased linearly (L, $p<0.05$) with increasing dietary molasses level, while the other individual VFAs including acetate (C2), propionate (C3), iso-butyrate, valerate, isovalerate and C2:C3 were similar ($p>0.05$) among the experimental diets.

Increasing the level of molasses in the diet up to 10% linearly increased (L, $p=0.05$) plasma TP concentration, while linearly reduced BUN concentration (L, $p<0.05$). Concentration of other plasma metabolites including albumin, creatinine, triglyceride and glucose remained unchanged ($p>0.05$) with increasing the level of molasses in the diets.

Discussion

Digestibility of nutrients

We found that adding molasses to the diets containing CSL improved apparent total tract digestibly of OM and NDFom, which might be attributed to the lower fibre contents (i.e., NDFom and ADFom) of molasses containing diets compared to the control diet as in 2. It has been reported that Water Soluble Carbohydrates (WSC) content of molasses are fermented in the rumen more rapidly compared to the starch of cereal grains [24]. This means that WSC supplied by molasses, as an energy source, may provide a better synchrony of protein and energy utilisation in the rumen when CSL, as a source of Rumen Degradable Protein (RDP), is included in the diet. Indeed, it has been shown that rumen fibrolytic enzyme activity and consequently fibre digestibility were improved when sheep fed diets synchronised for fermentable energy and RDP sources [25]. The findings of the present study agreed with those reported where adding 10% molasses to diet of sheep fed processed broiler litter, as a Non-Protein Nitrogen (NPN) source, increased apparent total tract digestibility of DM, CP and NDF [12]. Additionally, in another study OM digestibility was increased when barley was substituted with molasses in cattle fed silage based diet [26]. Additionally, adding molasses at levels of 0, 40, 80 or 120 g/kg of DM at the expense of corn in dairy cows' diets linearly increased apparent total tract digestibility of DM, OM, NDF and ADF [27].

Ruminal parameters and blood metabolites

In the present study, rumen pH values were in the normal physiological range, and remained unchanged by feeding diets containing different levels of molasses in fattening lambs [28]. Consistent to our results, in another experiment replacing corn with molasses at levels of 10 or 15% in the diet had no effect on ruminal pH of steers [29]. In the literature, effects of molasses on ruminal pH are controversial. In several studies, dietary supplementation of molasses decreased ruminal pH [30-32], while in some other studies rumen pH was increased by adding molasses to the diet [33-35].

Reduction of ruminal NH3-N with increasing dietary molasses level in the present study was likely due to a more synchrony between WSC content of molasses and SP of CSL which may have raised the incorporation of produced ammonia for MPS. In the present study, however, MPS was not estimated. Consistent to our results, it has been stated that sugars, particularly sucrose, as a fermentable carbohydrate source reduced ruminal NH3-N concentration compared to wheat starch in sheep fed grass silage-based diets [24]. They also reported that intestinal supply of Microbial Protein (MP) in sheep fed sucrose supplemented diet was 2.8 g/d higher than those fed starch supplemented diets. Similar findings were recorded with intraruminal infusion of urea and sucrose in sheep fed chopped alfalfa hay [36]. However, in a study ruminal ammonia was increased as the level of molasses was increased in the diet. They stated that this was probably due to a decrease in NH3-N absorption across the rumen wall due to a sudden drop in pH as a result of rapid fermentation in animals fed with the higher levels of molasses.

Volatile fatty acids represent the main supply of ME for the ruminant, and a decrease in their production would be nutritionally unfavorable for the ruminants [37]. In the present study, except for total VFA and molar proportion of butyrate which were increased with increasing molasses level in the diets, dietary treatments had no effect on total VFA and other VFAs. Increased total VFA concentration in the lambs fed increasing levels of molasses was probably attributed to the improved OM and NDF digestibility in them compared to the control lambs. Enhanced molar proportion of butyrate by supplementing diet with molasses was likely due to the fact that molasses shifts rumen fermentation towards production of more butyrate at the expense of propionate. Also, it has been demonstrated that replacing corn and barley with molasses increased the molar proportion of butyrate at the expense of propionate [38]. Consistent to these results, when comparing

different dietary fermentable energy sources, it has been reported that adding 24% molasses in the sheep diet increased total VFA and butyrate production compared to the diets containing the similar amounts of corn or barley. In another study, adding different levels of molasses up to 10% in the diet of sheep fed 24% processed broiler litter, as a NPN source, increased butyrate concentration, while the other individual VFAs were unchanged.

Increased blood TP concentration by increasing the level of molasses in the diet might be due to increased intestinal supply of MP, which enhanced the availability of amino acids for absorption [39]. Decreased BUN in lambs fed increasing levels of molasses was due to their lower rumen ammonia concentration. Based on the previous experiments, there is a positive correlation between rumen concentration of NH3 N and BUN concentration [40]. Blood glucose concentration was similar among the dietary treatments which was probably due to the same ruminal propionate production in the experimental groups as propionate is the main glucose precursor in ruminants and accounted for over than 75% of total blood glucose concentration [41] (Table 4).

Feed intake and performance

Increased TWG, ADG and improved FCR in lambs fed CSL containing diets supplemented with increasing molasses levels were not accompanied with increasing feed intake in them. Therefore, our results highlight and confirm the claim that synchronous supply of ruminal fermentable energy and protein has beneficial effects on growth performance of the ruminants *via* increasing MPS as previously reported by several researchers [42-44]. Based on Cornel Net Carbohydrate and Protein System (CNCPS) model, microorganisms that ferment soluble sugars (i.e., in molasses) could produce approximately 18% more MPS than those ferment starches (i.e., in high moisture corn). Enhanced OM and NDFom digestibility and increased total VFA production in lambs fed M5 and M10 diets would be the other reasons for the improved TWG, overall ADG and FCR in them. Similar to our results, it has been shown that synchronised supply of ruminal fermentable energy and protein in growing lambs did not affect their feed intake which agreed with our finding [45]. Other researchers also demonstrated that synchronized supply of starch sources and NPN in the rumen of lactating dairy cows did not affect feed intake, but improved milk production [46]. In another study, feeding different levels of molasses (up to 30% dietary DM) to male crossbred cattle calves at the expense of de-oiled rice bran had no effect on DMI, wheat straw and concentrate intakes (Table 5).

Table 4. Ruminal parameters and plasma metabolites of lori-male lambs fed experimental diets containing Corn Steep Liquor (CSL).

Item	Experimental diets (energy sources)			SEM	Contrast	
	Control	M5	M10		L	Q
Ruminal parameters						
pH	6.26	6.20	6.18	0.036	0.11	0.67
Ammonia (mg/dl)	21.8	18.2	17.6	0.589	<0.01	0.54
Total VFA (mmol/l)	101	106	113	3.22	0.05	0.78
Acetate (C2)	57.6	60.3	61.7	3.03	0.24	0.67
Propionate (C3)	20.4	19.8	20.1	0.634	0.53	0.78
Butyrate	17.7	19.1	19.9	0.571	0.02	0.93
Iso-butyrate	2.27	2.24	2.29	0.375	0.73	0.84
Valerate	2.66	2.57	2.72	0.165	0.44	0.68
Iso-valerate	1.89	1.93	1.96	0.136	0.76	0.92
C2:C3	2.85	3.08	3.07	0.149	0.41	0.79
Plasma metabolites						
Total protein (g/dl)	7.55	7.61	8.15	0.201	0.05	0.87
Albumin (g/dl)	3.60	3.65	3.90	0.122	0.15	0.67
Blood urea-N (mg/dl)	3.88	3.48	3.37	0.073	<0.01	0.39
Creatinine (mg/dl)	0.543	0.415	0.531	0.125	0.94	0.43
Triglyceride (mg/dl)	28.8	30.2	29.5	2.72	0.84	0.74
Glucose (mg/dl)	77.8	77.5	76.8	2.69	0.79	0.87

M5, diet containing 5% molasses; M10, diet containing 10% molasses; SEM: Standard Error of Means; L: Linear; Q: Quadratic

Table 5. Effect of different energy sources on feed intake and growth performance of lori-male lambs fed experimental diets containing Corn Steep Liquor (CSL).

Item	Experimental diets (energy sources)			SEM	Contrast	
	Control	M5	M10		L	Q
Day 1-75						
Initial body weight (kg)	27.5	27.4	28.4	0.865	0.41	0.71
Final body weight (kg)	43.2	44.4	45.6	0.881	0.11	0.43
Total weight gain (kg)	15.8	17.0	17.3	0.406	0.04	0.37
Average daily gain (g)	212	229	230	3.65	0.02	0.23
Feed conversion ratio	6.32	6.10	6.13	0.042	0.04	0.24
Dry matter intake (g)	1385	1380	1400	45.4	0.56	0.72
Organic matter intake (g)	1235	1223	1244	7.90	0.33	0.16
Crude protein intake (g)	220	218	219	5.29	0.84	0.91
NDF intake (g)	402	366	389	9.41	0.13	0.67
ADF intake (g)	218	209	214	4.34	0.23	0.92
Day 1-30						
Total weight gain (kg)	5.30	5.88	6.02	0.247	0.15	0.15
Average daily gain (g)	174	197	202	9.21	0.04	0.35
Feed conversion ratio	6.27	5.90	6.06	0.249	0.29	0.95
Dry matter intake (g)	1090	1144	1205	35.2	0.10	0.44
Day 31-45						
Total weight gain (kg)	3.47	3.47	3.73	0.199	0.26	0.57
Average daily gain (g)	226	229	247	9.85	0.15	0.45
Feed conversion ratio	5.97	5.90	5.54	0.274	0.84	0.21
Dry matter intake (g)	1426	1355	1418	57.2	0.31	0.72

Day 46-60						
Total weight gain (kg)	3.61	3.68	3.82	0.319	0.34	0.66
Average daily gain (g)	238	244	254	6.48	0.13	0.55
Feed conversion ratio	6.07	6.11	5.89	0.143	0.32	0.34
Dry matter intake (g)	1450	1498	1465	52.9	0.53	0.59
Day 60-75						
Total weight gain (kg)	3.31	3.42	3.67	0.256	0.23	0.55
Average daily gain (g)	221	228	245	17.1	0.24	0.65
Feed conversion ratio	6.54	6.55	6.22	0.206	0.85	0.19
Dry matter intake (g)	1470	1500	1507	52.2	0.45	0.73

M5, diet containing 5% molasses; M10, diet containing 10% molasses; SEM: Standard Error of Means; L: Linear; Q: Quadratic; NDF: Neutral Detergent Fibre; ADF: Acid Detergent Fibre.

Conclusion

Results of present study showed that dietary replacement of a corn/barley mixture with molasses up to 100 g/kg DM increased OM and NDFom digestibility, total ruminal VFA and production performance of fattening lambs fed a diet containing CSL, while feed intake was unchanged. More works especially on *in vivo* animals is needed to improve utilisation efficiency of corn steep liquor as a cheap protein source for ruminants.

Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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