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Substitution of Polymer Coated Urea for Soybean Meal on Digestibility, Rumen Fermentation, and Microbial Nitrogen Yield in Sheep Fed High Level of Concentrate

Research Article

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ABSTRACT

An experiment was conducted in a 4 × 4 Latin square design experiment to evaluate the effects of Optigen as polymer-coated urea (PCU) and sodium bentonite (SB) on intake, nutrient digestibility, nitrogen retention, rumen fermentation and microbial nitrogen yield in sheep fed high levels of concentrate. Four isonitrogenous and isocaloric diets with 60% concentrate and 40% corn stalks (dry matter (DM)) were offered twice daily to ensure about 5% ort. The treatments were control (CON), soybean meal replaced by Optigen (OPT), SB (CON+2% SB), and OPTSB (OPT+2% SB). Sheep on SB treatment showed a lower nitrogen ($P<0.02$) and organic matter (OM) true digestibility ($P=0.04$). Nitrogen (N) intake, and its fecal excretion were not affected by different treatments, but N retention was lower ($P=0.04$) in sheep fed Optigen. Irrespective of nitrogen source, SB decreased purine derivates ($P=0.03$) and microbial nitrogen yield ($P=0.03$). Sheep fed Optigen had greater ($P<0.03$) ruminal NH_3-N concentration compared with SB treatment. The pH was not affected by different treatments. The volatile fatty acids (VFA) tended to be higher ($P=0.052$) for treatments that included Optigen. The OPTSB had lower proportion of acetate ($P=0.03$), but had higher propionate and valerate ($P<0.02$) compared to other treatments. The ruminal NH_3-N for sheep fed Optigen continuously increased, with the highest level achieved 3 h after feeding. No differences were discerned in ruminal kinetics and effective degradability of corn stalk DM for different treatments. It was concluded that replacing soybean meal with Optigen for sheep fed high level of concentrate had no adverse effect on nutrient digestibility, microbial nitrogen yield, rumen fermentation and effective degradability (ED). Adding SB to Optigen had no beneficial effects on nutrients digestibility, N retention, microbial N yield and ED of corn stalk.

KEY WORDS corn stalk, digestibility, polymer-coated urea, ruminal fermentation, sheep, sodium bentonite.

INTRODUCTION

The global importance of livestock and their products is increasing as consumer demand in the developing countries expands with population growth and rising incomes (Sal, 2011). According to FAO report food production must increase by 70 percent tail 2050, annual meat production will need to rise by over 200 million tones to reach 470 million tones (FAO, 2009). Ruminant animals are one of the man's most valuable and renewable resources. As protein is one of the most limiting in human alimentation, the ruminants are indispensable utilizing marginal lands, crop residues and by products inedible by humans (Sal, 2011). The nitrogenous components of the diet support the protein metabolism of the rumen organisms and their host (Van Soest, 1994). High quality plant and animal proteins are some of the best pro-

tein sources to be used, but they are usually expensive and not always economically justified (Jooste, 2012). Urea is probably the most popular source of non-protein nitrogen (NPN) in ruminants (Jayasuriya and Perera, 1982). Urea nitrogen is converted into microbial protein via ammonia, supplying additional protein to the host animal (Castro *et al*. 1999). However, it could be rapidly hydrolyzed to NH_3, making urea inefficient for microorganisms in the rumen (Satter and Roffler, 1975), which can cause ammonia toxicity and depress feed intake and animal performance or even lead to animal's death (Huntington *et al*. 2006). One approach to solve this problem could be to modify urea to control its rate of degradation so that NH_3 release more closely parallels to carbohydrate digestion in the rumen (Pinos-Rodríguez *et al*. 2010). Optigen II (OPT, Alltech Inc. Nicholasville, KY) is polymer-coated urea which allows the diffusion of urea through micro-pores and thus slows down the rate of nitrogen release in the rumen (ICF Consulting, 2004). It is potentially useful in sheep diet formulation when the soybean meal price is high. In addition, sodium bentonite (SB) is an expanding lattice clay of the montmorillonite group of minerals (Bates and Jackson, 1980) that decreases the ruminal NH_3 concentration and increases bacterial protein supply to the small intestine of host animal (Ivan *et al*. 1992). Therefore, replacing soybean meal with OPT in high-concentrate diets is expected to be beneficial for sheep under productive conditions, when sheep diets are comprised of high level of concentrate and limited protein sources.

However, no published studies have evaluated the effect of dietary OPT and SB on digestibility microbial N yields and rumen fermentation in sheep fed high concentrate diets. We hypothesized that replacing soybean meal with OPT could influence the rumen fermentation, increase the digestibility of diet, and increase microbial protein synthesis in the rumen. Also adding SB to OPT in sheep fed high concentrate diets could have more beneficial effects on the nutritional value, rumen fermentation or microbial protein synthesis in the rumen.

MATERIALS AND METHODS

Animals, diets, housing, and experimental design

Animals care, handling, and surgical techniques followed the protocols approved by the Animal Ethics Committee of Chinese Academy of Agricultural Science in compliance with directive on the protection of animals used for scientific purposes (2010/63/EU). The current experiment was conducted from July to October 2011 at the Experimental Station of the Chinese Academy of Agricultural Sciences (CAAS) in Nankou, Beijinig, China. Four mature thin-tailed Han × Dorper crossbreed wethers (initial BW 52±0.15 kg) with ruminal cannula were housed in individual pens in an enclosed, climate-controlled room, and randomly assigned to 1 of 4 dietary treatments in a 4 × 4 Latin-square design balanced with 4 trial periods. Each period lasted 24 days (d) with 14 d of diet adaptation and 10 d of sampling (5d for digestibility and 1 d of rest before rumen fluid sampling to prevent interference with the digestibility trial, 1 d for rumen fluid collection, and 3 d for the in situ trial). Four days prior to the digestibility trial, animals were placed individually in metabolism cages and allowed to adapt to their cages. Four isonitrogenous and isocaloric dietary treatments composed of 40% corn stalk (DM basis) and 60% concentrate (DM basis) were formulated (Table 1). The treatments were control (CON), soybean meal isonitrogenously replaced by Optigen (OPT), CON diet was supplemented with 2% SB (SB), and OPT diet with an additional 2% SB (OPTSB). The OPT (Optigen II, Alltech Inc. Nicholasville, KY), was a prilled urea coated with a porous biodegradable polymer with a controlled release property containing 97% urea and 3% coating (by weight) with ≥ 41% (by weight) nitrogen content. Corn stalk (Qingdao Jiahong Import and Export Co. Qingdao, Shangdon, China) was a milled and pelleted product containing ≥ 5% protein, ≤ 8% moisture, and ≤ 10% ash. Animals were fed ad libitum a mixture of the concentrate and corn stalk pellets twice daily in two equal portions at 7:00 and 19:00 h to ensure approximately 5% ort. All animals had free access to water throughout the trial. The animals were weighed at the beginning and the end of each period to record live weight changes.

Sample collection

The feed samples were collected weekly and composited for each animal in each period. Samples were oven-dried at 55 °C for 48 h and ground through a 1 mm screen (standard model 4, Arthur Thomass Co., Philadelphia, PA) for further chemical analysis. Orts were weighed and recorded daily before morning feeding, bulked by animal and subsamples, dried at 55 °C for 48 h, and then composited by animal and period for subsequent chemical analysis.

Urine and feces were collected and weighed separately for 5 consecutive days (d 15-20) using metabolism cages. Ten percent (by weight) of total feces were sampled and stored at -20 °C for each sheep within the period for further analysis. Daily urine was collected in plastic buckets containing 100 mL of 10% (vol/vol) sulfuric acid to ensure ammonia preservation by reducing pH to below 2.5. Excreted urine volumes for each sheep within the period were diluted to 5 L with distilled water, and then 20 mL of subsample was immediately taken and stored at –20 ºC for analyzing total nitrogen and PD components for each sheep within the period.

Table 1 Ingredients (% of DM) and nutritive composition of experimental diets

Ingredients	Treatments			
	CON	SB	OPT	OPTSB
Corn stalk	40.3	40	40	40
Barley	18.7	16.6	15.2	11.53
Wheat bran	12.6	11.6	8.8	9.6
Corn	16	17.5	33	34
Soy bean meal	11	11	0	0
Polymer coated urea (Optigen)	0	0	1.8	1.8
Sodium bentonite	0	2	0	2
Mineral and vitamin premix[1]	0.2	0.2	0.3	0.3
Calcium carbonate	0.6	0.6	0.6	0.5
Nutrient composition of diet				
Dry matter (DM)	90.16	90.28	90.26	90.63
Crude protein (CP)	12.90	12.57	12.90	12.69
Metabolizable energy (ME, Mj/kg)[2]	8.33	8.20	8.25	8.04
Organic matter (OM)	89.30	87.72	90.34	88.77
Neutral detergent fiber (NDF)	37.30	36.68	34.34	35.08
Acid detergent fiber (ADF)	18.54	18.33	17.27	17.21
Acid detergent lignin (ADL)	3.92	3.87	3.74	3.72
Hemi cellulose	18.76	18.35	17.07	17.87
Cellulose	14.62	14.46	13.53	13.49
Calcium	0.52	0.44	0.40	0.36
Phosphorus	0.38	0.37	0.30	0.29
Corn stalk/concentrate	2/3	2/3	2/3	2/3

[1] Manufactured by Precision Animal Nutrition Research Centre, Beijing, China. The premix contained (per kg): Fe: 22.1 g; Cu: 13.0 g; Mn: 30.2 g; Zn: 77.2 g; Se: 19.2 g; I: 53.5 g; Co: 9.10 g; vitamin A: 56.0 g; vitamin D_3: 18.0 g and vitamin E: 170 g.
[2] NRC (2007).
CON: control; SB: control + sodium bentonite; OPT: polymer coated urea (Optigen); OPTSB: polymer coated urea + sodium bentonite.

Ruminal fluid samples were taken on d 21 of each period at -1, 1, 3, 5, 7, and 9 h post-feeding. Approximately 0.2 L of ruminal contents was obtained from several sites within the rumen and strained through 4 layers of cheesecloth. Ten mL of filtrate sub-sample were preserved with 3 mL chilled 25% (wt/vol) HPO_3, and 5 mL of filtrate sub-sample was preserved with 1 mL 1% H_2SO_4 for determining VFA and NH_3-N concentrations, respectively. The samples were stored at -20°C until they were analyzed. Ruminal pH values were measured immediately after collecting ruminal content samples by using a portable pH meter (Sartorius PB-10, Sartorius Co., Germany) at each time point.

In situ ruminal incubations were conducted to determine nutrient degradation of corn stalk in the rumen during the last 3 d of each experimental period. Two grams (DM basis) of milled corn stalk (milled through a 2 mm screen) were, placed into nylon bags (6×10 cm^2, nitrogen-free polyester, pore size 50±15 µm, Gonglu Instrument Company, Zhejiang, China), and sealed. Duplicate bags for each incubation time were placed in the rumen of each fistulated sheep for 0, 6, 12, 24, 48, and 72 hours (Osuji *et al*. 1993) in reverse order of incubation time so that all bags were removed simultaneously. The 0 hour samples were not placed in the rumen, but treated in the same rinsing procedure described for the other bags. Upon removal, nylon bags were thoroughly washed with running tap water until no further colored liquid was extruded and then dried at 55°C for 48 hours. The bags and contents were weighed to calculate DM loss. Residues from duplicate bags for each time point were pooled according to the animal and ground through a 1 mm screen for chemical analysis.

Chemical analyses

The DM content of samples was determined by oven drying at 105°C for 16 h (AOAC, 1990; method 967.03). The ash concentration was determined after 5 h of combustion at 550°C in a muffle furnace. OM was calculated as the difference between 100 and the ash percentage (AOAC, 1995; method 942.05). Total nitrogen was determined using an auto micro-Kjeldahl nitrogen analyzer (model KDY-9830, Tongrunyuan Electromechanical Technology Co., Ltd, China). Calcium content in feed was determined using an atomic absorption spectrometric method (AOAC, 1990; method 968.08D). The colorimetric assay adapted from AOAC (method 965.17) was used to measure the P concentrations in feed (AOAC, 1990). The neutral detergent fibre (NDF) and acid detergent fiber (ADF) fractions were determined according to the Ankom A200 (Ankom Technology Corp., Fairport, NY) filter bag technique. ADL was determined by the Ankom Technology filter bag technique in beakers (Ankom Technology Method for determining Acid Detergent Lignin in Beakers-AK8/05; Corp., Fairport, NY, USA). Digestion coefficients were calculated by Schneider and Flatt's formula (Schneider and Flatt, 1975).

As high concentrations of protein and starch were present in some feed ingredients and orts, pepsin (P7000, Sigma-Aldrich Co. LLC., USA), and heat-stable α-amylase (A4551, Sigma-Aldrich Co. LLC., USA) pre-treatments were carried out prior to NDF and ADF determination (Van Soest *et al*. 1991). Urinary PD concentrations, including allantoin, uric acid, xanthine, and hypoxanthine were quantified separately by a colorimetric method (Chen and Gomes, 1992). Ruminal VFA was measured with a flame ionization detector in a GC (GC522, Wufeng Instruments, China) using a 15-m semi capillary glass column (0.53 mm in diameter) packed with Chromos orb 101 and with N_2 as the carrier gas at a 120°C column temperature. Before analysis, caproic acid was added to each sample as an internal standard (Cao Yang, 2011). The concentration of NH_3-N in the ruminal contents was determined by centrifuging the supernatant at 10000 × g for 10 min and measuring a portion by spectrophotometry (Spectra Max Plus384 Absorbance Microplate Reader California, USA) (Broderick and Kang, 1980). Urine samples were also analyzed for total nitrogen (AOAC, 1995; ID 984.13).

Calculations and statistical analysis

Digested DM, OM, and nitrogen were calculated by taking the difference between intake and fecal output. Nitrogen retention was calculated as nitrogen intake minus fecal and urinary nitrogen. Hemicellulose content was determined by calculating the difference between NDF and ADF, and the cellulose amount was calculated by subtracting ADL from ADF. OM true digestibility was estimated according to Mulligan *et al*. (2001) by only considering the NDF fraction of faeces originating only from feed (Van Soest, 1994): [OM intake (g/d)–fecal NDF (g/d) /OM intake (g/d)]. The microbial nitrogen yield estimation purine absorption and urinary PD excretion equations was adapted for sheep to quantify the relationship between microbial purine absorption (X mmol/d) and urine PD excretion as follows:

Y mmol/d:

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

Where:

$W^{0.75}$: metabolic body weight (kg) of the animal.

The microbial nitrogen (MN) was estimated as follows:

MN (g/day)= 70X/ (0.116×0.83×1000) = 0.727X (Chen and Gomes, 1992)

Where:

X: microbial purines absorption.

The kinetics of DM disappearance in situ were estimated by the nonlinear regression procedure of SAS (SAS, 1999) according to the exponential equation of McDonald (1981):

$$Y= a + b\ (1–e^{-c(t-lag)})$$

Where:

Y: DM disappearance in the rumen at time *t*.

a: highly soluble and readily degradable fraction.

b: insoluble and slowly degradable fraction.

c: rate constant for degradation.

t: time of incubation (h).

Effective ruminal degradability (ED) of corn stalk DM was estimated according to Ørskov and McDonald's equation (Ørskov and McDonald, 1979):

$$ED= a + [bc / (c+k_p)]$$

Where:

k_p: rumen outflow rate of 2, 4, and 6 % per hour.

a, *b*, and *c*: defined by the previous McDonald (1981) equation.

Data were analyzed by analysis of variance for a 4 × 4 Latin square design using the MIXED procedure of SAS (1999) with variance components and a covariance structure. The best covariance structure was selected for the final analysis for each dependent variable according to the lowest Akaike's Information Criterion (AIC) value. Least squares means were calculated for each treatment, and Fisher's protected LSD test (Steel and Torrie, 1980) was used to evaluate differences between means for significance. Data for ruminal pH, VFA, and NH_3-N were analyzed using the PROC MIXED procedure for repeated measures. Sampling time was considered as the repeated variable. The model included the fixed effects of treatment, sampling time, and the interaction of treatment × sampling time. Random effects consisted of sheep and the period. Differences were declared significant at $P < 0.05$. Trends were discussed at $0.05 < P < 0.10$ unless otherwise stated.

RESULTS AND DISCUSSION

Table 2 presents dry mater intake (DMI), total tract digestibility of DM, OM, and NDF, ADF, hemicellulose, cellulose, nitrogen, OM true digestibility, and nitrogen balance. DMI was not significantly different between treatment groups (P=0.38). Total tract digestibility of DM, NDF, ADF, hemicelluloses and cellulose were not affected by treatments. The digestibility of OM tended to be lower (P=0.06) for SB and OPTSB.

Table 2 Effect of polymer coated urea (Optigen) and sodium bentonite (SB) on DM intake, total tract digestibility of nutrients, and nitrogen balance for sheep fed high levels of concentrate with corn stalk as basal roughage

Item	Treatment				SEM	P-value
	CON	SB	OPT	OPTSB		
DMI (kg)	1.9	1.9	1.7	1.6	0.22	0.38
Total tract digestibility						
Dry matter (DM)	0.586	0.551	0.556	0.537	0.002	0.18
Organic matter (OM)	0.604	0.581	0.601	0.584	0.001	0.06
Neutral detergent fiber (NDF)	0.388	0.334	0.374	0.371	0.003	0.47
Acid detergent fiber (ADF)	0.309	0.272	0.317	0.296	0.003	0.67
Hemi cellulose	0.462	0.428	0.497	0.411	0.004	0.35
Cellulose	0.417	0.329	0.373	0.359	0.007	0.43
N	0.690 ^{a}	0.638^{b}	0.725^{a}	0.688^{a}	0.002	0.02
OMTD[1]	0.752^{a}	0.705^{b}	0.773^{a}	0.737^{ab}	0.001	0.04
N g/d						
intake	40.3	37.8	35.5	34.3	4.5	0.62
fece excretion	12.4	13.8	10.0	9.7	1.7	0.25
urine excretion	13.7	11.9	15.5	16.9	1.17	0.06
Retention[2]	14.2^{a}	12.1^{ab}	10.0^{bc}	7.7^{c}	1.8	0.04

[1] Organic matter true digestibility estimated according to Mulligan *et al*. (2001), considering that only the NDF fraction of feces originated from feed (Van Soest,1994), as: (OM intake–fecal NDF) / OM intake.
CON: control; SB: control + sodium bentonite; OPT: polymer coated urea (Optigen); OPTSB: polymer coated urea + sodium bentonite.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The nitrogen digestibility was lower compared to other treatments (P=0.02), and OM true digestibility was greater in CON and OPT compared to SB and OPTSB treatments (P=0.04).

Total nitrogen intake and fecal nitrogen excretion were not affected by treatments (P>0.05), although urinary nitrogen excretion tended to be lower for SB treatment (P=0.06), and nitrogen retention was significantly affected by treatments (P=0.04), thereby OPT and OPTSB-fed sheep had lower N retention compared to CON, and was differ for SB *vs*. OPTSB treatment.

Urinary PD excretion and predicted microbial nitrogen yields are presented in Table 3. No difference in urinary uric acid, and xanthin + hypoxanthin output were found among treatments. Urinary allantoin excretion in SB and OPTSB was lower compared to CON (P=0.04), thereby, total PD (P=0.03) and microbial nitrogen yields (P=0.03) were decreased for SB and OPTSB compared to CON.

Ruminal NH_3-N concentrations at different sampling times are present in Figure 1. Ruminal pH values and VFA concentrations are shown in Table 4. An interaction between time and treatments for ruminal NH_3-N concentrations was observed (Figure 1), whereby at 1 h post-feeding, NH_3-N concentrations increased significantly in treatments that included Optigen (P<0.05). In OPT, ruminal NH_3-N concentration increased (P<0.05), with the highest level achieved 3 h post-feeding, and remained high for almost 9 h post-feeding compared to CON and SB treatments. The ruminal pH value was not affected by treatments.

No treatment × sampling time interactions were observed for ruminal pH, total VFA, acetate, propionate, butyrate, isovalerate, and valerate; therefore, only mean values across sampling time are reported (Table 4). Total ruminal VFA concentration tended to be higher (P=0.052) for Otigen-fed sheep. The molar proportion of acetate (P=0.03), propionate (P=0.02), butyrate (P=0.05), isovalerate (P<0.01), valerate (P=0.02) and acetate to propionate ratio (P<0.01) were different between the treatment groups. Sheep fed OPTSB had greater molar proportion of propionate and valerate (P=0.02; and 0.02, respectively) compared to other groups, whereas molar proportion of acetate, butyrate and acetate to propionate ratio for OPTSB were lowest (P=0.03, 0.05, and 0.01, respectively). The molar proportion of isovalerate in groups SB was greater (P<0.01) compared with CON or OPTSB, and that in group OPTSB was greater (P<0.01) compared with CON.

The DM degradation kinetics and effective degradability of corn stalk after incubation in the rumen of sheep fed experimental diets are shown in Table 5. There were no significant (P>0.05) differences between treatments for DM degradation kinetics (*a*, *b*, and *c* fractions) and ED of corn stalk DM.

Replacing soybean meal with Optigen did not affect DMI of sheep in the present study. This is consistent with the findings by Galo *et al*. (2003), and Highstreet *et al*. (2010) who found that slow-releasing urea did not alter DMI in dairy cows. Pinos-Rodríguez *et al*. (2010) reported that substitution soybean meal with coated slow-releasing urea did not alter DMI in steers.

Table 3 Effect of polymer coated urea (Optigen) and sodium bentonite (SB) on purine derivates (PD) excretion and microbial nitrogen (MN) yields estimated from PD for sheep fed high levels of concentrate with corn stalk as basal roughage

Item	Treatments				SEM	P-value
	CON	SB	OPT	OPTSB		
Purine derivatives (PD) excretion(mmol/d)						
Allantoin	18.1[a]	13.7[b]	16.5[ab]	13.2[b]	2.1	0.04
Uric acid	2.5	2.2	2.2	2.3	0.4	0.56
Xanthin + Hypoxanthin	1.2	1.1	1.1	1.1	0.17	0.98
Total PD	21.9[a]	17.0[b]	19.8[ab]	16.7[b]	2.5	0.03
Microbial nitrogen yield g/d	18.7[a]	14.5[b]	16.9[ab]	14.3[b]	2.1	0.03

CON: control; SB: control + sodium bentonite; OPT: polymer coated urea (Optigen); OPTSB: polymer coated urea + sodium bentonite.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

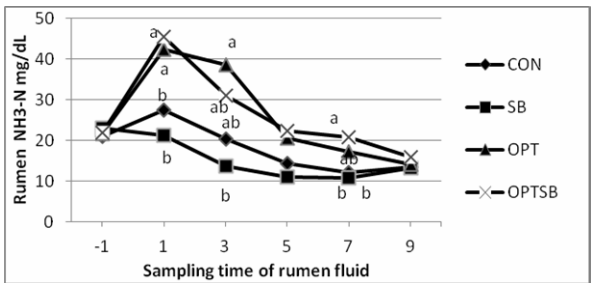

Figure 1 Ruminal ammonia nitrogen concentrations at different sampling times in the ruminal fluid of sheep following treatments composed with 40% corn stalk (DM basis) and 60% concentrate (DM basis). Concentrate of control (CON) including barley, wheat bran, soy bean meal; SB: CON plus 2% sodium bentonite (DM basis); OPT: including barley, wheat bran, corn that replaced 1.8% (DM basis) polymer coated urea (Optigen) with soy bean meal; OPTSB: polymer coated urea + 2% sodium bentonite (DM basis). Treatment × time; P < 0.03. Means differ for OPT and OPTSB *vs.* CON and SB at time 1 (P<0.05), for SB *vs.* CON, OPT and OPTSB at time 3 (P<0.05), and for OPTSB *vs.* CON and SB at time 7 (P<0.05). Pooled SEM= 4.24

Likewise, Huston *et al.* (1974) reported that the lambs fed the slow-releasing urea had similar feed intake to those of cottonseed meal-fed group. Furthermore, Rezende *et al.* (2008) observed that DMI decreased in cows fed conventional urea when dietary CP was higher than 105 g/kg of diet. However, DMI in OPT treatment did not decrease when CP=125 g/kg of diet in the current study, suggesting that the amount of nitrogen from Optigen was sufficient to improve rumen fermentation (Tedeschi *et al.* 2002). This may be due to the digestion rate of feed, particularly roughage feed, and the residue passage rate from rumen, both of which are important determinants of voluntary feed intake and productivity (Miller, 2002). Nutrient digestibility in OPT-fed sheep was similar to those of the soybean meal-fed animals in the present study, and no negative effects on fiber digestion duo to replacement soybean meal with Optigen were observed. Russell *et al.* (1992) reported that the replacement of amino acids in soybean and rapeseed meal with urea was predicted to have little detrimental effect on fiber digestion, as the bacterial populations associated with fiber in the rumen have been shown to exclusively use ammonia for their growth requirements. Likewise, Owens *et al.* (1980) found that digestibility of DM did not change in steers when slow- releasing urea replaced isonitrogenously with soybean meal in the diet. In the current study, replacement soybean meal with Optigen did not alter whole-tract fiber digestion, which is in agreement with findings by Sinclair *et al.* (2012) who reported that the partial replacement of soybean meal with feed grade urea or slow-releasing urea did not affect whole-tract apparent digestibility of DM, OM, NDF and N. Likewise, Galo *et al.* (2003) added a polymer-coated urea source to a basal diet and found no effect on NDF digestibility, although ADF digestibility was decreased. In contrast, Galina *et al.* (2003) and Xin *et al.* (2010) evaluated the controlled-release urea source and reported a significant improvement in ruminal digestion of fiber in cows. Additionally, Akay *et al.* (2004) reported that Optigen improved rumen function by supplying nitrogen to rumen bacteria at a rate that optimizes the conversion of nitrogen into bacterial protein. Additionally, Optigen improved nutrient imbalance for rumen bacteria by increasing availability of energy from simple carbohydrates (Cherdthong and Wanapat, 2010).

The replacement of soybean meal with Optigen did not affect nitrogen intake, and fecal nitrogen excretion, whereas urinary nitrogen excretion tended to be higher in Optigen-fed sheep. Therefore, nitrogen retention was significantly decreased for Optigen-fed sheep compared with CON. Nitrogen intake, and its retention in our result are similar to those reported by Owens *et al.* (1980) in steers when soybean meal replaced with urea or slow-releasing urea. Huston *et al.* (1974) found that nitrogen retention of the lambs fed slow-releasing urea was greater than that of those fed urea or untreated urea. The results of the current study in terms of nitrogen intake, and its urinary were similar to findings of Galo *et al.* (2003) in cows. Tendency for higher urinary nitrogen excretion in Optigen-fed sheep might explained this occurrence by suggesting that the higher nitrogen hydrolyze with the Optigen compared to soybean meal in rumen.

Table 4 Effect of polymer coated urea (Optigen) and sodium bentonite (SB) on ruminal pH and volatile fatty acids (VFA) for sheep fed fed high levels of concentrate with corn stalk as basal roughage

Item	Treatment				SEM	P-value		
	CON	SB	OPT	OPTSB		Diet	Time	Diet*Time
PH	5.58	5.57	5.64	5.57	0.14	0.97	<0.01	0.54
Total VFA (mmol/L)	98.4	107.0	113.2	124.0	6.85	0.052	0.91	0.41
Acetate (%)	69.03^{a}	71.11^{a}	69.87^{a}	64.65^{b}	1.59	0.03	0.12	0.30
Propionate (%)	18.14^{b}	15.59^{b}	18.55^{b}	25.98^{a}	2.41	0.02	0.25	0.28
Butyrate (%)	11.20^{a}	11.37^{a}	9.60^{ab}	7.03^{b}	1.09	0.05	0.09	0.46
Iso valerate (%)	0.710^{c}	1.037^{a}	0.941^{ab}	0.867^{b}	0.13	<0.01	<0.01	0.70
Valerate (%)	0.91^{b}	0.89^{b}	1.03^{b}	1.47^{a}	0.12	0.02	<0.01	0.23
Acetate: propionate[2]	4.17^{a}	4.95^{a}	4.21^{a}	2.61^{b}	0.59	<0.01	0.01	0.26

CON: control; SB: control + sodium bentonite; OPT: polymer coated urea (Optigen); OPTSB: polymer coated urea + sodium bentonite.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 DM degradability constants and calculated effective degradability of corn stalk after incubation in rumen of thin-tailed Han × Dorper cross breed sheep fed high levels of concentrate with corn stalk as basal roughage

Item	Treatment				SEM	P-value
	CON	SB	OPT	OPTSB		
A[2]	0.153	0.164	0.174	0.158	0.001	0.59
B[2]	0.386	0.396	0.408	0.371	0.003	0.82
c[2]	0.025	0.026	0.026	0.029	0.002	0.52
ED[3] (Kp=0.02)	0.365	0.386	0.402	0.370	0.002	0.55
ED (Kp=0.04)	0.308	0.319	0.333	0.314	0.001	0.45
ED (Kp=0.06)	0.265	0.283	0.294	0.279	0.001	0.58

[2] For each sample, the model: $Y= a + b(1–e^{-ct})$ was fitted to the % disappearance of DM;
Where:
a: soluble (rapidly degradable) fraction.
b: insoluble and slowly degradable fraction.
c: fractional rate of disappearance (per hour).
t: time of incubation (hours).
[3] $ED= [a + b \times c / (c+K_P)]$ was used to calculate effective degradability (ED). In this equation, K_P represents the flow rate of particles out of the rumen that was considered equal to 0.02, 0.04, or 0.06.
CON: control; SB: control + sodium bentonite; OPT: polymer coated urea (Optigen); OPTSB: polymer coated urea + sodium bentonite.
SEM: standard error of the means.

Urine is the main route for nitrogen excretion in cows, followed by faces. Due to a protein surplus or an unbalanced amino acid profile, urinary nitrogen excretion increases with an accumulation of ruminal ammonia or high levels of deamination in the body (Galo *et al*. 2003). In the present study, sheep from OPT did not achieve a higher nitrogen retention compared with those of CON it may be explained by higher urinary nitrogen losses in OPT-fed sheep compared to CON. On the other hand, microbial protein synthesis appeared to be limited by fermentable energy and rumen turnover time rather than by nitrogen availability (Owens *et al*. 1980).

Microbial protein synthesis in the rumen provides the majority of protein supplied to the small intestine of ruminants, accounting for 50 to 80% of total absorbable protein (Firkins *et al*. 2007). In the current study, the substitution of soybean meal with Optigen did not affect urinary PD excretion and rumen microbial nitrogen calculated from urinary PD excretion. Jooste (2012) found no differences in urinary purine excretion and microbial nitrogen production among lambs fed urea or urea plus Optigen. Also Chegeni *et al*. (2013) reported that Optigen did not affected urinary PD excretion and rumen microbial yield when sheep fed low levels of concentrates. Likewise, Xin *et al*. (2010) found that microbial protein synthesis efficiency (*in vitro)* of the polyurethane coated urea diet was significantly higher than the feed grade urea diet. Proteins with a lower rate of ruminal degradation tend to improve the efficiency of microbial protein synthesis, probably because of the better capture of released nitrogen by rumen microbes (Karsli and Russelr, 2001). On the other hand, when the rate of carbohydrate fermentation exceeds that of protein degradation, inefficient microbial protein synthesis may occur (Bach *et al*. 2005). Therefore, nutrient synchrony between the supply of energy and nitrogen to the rumen microorganisms in treatments had Optigen might have improved the efficiency of rumen microbes in capturing nitrogen and use of energy for microbial growth similar to CON (soybean meal source nitrogen). It seems that soybean meal can be substituted with Optigen without having any negative effects on microbial nitrogen production.

By evaluating the NH_3-N curves (Figure 1), in general, we noticed a greater NH_3-N concentration for treatments that included Optigen. For treatments with Optigen, NH_3-N levels were continuously high with maximum concentration up to 3 h post feeding, and then were reduced to the same

level of other treatments six hours later. Ammonia concentration can influence fiber degradation, Belasco (1954) suggested that the maximum digestion of cellulose occurs when ammonia concentration was about 43 mg/100 mL in the rumen, However, Van Soest (1994) reported that an optimal NH_3 concentration exists for each diet because the capacity of protein synthesis and ammonia use depends on a carbohydrate-fermentation rate. Greater fermentation of carbohydrates causes an increase in ammonia levels. Similar findings by Pinos-Rodríguez *et al*. (2010) showed that overall ruminal ammonia nitrogen concentrations of steers fed diets with coated urea was greater significantly compared to soybean meal. The ruminal NH_3-N results in our experiment were similar to those obtained by both Owens *et al* (1980) and Ribeiro *et al*. (2011). Both studies showed that NH_3-N concentration in a slow releasing urea diet was higher than a control diet but lower than in a conventional urea diet. Additionally, De Paula *et al*. (2009) evaluated ammonia release in situ from both urea and a urea protected with polymers and discerned a NH_3-N peak during the first hour after supplementation (30.54 mg/dL for urea) that reduced after 4 h to 14.02 mg/dL. Protected urea exhibited greater stability in NH_3-N concentrations compared to urea, which were 39.59 mg/dL and 29.64 mg/dL for 1 and 4 h post-feeding, respectively. Our results show that the NH_3-N concentration was adequately stable in OPT compared to CON, and the Optigen supplied sufficient nitrogen (ammonia) for microbial protein synthesis as well as soybean meal.

Replacing soybean nitrogen with Optigen did not affect pH, which are similar with findings by Xin *et al*. (2010) and Pinos-Rodríguez *et al*. (2010) who replaced soybean N with coated urea in diets of dairy cows and steers, respectively. VFA concentrations in the OPT treatment tend to be higher compared to CON (113.22 mmol/L *vs*. 98.44). Also as a result of the substitution of soybean nitrogen with Optigen, showed that proportion of acetate, propionate, butyrate, valerate and ratio of acetate to propionate did not alter, whereas isovalerate was greater for OPT treatment compared to CON. Cherdthong *et al*. (2011) reported that slow releasing urea did not alter the total VFA produced but led to an increase in the proportion of propionate. However, Xin *et al*. (2010) showed that total VFA concentration or individual VFA proportions were not changed in diets with slow releasing urea compared to diets with conventional urea and isolated soybean protein, because ruminal VFA is mainly derived from dietary carbohydrate fermentation (Firkins, 1996). In our experiment increase total VFA concentrations at 1 up to 3 h post feeding and Similarity total VFA concentration values between CON and OPT treatments reflected no adverse fermentation by replacing of soybean meal with Optigen in the diet. Kim *et al*. (2007) reported that urea supplementation in steers significantly produced greater pH values ($P<0.05$) and greater concentrations of NH_3-N ($P<0.01$) and total VFA ($P<0.10$) than supplementation with soybean meal and expeller soybean meal. The significantly higher Isovalerate in treatments with Optigen in the present study was not clear.

No differences were discerned in ruminal kinetics and ED of corn stalk for treatments supplied with Optigen. These results are in agreement with *in situ* findings of Ribeiro *et al*. (2011) who found no differences ($P>0.05$) between treatments for in situ ruminal DM degradation of hay when hay supplemented with urea or polymer-coated urea in the rumen of cattle. In our study similarity of results in situ trail in CON and OPT treatments reflected no adverse effects by replacing of soybean meal with Optigen in the diet, and Optigen can provide appropriate conditions for the fiber degradation as well as soybean meal in the rumen. Dietary SB did not affect DMI in the current study. Previous reports regarding the effects of bentonite on DMI intake are inconsistent, so that, DMI was not affected by feeding bentonite to steer, goats and sheep with high level of concentrate (Martin *et al*. 1969; Gaber *et al*. 2003; Aguilerasoto *et al*. 2008; Jeronimo *et al*. 2010). In contrast, Helal and Abdelrahman (2010) and Walz *et al*. (1998) found that DMI increased with dietary SB in ewes and lambs. However, Jacques *et al*. (1986) reported that dietary SB reduced feed intake of cows on pastures. The variation in the effect of SB on DMI may be related to basal diets, supplementary bentonite levels and other factors.

Dietary SB did not affect digestibility of DM, NDF, ADF, hemicellulose and cellulose, whereas tended to be lower for OM digestibility in SB treatment. OM true digestibility and nitrogen digestibility were decreased by SB. Published studies shows that adding SB to ruminants´ diets with high level of concentrate are inconsistent. For example, Lemerle *et al*. (1984) found that no significant differences were found in rumen dilution rate or in nitrogen degradability, and Aguilerasoto *et al*. (2008) reported that adding SB to growing lambs diet did not alter apparent digestibility of DM, OM, CP (crud protein), NDF, and ADF. In contrast, Helal *et al*. (2010) observed an increase in apparent digestibility of DM, OM, CP, CF (crud fiber), EE (ether extract), and NFE (nitrogen free extract) in ewes supplemented with SB. However, nutrient digestibility in the current study are in agreement with the findings of Rindsig and Schultz (1970) who recorded a depression in nitrogen digestibility without changes in dry matter digestibility or nitrogen retention when SB was included in the diet of dairy cows. Likewise, Fisher and Mackay (1983) reported that apparent digestibility of DM and ADF was decreased by dietary SB in lactating cows. In addition, Aitchison *et al*. (1986) found that inclusion of bentonite

significantly decreased OM digestibility in sheep fed with high concentrate diet. Aitchison *et al.* (1986) concluded that the decrease in diet digestibility with bentonite would be expected to reduce the performance of animals over long periods of feeding. In the current study the complexing of soybean meal with SB did not affect N retention, whereas dietary SB numerically reduced nitrogen intake, its urine or retention. Aitchison *et al.* (1986) found a significant reduction for nitrogen retention in sheep fed high concentrate diet supplemented by bentonite. However, the mode of action is unclear.

The complexing of soybean meal with SB decreased total urinary PD excretion, and microbial nitrogen production in the present study. Galyean *et al.* (1981) reported that microbial protein yields from the rumen did not affected by dietary SB in steer. Incorporation of bentonite into diets containing soybean meal supplements may have increased the proportion of dietary protein that escapes microbial degradation in the rumen (Ivan *et al.* 1992). Therefore, similar to the suggestion of Ivan *et al.* (1992), in our results bentonite may decreased the degradation of dietary protein in the rumen.

The complexing of soybean meal with SB did not affect ruminal pH, total VFA and ammonia concentrations in the present study. These results are in accordance with those by Helal *et al.* (2010) who reported that ruminal pH values, and NH_3-N concentration were not altered by supplementary bentonite at 3 and 6 h post feeding in lactating ewes. In the current study, ruminal concentrations of VFA components (except isovalerate) were not altered by complexing of soybean meal with SB, which is similar to the results of Aguilerasoto *et al.* (2008) who reported that no influences of dietary bentonite on ruminal fermentation characteristics in growing lambs. However, Walz *et al.* (1998) reported that total ruminal VFA was increased by supplementary SB in lambs fed high concentrate diets. Colling *et al.* (1979) observed no interactions between SB and concentrate level in lambs, the authors also found that SB supplemented diets had greater ($P<0.05$) acetate and butyrate and lower ($P<0.01$) propionate. The alteration in VFA may be a result of bacterial selection or altered liquid turnover rate (Hodgson and Thomas, 1975). Concentrations of NH_3-N, and VFA in ruminal fluid indicated no appreciable effects of dietary supplementation of SB in our study. Due to relatively high post-ruminal protein digestibility of soybean meal (Stern *et al.* 1994), in our experiment rumial NH_3-N concentrations were uniformly same in CON and SB treatments (Figure 1), therefore lower numerically rumen ammonia nitrogen concentration with bentonite supplementation may be duo to ability of betonite to absorb NH_3-N from rumen fluid and release it later when the level falls (Helal *et al.* 2010). The significance of isovalerate accumulation with the SB diet in the present study was not clear.

Adding SB to soybean protein source (in SB treatment) did not change in ruminal kinetics and ED of corn stalk DM. Research comparing the effect of SB on rumen degradability of cellulosic forage is scarce. However, Jacques *et al.* (1986) reported that bentonite did not change in situ degradation rate constants of NDF when nylon bag method was used for comparing NDF degradability of fresh sorghum silage.

The results of the present study showed no advantages in adding SB to Optigen (OPTSB treatment) in sheep diets with a high concentrate ratio. Adding SB to Optigen did not alter nitrogen intake, urinary excretion, fecal excretion or retention compared to OPT. None of previous studies did not report regarding the effects of Optigen combination and SB in ruminants with high concentrate diet. Mohsen and Tawfik (2002) found when SB was supplemented to a high concentrate diet containing conventional urea, the digestibility of DM, OM, and CP, as well as N retention were increased. Mohini *et al.* (1999) reported that inclusion of betonies with or without urea did not have any significant effect on DMI, digestibility of DM OM, CP, EE, NFE and growth performance in calves. However, nitrogen retention in the present study is consistent with findings of Martin *et al.* (1969) when who investigated nitrogen retention in lambs fed high concentrate diet with 2% bentonite and 1.15% conventional urea.

In the present study, adding dietary SB to Optigen numerically reduced urinary excretion of allantoin and total PD, and microbial nitrogen yields compared with OPT treatment, whereas they were significantly lower compared with CON. Ruminal microbial protein synthesis depends on the supply of adequate amounts and type of carbohydrate as an energy source for the synthesis of peptide bonds (Bach *et al.* 2005). In our study, it seems adding SB to Optigen has an adverse effect on PD and microbial nitrogen in sheep fed high concentrate diet.

Sheep fed in treatment which included Optigen complexed with SB in high concentrate ratio tend to be higher for total VFA concentrations compared to OPT treatment, whereas pH values and NH_3-N concentration in different time point sampling did not alter. Likewise, duo to adding SB to Optigen significantly reduced molar proportion acetate, and acetate to propionate ratio, whereas propionate and valerate proportion significantly increased. Mohsen and Tawfik (2002) found that addition of bentonite at the rate of 2.5 and 5 % to high concentrate diet containing urea-treated rice straw did not change pH and total VFAs concentration, whereas NH_3-N concentration significantly decreased. Direct comparition to other reports with Optigen or

slow releasing urea in animal trials is difficult because in all case no bentonite was added to the diets and not complexed with a protein as in this experiment. However, in our study adding SB to diet containing Optigen (in OPTSB treatment) with high concentrate ratio had no discernible benefit on nutrient digestibility, nitrogen retention, microbial nitrogen, pH, total VFA concentration and effective degradability (ED).

CONCLUSION

Replacement of soybean meal with Optigen (slow-releasing urea) in sheep diets with a high concentrate ratio did not alter DMI, nutrient digestibility, and ED, but decreased nitrogen retention. Dietary supplementation of SB did not affect DMI, rumen fermentation characteristics, and ED, but decreased apparent digestibility of OM and nitrogen, urinary excretion of PD, and microbial nitrogen production in the rumen. Adding SB to Optigen however, had no beneficial effects on nutrient digestibility, nitrogen retention, microbial nitrogen yield, and ED, In general, Using Optigen to replace soybean meal in sheep fed high levels of concentrate, and corn stalk as basal roughage did not have a negative effect on digestibility, rumen fermentation, and microbial protein yields.

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