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The influence of the different carbohydrate sources on utilization efficiency of processed broiler litter in sheep

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ABSTRACT

In a completely randomized design, fifteen male Moghani sheep were used to determine the influence of supplementing processed broiler litter (BL) with different carbohydrate sources (*i.e.*, corn, barley or molasses) on the nutrients digestibility, microbial protein (MP) production, ruminal parameters and blood metabolites. The three dietary treatments, which were iso-caloric and iso-nitrogenous, were corn diet (alfalfa hay, wheat straw, processed BL, corn grain), barley diet (alfalfa hay, wheat straw, processed BL, barley grain) and molasses diet (alfalfa hay, wheat straw, processed BL, molasses). The digestibility of dry matter, crude protein and neutral detergent fiber and MP in sheep fed molasses diet were higher ($P<0.05$) compared with those fed with diets containing corn or barley. However, sheep fed molasses diet had lower ($P<0.05$) ruminal pH ammonia concentration than those fed with other diets. Including various carbohydrate sources in the diets had no effect on volatile fatty acid (VFA) concentrations ($P>0.05$), except for total VFA and molar proportion of butyrate which increased ($P<0.05$) by molasses feeding. From blood metabolites only the blood urea-N concentration in sheep fed diet containing molasses was lower ($P<0.05$) than diet containing corn. In conclusion, adding molasses to processed BL-containing diet led to improved nutrient digestibility and MP production in sheep.

1. Introduction

The broiler litter (BL) is a solid waste consisting of poultry excreta (urine and feces), bedding material, feathers and spilled feed. In Iran, production of dry BL exceeds 1.3 million t/year. The proper use of inexpensive agro-industrial co-products such as BL is important to beneficial livestock production (Negesse et al., 2007). The commercial value of poultry litter as a feedstuff is based usually on its crude protein (CP) (150–350 g/kg dry matter (DM), Obeidat et al., 2011) and minerals content (Jordaan, 2004). However, Van Ryssen (2000) reported that 400–600 g/kg of the CP in BL is in the form of non-protein N (NPN) which is quickly degraded in the rumen (Animut et al., 2002). The synchronization of degradation rate of NPN and carbohydrate may lead to improve microbial protein (MP) synthesis in the rumen, decrease urinary N excretion and animal performance (Cole and Todd, 2008). Sugars are considered to have a fast degradation rate, and starch an intermediate rate (Sniffen et al., 1992). Molasses is rich in water-soluble carbohydrate (WSC) (Oba, 2011), which is cost-effective source of

Abbreviations: ADFom, ash-free acid detergent fiber; BL, broiler litter; BUN, blood urea-N; CP, crude protein; DM, dry matter; DMD, *in vivo* DM digestibility; DOMD, digestible OM in DM; Lignin(sa), lignin measured by solubilization of cellulose with sulphuric acid; ME, metabolizable energy; MP, microbial protein; NDFom, ash-free neutral detergent fiber; OM, organic matter; PD, purine derivatives; TPD, total purine derivatives; VFA, volatile fatty acids; WSC, water-soluble carbohydrate

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energy in comparison to starchy energy sources such as corn and barley. Because corn, barley or molasses differ in their rates and extents of ruminal degradation, our hypothesis was that the supply of N from processed BL to the rumen and different dietary energy source to support MP synthesis in the rumen would differ. Therefore, the objective of the study was to evaluate the effect of dietary inclusion of corn, barley or molasses as a source of energy on nutrients digestibility, ruminal parameters, blood metabolites and microbial N flow to the duodenum in sheep fed processed BL.

2. Materials and methods

2.1. Broiler litter

Broiler litter was obtained from the main factory in Sabzevar city, in which BL was humidified up to 23%, and then the material was thermally processed by indirect vapor pressure at 80 °C for 20 min.

2.2. Animal study

Fifteen mature-male Moghani sheeps with average live weight of 62 ± 2.3 kg were allocated individually in metabolic cages to allow total collection of feces and urine. The animals were assigned to a balanced completely randomized design with 5 animals in each diet. The three experimental diets which are shown in Table 2 consisted of 260 g alfalfa hay, 260 g wheat straw and 240 g processed BL per kg of DM, and were supplemented with either 240 g corn (corn diet), barley (barley diet) or sugar beet molasses (molasses diet) per kg DM. The ingredients and nutrient composition for the experimental diets, which were formulated according to NRC (2007), are shown in Table 2. The diets were provided in two equal meals each day at 08:00 and 17:00 h with free access to clean water and mineral–vitamin block. All sheeps were fed with equal amounts of the diets, which had no differences in type and proportion of ingredients except for main carbohydrate source (*i.e.,* corn, barley or molasses) in order to avoid the influences of ingredient characteristics and intake level on the rumen parameters (Seo et al., 2010).

Table 1
Mean chemical composition and metabolizable energy (ME) of processed BL (g/kg DM or as stated) (n=4).

Item	Processed BL
DM (g/kg fresh weight)	930
CP	238
NPN (g/kg CP)	451
TP (g/kg CP)	549
EE	22.3
NDFom	353
ADFom	185
Lignin(sa)	75
Ash	184
ME[a] (MJ/kg DM)	9.3

DM, dry matter; CP, crude protein; NPN, non-protein nitrogen; TP, true protein; EE, ether extract; NDFom, ash-free neutral detergent fiber; ADFom, ash-free acid detergent fiber.

[a] The metabolizable energy calculated by the following equation (Deshck et al., 1998); ME (MJ/kg DM)=digestible OM (g/g DM) × 18.5 (MJ/kg DOM) × 0.80.

Table 2
Ingredients and nutrient composition and metabolizable energy (g/kg DM or as stated) for the experimental diets given to sheep.

	Source of carbohydrate				
	Corn	Barley	Molasses	SEM	P-value
Ingredients					
Alfalfa hay	260	260	260		
Wheat straw	260	260	260		
Processed BL	240	240	240		
Ground corn	240	0	0		
Barley	0	240	0		
Molasses	0	0	240		
Nutrient composition					
DM (g/kg fresh weight)	928	933	923	6.1	0.16
CP	123	125	121	2.4	0.44
NDFom	422[b]	452[a]	377[c]	3.5	0.011
ADFom	269[a]	271[a]	252[b]	2.9	0.017
Lignin(sa)	62.9[ab]	65.2[a]	60.4[b]	1.5	0.032
Ash	99[b]	103[b]	122[a]	3.05	< 0.01
Ca	6.6	6.7	6.9	0.45	0.90
P	3.4	3.5	2.6	0.34	0.63
ME (MJ/kg DM)	9.04	8.91	8.70	0.24	0.54

DM, dry matter; CP, crude protein; NDFom, ash-free neural detergent fiber; ADFom, ash-free acid detergent fiber; ME, metabolizable energy. Means in the same row with different superscripts differ ($P < 0.05$).

The apparent nutrients digestibility period lasted for 28 days with 21 days for adaptation period to metabolic cages and diets and 7 days for samples collection (Givens et al., 2000). During the last week, samples of feeds and feces from each sheep on each treatment were weighed and 10% sample was frozen for later analysis. At the same time, urine sample was collected in a bucket containing 100 ml of sulfuric acid solution (containing 10 ml of concentrated sulfuric acid in 100 ml of distilled water), to keep the final pH below 3, which was placed below the urine outlet in the metabolic cages. The collected urine from an individual animal was measured and a subsample of 20 ml was stored at −20 °C for the estimation of purine derivatives (PD) (Chen and Gomes, 1995). The DOMD (digestible organic matter (OM) in the DM) was calculated using the following equation:

$$\text{DOMD}\ (g/\text{kg DM}) = [\text{OM intake}\ (g) - \text{faecal OM}\ (g) / \text{DM intake}\ (\text{kg})]$$

The metabolizable energy (ME) values of the experimental diets were calculated using the following equation (Agricultural and Food Research Council 1993):

$$\text{ME}\ (\text{MJ/kg DM}) = 0.0157 \times \text{DOMD}$$

2.3. Rumen liquor samples

Samples of rumen liquor were withdrawn 0, 3 and 6 h after morning feeding on the day 6 of last week of experiment by stomach tube, strained through two layers of muslin and pH was measured immediately. Samples

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(5.0 ml) of rumen liquor collected into 1 ml of HCl 0.2 N, were transported to the laboratory for ammonia analysis. For analysis of ruminal volatile fatty acids (VFA), 1 ml of rumen liquor was added to 0.25 ml of an acid solution containing 20% orthophosphoric acid and 20 mM 2-ethyl-butyric acid, and then frozen at −20 °C.

2.4. Blood samples

Individual blood samples (10 ml) for plasma production were collected by jugular venepuncture (Scaife et al., 1982) on day 6 of sampling period, 0 (just before the morning feeding), 3 and 6 h after feeding. Plasma sample was harvested after centrifugation at 1500 × *g* at room temperature for 15 min and stored at −20 °C until analyzed.

2.5. Laboratory analyses

Before laboratory analysis, processed BL, diets and feces were oven-dried at 55 °C to reach a constant weight, and then ground to pass a 1 mm diameter sieve (Wiley mill, Swedesboro, USA) and following (AOAC, 1990) procedures, DM (# 930.15), ash (# 924.05) and N (# 984.13) were analyzed. The determination of ash-free neutral detergent fiber (NDFom) was performed without sodium sulphite and expressed exclusive of residual ash according to Van Soest et al. (1991). Ash-free acid detergent fiber (ADFom) was determined and expressed exclusive of residual ash (AOAC, 1990; # 973.18). Lignin(sa) was measured by solubilization of cellulose with 720 g/kg sulphuric acid (Robertson and Van Soest, 1981). Calcium was determined by atomic absorption (Temminghoff and Houba, 2004) and *P* by spectrophotometer (Chapman and Pratt, 1961). Rumen liquor was analyzed for ammonia N as described by (Broderick and Kang, 1980). Urinary PD was estimated by spectrophotometric method, as described by Chen and Gomes (1995). Allantoin was measured in urine by colorimetric method at 522 nm after its conversion to phenyl hydrazone. The sum of xanthine and hypoxanthine were calculated by their conversion to uric acid with xanthine oxidase (Sigma; Catalog no. X-1875, 5 Units, Germany), with subsequent optical density at 293 nm. The uric acid was measured from the reduction in optical density at 293 nm following degradation of uric acid to allantoin with uricase (Sigma; Product no. U-9375, Germany). Based on Chen and Gomes (1995) technique, non-linear equation to describe in quantitative relationship between absorption of microbial purines and excretion of PD in urine is:

$$Y = 0.84X + (0.150W^{0.75}e^{-0.25x})$$

where *Y* is the daily urinary PD excretion in mmol/d, *X* is the daily absorbed exogenous purines in mmol/d, and $W^{0.75}$ the metabolic body weight (kg) of animal.

The calculation of X from Y based on above equation can be performed by means of the Newton–Raphson iteration process as given below:

$$X(n+1) = Xn - \frac{f(Xn)}{f'(Xn)}$$

where $f(X) = 0.84X + 0.150W^{0.75}e^{-0.25X} - Y$ and the derivatives of $f'(X) = (0.84 - 0.038W^{0.75}e^{-0.25x})$.

Finally, produced microbial nitrogen was estimated by the following equation:

$$\text{Microbial}N\ (g/d) = \frac{X\ (\text{mmol}/d) \times 70}{0.116 \times 0.83 \times 1000} = 0.727X$$

After thawing, acidified samples of rumen liquor were centrifuged (14000 rpm, 5 °C, 15 min) and VFA were determined by gas liquid chromatography using ethyl-butyric acid as the internal standard as described by Stewart and Duncan (1985). Plasma biochemical parameters including total proteins, albumin, blood urea-N (BUN), creatinine, glucose and cholesterol were determined by the use of spectrophotometer.

2.6. Statistical analysis

A completely randomized design was used to determine the effect of carbohydrate source on various parameters. All data were analyzed using the GLM procedure in SAS (SAS Institute, 2001), based on the statistical model:

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

where Y_{ij} is the observation (digestibility of nutrients, microbial protein, parameters of rumen fermentation and plasma), μ is the general mean, T_i is the effect of energy source and e_{ij} is the standard error of term. Differences among means were tested using Duncan method (Steel and Torrie, 1980).

3. Results

3.1. Chemical composition of processed BL and nutrients digestibility

The chemical analysis and ME value of processed BL is shown in Table 1. It can be seen in Table 3 that the apparent digestibility of DM, CP and NDFom of diet contained molasses was significantly ($P < 0.05$) higher than those diets which contain corn or barley. However, the digestibility of OM, DOMD and estimated ME were not influenced by dietary treatments.

3.2. Microbial protein production

Sheep fed molasses diet had higher ($P < 0.05$) urinary allantoin in comparison to those offered other diets (Table 4). The value of xanthine plus hypoxanthine in sheep fed molasses diet increased ($P < 0.05$) compared with barley diet but, experimental diets did not affected uric acid ($P > 0.05$). Total purine derivatives (TPD) excreted, TPD absorbed and MP increased ($P < 0.05$) in sheep fed molasses diet compared with those fed with other diets.

3.3. Ruminal parameters and plasma metabolites

Ruminal pH and ammonia concentration decreased in sheep fed with molasses diet compared to corn or barley

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Table 3
Total tract nutrient digestibility and estimated metabolizable energy in sheep fed the experimental diets containing processed BL with different carbohydrate sources.

	Source of carbohydrate				
	Corn	Barley	Molasses	SEM	*P*-value
Digestibility					
DM	650[b]	645[b]	673[a]	6.9	0.043
OM	678	670	689	7.8	0.17
CP	671[b]	682[b]	706[a]	6.4	0.025
NDFom	601[b]	588[b]	638[a]	7.3	0.021
ADFom	573[ab]	538[b]	586[a]	9.6	0.040
DOMD	618	609	612	5.3	0.51
ME (MJ/kg DM)	9.70	9.56	9.61	0.077	0.53

DM, dry matter (g/kg DM); OM, organic matter (g/kg OM); CP, crude protein (g/kg CP); NDFom, ash-free neural detergent fiber (g/kg NDFom); ADFom, ash-free acid detergent fiber (g/kg ADFom); DOMD, digestible organic matter in dry matter (g/kg DM); ME, metabolizable energy. Means in the same row with different superscripts differ ($P<0.05$).

Table 4
Urinary purine derivatives excretion and microbial protein (MP) production in sheep fed the experimental diets containing processed BL with different carbohydrate sources.

	Source of carbohydrate				
	Corn	Barley	Molasses	SEM	*P*-value
Purine derivatives (mmol/d)					
Allantoin	10.7[b]	11.2[b]	12.5[a]	0.38	0.011
Uric acid	2.05	2.17	2.09	0.214	0.27
X+H	0.92[ab]	0.84[b]	1.04[a]	0.055	0.021
TPD excreted	13.7[b]	14.2[b]	15.6[a]	0.76	0.013
TPD absorbed	15.4[b]	16.1[b]	17.8[a]	0.83	0.014
MP (g/d)	70.2[b]	72.9[b]	80.7[a]	4.13	0.015

X+H, xanthine+hypoxanthine; TPD, total purine derivatives. Means in the same row with different superscripts differ ($P<0.05$).

Table 5
Ruminal parameters and plasma metabolites in sheep fed the experimental diets containing processed BL with different carbohydrate sources.

	Source of carbohydrate				
	Corn	Barley	Molasses	SEM	*P*-value
Ruminal parameters					
pH	6.29[a]	6.27[a]	6.12[b]	0.050	0.048
Ammonia (mg/dl)	22.1[a]	20.4[ab]	18.1[b]	1.50	0.037
Total VFA (mmol/l)	101.1[b]	99.2[b]	106.8[a]	3.50	0.032
VFA (mmol/l)					
Acetate (C2)	64.7	64.4	66.6	2.70	0.19
Propionate (C3)	22.5	21.8	20.1	2.20	0.30
Butyrate	11.9[b]	13.1[b]	16.6[a]	0.59	0.002
Isovalerate	1.29	1.46	1.12	0.171	0.60
Valerate	1.54	1.60	1.38	0.202	0.73
C2:C3	2.96	3.01	3.07	0.169	0.47
Plasma metabolites					
Total protein (g/dl)	7.47	7.97	7.49	0.355	0.28
Albumin (g/dl)	3.16	3.42	3.37	0.171	0.45
Blood urea-N (mg/dl)	3.74[a]	3.50[ab]	3.20[b]	0.109	0.040
Creatinine (mg/dl)	1.02	0.90	1.00	0.040	0.12
Cholesterol (mg/dl)	75.3	74.7	72.0	1.52	0.21
Glucose (mg/dl)	68.3	67.6	66.4	1.24	0.38

Values of ruminal and plasma parameters are means across sampling times of 0, 3 and 6 after morning feeding. Means in the same row with different superscripts differ ($P<0.05$).

diets (Table 5). However, animal consumed the former diet had higher ($P<0.05$) total VFA concentration and molar proportion of butyrate. Apart from BUN concentration, dietary treatments had no significant effect on the plasma metabolites.

4. Discussion

4.1. Chemical composition of processed BL and nutrients digestibility

The high DM content (930 g/kg fresh weight) of processed BL was due to the thermal processing and is similar to the value reported by Muia et al. (2000). Processed BL with more than 250 g/kg DM moisture content may be sticky and unable to mix with other ingredients of the ration (Jordaan, 2004). The processed BL was found higher in CP (238 g/kg DM) than values reported by Jackson et al. (2006) and Mavimbela and Van Ryssen (2001). The variations in the CP contents of the litter are probably due to age of the litter, type of bedding material and the level of feeding. The obtained CP content in this study suggests a good quality of BL as a ruminant feed, as Bagley and Evans (1998) mentioned that good quality of BL should contain 200–300 g CP per kg DM. Litter can be low in CP because of very high ash content. The NPN fraction of BL was about half of the CP content, however, uric acid, main part of NPN in BL, is broken down in the rumen at a slower rate compared to urea and consequently most of the ammonia is captured by the rumen microbes (Oltjen et al., 1968). The content of ether extract was similar to the amount reported by Obeidat et al. (2011). The mean value for NDF in BL was higher than those reported by Mekasha et al. (2004) and Obeidat et al. (2011). The NDF content of litter is quite variable, affected by type of bedding material, the number of growing periods before harvest and extent of heat damage (Goetsch and Aiken, 2000). The ash concentration of BL (184 g/kg DM) was within the acceptable range for ruminant feed (*i.e.*, between 150 and 250 g/kg DM) as reported by Jacob et al. (1997). Consequently, this has led to high ME content in BL (Table 1).

Table 3 showed that the digestibility of nutrients in sheep fed with molasses diet was the highest among treatments which may be due to the availability of sucrose which is more rapid fermentable carbohydrate than starch in other treatments (Chamberlain et al., 1993). The lower fiber fractions (NDFom, ADFom and lignin(sa)) may, also, improve the nutrients digestibility in animal fed molasses-contained diet in comparison to others. In agreement to our results, Broderick and Radloff (2004) reported an increase in the digestibility of DM, OM, NDF and ADF when corn was substituted with molasses in the ration of dairy cows.

4.2. Microbial protein production

The improvement of MP production in sheep fed molasses diet than those fed with others diets was probably

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related to the supply of more readily fermentable energy source (*i.e.*, sucrose in molasses) with soluble N of BL for the synthesis of MP (Richardson et al., 2003; Van Soest, 1994). Synchronous supply of ruminal fermentable carbohydrates (sucrose in molasses) and rumen degradable protein of BL is a possible reason to enhance the production of MP (Sinclair et al., 1993).

Moreover, Chamberlain et al. (1993) concluded that sugars, particularly sucrose, were better than starch as an energy source for the production of MP in sheep. They observed that the microbial N entering the small intestine in sheep fed diet supplemented with sucrose (14.8 g/d) was higher than sheep fed starch supplemented diet (11.9 g/d). In the contrary to our findings, replacement of corn with sucrose has resulted in decrease of the MP synthesis in dairy cows diet (Sannes et al., 2002).

4.3. Ruminal parameters and plasma metabolites

Mean ruminal pH values in all experimental diets were within the normal physiological range of 6.1–6.8 as reported by Van Soest (1994) and sheep fed molasses-contained diet had the lowest pH value. Similarly, Sahoo et al. (1999) have noted a decline in ruminal pH when de-oiled rice bran was replaced with molasses in cattle calves ration. However, inconsistent with the results of Preston et al. (1971) and Marty and Henderickx (1973) who found that ruminal pH was higher for molasses than for cereal grain diets which may be due to higher content of cations (mainly K^{+}, Ca^{++} and Mg^{++}) that led to high rumen buffering capacity in molasses-containing diets (Araba et al., 2002).

In all diets, ruminal ammonia concentration was more than 5 mg/dl, which according to Satter and Slyter (1974) is the minimum level required by rumen microorganism to support their optimum growth. Lower rumen ammonia concentration in molasses diet can be justified by the raise in the production of MP (Table 4). These results are in agreement with results achieved by Obara and Dellow (1993) in sheep. Dissimilar to our results, Sahoo et al. (1999) observed a raise in the ruminal ammonia concentration with increasing levels of dietary molasses, which is probably due to lower ruminal pH obtained with molasses diet.

The higher NDFom digestibility in animals fed molasses diet may have led to improve the total ruminal VFA concentration in comparison to other animals. Similar findings noted by Sahoo et al. (1999) where total ruminal VFA concentration that had significantly increased due to feeding levels of molasses at the expense of de-oiled rice bran. On the other hand, a reduction in the total VFA concentration was recorded by Araba et al. (2002) when dietary levels of molasses substituted with ground barley, which is possibly due to the presence of protozoa in the rumen of cattle fed molasses that reduce the concentration of VFA. Additionally, the increase in the concentration of butyrate in molasses diet consistent with other observations (Araba et al., 2002; Hristov et al., 2005) is possibly due to stimulation of large numbers of small important protozoa population that produce butyrate than other microbes as the main end products of their fermentation. Numerous studies concluded that replacing cereal grain with molasses had led to increase in the molar proportion of butyrate instead of propionate (Pate, 1983).

In all animals, the BUN concentration show parallel pattern to the ruminal ammonia concentration and was consistent with results of Sannes et al. (2002) in dairy cows. It is reported that concentrations of BUN are usually positively associated with ruminal ammonia concentrations (DePeters and Ferguson, 1992).

The concentration of plasma glucose was not affected by diets which may be due to similar ruminal propionate concentration, main glucose precursor, obtained in all animals (Brockman, 1993).

Despite favorite chemical composition, nutrients digestibility and other parameters, feeding raw BL to livestock can cause serious hazards to human and animal health due to presence of pathogenic organisms, pesticides and drug residues in the litter (Al-Rokayan et al., 1998). However, the thermal processing method used in the current study significantly decreased total population of pathogenic bacteria, *Escherichia coli* and *Salmonella* (Badiee Baghsiah, 2011).

5. Conclusion

Molasses incorporation in BL-containing diet may lead to improved nutrient digestibility and MP in sheep. However, more work particularly on *in vivo* animal is required to support the usefulness of BL in the ruminant diets.

Conflict of interest

There is no conflict of interest existing.

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