Effects of dietary protein sources and nisin on rumen fermentation, nutrient digestion, plasma metabolites, nitrogen utilization, and growth performance in growing lambs¹

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ABSTRACT: This study investigated the effects of dietary protein sources and nisin on rumen fermentation, nutrient digestion, plasma metabolites, N utilization, and growth performance in growing lambs. Thirty-two male Hu lambs (23 ± 2 kg initial BW) were assigned to four dietary treatments in a randomized block design with a 2×2 factorial arrangement. Two protein sources, soybean meal (SBM) and dried distillers grains with solubles (DDGS), and two levels of nisin, 0 and 30.5 mg of nisin/kg of feed, were used to formulate four isonitrogenous and isoenergetic diets. No interaction ($P \geq 0.16$) of protein $\times$ nisin was found except on apparent digestibility of DM, OM, NDF, and ADF ($P \leq 0.02$). Lambs receiving DDGS had lower ($P \leq 0.04$) concentrations of ruminal acetate and butyrate, but propionate concentrations did not differ ($P = 0.39$), compared with those fed SBM, leading to a trend for reduced total VFA concentration ($P = 0.07$). Ruminal NH_3 -N and total branched-chain VFA concentrations were lower ($P \leq 0.01$) in the lambs fed DDGS than in those fed SBM. The DDGS-fed lambs had less ($P < 0.01$) CP, but greater ($P < 0.01$) ether extract

apparent digestibility than those fed SBM. For plasma metabolites, only blood urea N and albumin concentrations were lower in the DDGS-fed lambs ($P < 0.01$) than in those fed SBM. Nitrogen excretion pathway was altered when DDGS replaced SBM, with fecal N excretion (% of N intake) being greater ($P < 0.01$), while urinary N excretion (% of N intake) tending to be less ($P = 0.06$) from the DDGS-fed lambs than those fed SBM. Protein sources affected growth performance in an age/time-dependent manner. From weeks 1 to 4, DDGS resulted in less ($P = 0.03$) DMI and ADG than SBM. From weeks 5 to 8, DDGS did not affect ($P \geq 0.23$) DMI or ADG but resulted in a greater ($P = 0.04$) G:F than SBM. Final BW did not differ ($P = 0.58$) due to protein source. Providing nisin had no impact on DMI ($P = 0.44$), ADG ($P = 0.84$), or G:F ($P = 0.73$). Nisin addition only affected plasma uric acid concentration ($P = 0.04$). It was concluded that DDGS could substitute for SBM as a nitrogen source to growing Hu lambs to reduce N excretion via urine without adverse effects on animal performance, but nisin supplementation probably had no additional benefits.

Key words: dried distillers grains with solubles, growing lambs, growth performance, nisin, nitrogen utilization, soybean meal

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INTRODUCTION

Soybean meal (SBM) is an important source of protein for livestock (Dei, 2011). However, the use of this traditional feed ingredient increases feed cost (Held, 2006). Moreover, for ruminants, the high ruminal degradability of SBM can lead to rapid production of ruminal NH_3 (Kleinschmit et al., 2006), most of which is lost via urine (Hristov et al., 2004). Using agricultural byproducts, such as dried distillers grains with solubles (DDGS), as a protein source to replace SBM can reduce feed cost (Held, 2006) and ruminal protein degradation (Kleinschmit et al., 2007). Previous studies have demonstrated that DDGS does not affect the performance of finishing lambs when included at 50% (Van Emon et al., 2012) and 60% (Schauer et al., 2008) of dietary DM. However, a diet containing a large proportion of DDGS can increase N excretion, potentially polluting the environment (Felix et al., 2012; Salim et al., 2012; Walter et al., 2012). To date, few studies have examined the effects of feeding isonitrogenous diets comparing DDGS to SBM in growing lambs.

Nisin, a bacteriocin, has been shown as a potential alternative to monensin, an ionophore, in modulating rumen fermentation. Several *in vitro* experiments have shown that nisin can suppress AA deamination and microbial methanogenesis without decreasing DM digestibility (Shen et al., 2016) or VFA production (Callaway et al., 1997). However, *in vivo* feeding experiments are needed to further validate its usefulness and efficacy. It is hypothesized that: 1) substituting DDGS for SBM in an isonitrogenous diet for growing lambs would reduce urinary N excretion without adverse effects on growth performance, and 2) nisin addition would reduce ruminal NH_3 production and improve animal performance. Therefore, the objectives of this study were to evaluate the effects of dietary protein sources and nisin on rumen fermentation, nutrient digestion, plasma metabolites, N utilization, and growth performance in growing lambs.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

The experimental procedures used in this study were approved by the Animal Care and Use Committee of Nanjing Agricultural University.

Thirty-two male Hu lambs (23 ± 2 kg initial BW) were assigned to four dietary treatments in a randomized block design with a 2×2 factorial

arrangement. Two protein sources, SBM and corn DDGS, and two levels of nisin, 0 and 30.5 mg of nisin/kg of diet, were used to formulate four isonitrogenous and isoenergetic diets (Table 1). The DDGS replaced all the SBM and a portion of the ground corn in the diets. The dosage of nisin (1,000 IU/mg of solid, about 2.5% w/w purity; Zhejiang Silver-Elephant Bio-engineering Co., Ltd., Zhejiang, China) used *in vivo* was based on previous *in vitro* studies (Callaway et al., 1997; Shen et al., 2016). Nisin addition at 30.5 mg/kg of DM is approximately equivalent to 2 $\mu\text{mol/L}$ of ruminal content for lambs (assuming an average DMI = 1,150 g/d and rumen volume = 5 liters).

Thirty-two lambs were divided into two blocks (16 lambs per block) based on their BW (low and high). The lambs in each block were then randomly assigned to one of four diets, with each dietary treatment having eight lambs. Diets were formulated to meet the Feeding Standards of Meat-producing Sheep and Goats (Ministry of Agriculture of P. R. China, 2004; Table 1). All the lambs were fed a total mixed ration twice daily at 0700 and 1900 h, and there was approximately 10% feed refusal. Lambs were housed in individual pens (1.5 m $\times$ 4 m) with wooden slatted floors and had free access to drinking water. Feeding trials were conducted for 10 wk, consisting of 1 wk for adaptation followed by 9 wk of dietary treatment.

Sampling and Measurement

Ruminal fluid was collected using an oral stomach tube approximately 3 h after morning feeding on the seventh day of weeks 6 and 8. The first 80 mL of the rumen sample was discarded to minimize contamination by saliva. The pH of each ruminal fluid sample was measured immediately with a portable pH meter (Ecoscan pH 5, Eutech Instruments, Singapore). One milliliter of each ruminal fluid sample was preserved by adding 0.2 mL of 25% HPO_3 for VFA analysis using gas chromatography (7890A, Agilent, UK) according to the method described by Mao et al. (2008). Another 1 mL of each ruminal fluid sample was stored at -20°C for subsequent analysis for $\text{NH}_3\text{-N}$ using a colorimetric method (Chaney and Marbach, 1962).

Diet and ort samples were collected daily for 5 d (third day to seventh day) on weeks 2, 4, 6, and 8, or 3 d (first day to third day) in week 9. Fecal samples were collected twice daily from each lamb before each feeding on the first day to third day of week 9. Daily feed, orts, and fecal samples were composited per lamb for the whole experimental

Table 1. Ingredient and chemical composition of the experimental diets¹

Item	SBM-based diet	DDGS-based diet
Ingredient (% of DM)		
Corn silage	22.00	22.00
Chinese wild rye	24.00	24.00
Brewer's grains	7.00	7.00
Corn grain	24.00	16.00
Barley grain	4.00	4.00
SBM ²	12.00	–
DDGS ²	–	20.00
Wheat bran	3.00	3.00
Calcium carbonate	1.00	1.00
Calcium monophosphate	0.50	0.50
Sodium bicarbonate	1.00	1.00
Salt	1.00	1.00
Premix ³	0.50	0.50
Nutrient composition (% of DM)		
CP	15.42	15.35
NDF	39.33	43.49
ADF	21.35	23.06
EE	5.24	7.01
Ash	8.02	8.32
AIA	2.33	2.42
DE, MJ/kg	13.45	13.44

AIA, acid-insoluble ash.

¹Nisin (2.5% w/w purity) was added at 0 and 30.5 mg/kg of SBM-based or DDGS-based diet.

²SBM and DDGS contained (DM basis): 50.1% and 30.9% CP, 17.3% and 37.5% NDF, 9.0% and 18.3% ADF, and 3.1% and 12.7% EE, respectively.

³Formulated to provide (per kilogram of DM): vitamin A, 1,320,000 IU; vitamin D3, 264,000 IU; vitamin E, 7,200 IU; Cu, 4,800 mg; Co, 73 mg; I, 144 mg; Mn, 6,480 mg; Zn, 9,600 mg; Se, 84 mg; Fe, 6,480 mg; Mg, 7,920 mg.

period, subsampled, and were subsequently stored at -20°C until analysis. At the end of the experiment, all samples were thawed and dried at 65°C for 48 h. Dried samples were ground through a 1-mm screen using a Cyclotec mill (Tecator 1093; Tecator AB, Höganäs, Sweden) before analysis. All samples were analyzed for DM (method 967.03; AOAC, 1990), OM (method 942.05; AOAC, 1990), and CP (method 984.13; AOAC, 1990). Contents of ADF and NDF were determined according to Van Soest et al. (1991). The acid-insoluble ash in both the diet and the fecal samples were analyzed using the method described by Van Keulen and Young (1977). The results were used as internal markers for estimating apparent nutrient digestibility.

Blood samples (9 mL) were collected from the jugular vein of each lamb into heparinized evacuated tubes at approximately 3 h after morning feeding on the sixth day of weeks 6 and 8. The sample

was then centrifuged at $3,000 \times g$ for 15 min to obtain the plasma. All plasma samples were stored at -20°C for later analysis for blood urea nitrogen (BUN; Wang et al., 2007), glucose (Barham and Trinder, 1972), total protein and albumin (Kingsley, 1939), creatinine (Owen et al., 1954), uric acid (Kabasakalian et al., 1973), total cholesterol (Mann, 1961), triglyceride (Cole et al., 1997), and alkaline phosphatase (Keay and Trew, 1964) using commercial kits (Jiancheng Bioengineering Institute, Nanjing, China). Globulin was calculated using the difference between total protein and albumin.

Urine samples were collected using a commercial urine receptor (Hengkang, Hebei, China) twice daily before each feeding on the first day to third day of week 9. Fresh urine samples were acidified by adding an equal volume of 6 mol/L HCl to prevent NH_3 volatilization. Daily urine samples were pooled by lamb, and subsamples were stored at -20°C for later analysis. At the end of the experiment, all urine samples were thawed at 25°C and filtered through Whatman no. 1 filter paper. Filtrates were then analyzed for total N using the Kjeldahl method (method 984.13; AOAC, 1990), and creatinine using a picric acid assay (Oser, 1965). Urine volumes were computed using creatinine as a marker based on an assumption of a creatinine excretion of $0.334 \text{ mmol/kg of BW}^{0.75}/\text{d}$ (Ma et al., 2014).

The diet offered andorts were weighed for 5 consecutive days (third day to seventh day) in weeks 2, 4, 6, and 8, and then for 3 consecutive days (first day to third day) in week 9 to determine DMI. The lambs were weighed on 3 consecutive days with 14-d intervals in weeks 0, 2, 4, 6, and 8 at 0600 h before feeding. Data were used to calculate ADG (mean of ADG for the period of weeks 1 to 2, 3 to 4, 5 to 6, and 7 to 8). Lamb G:F was calculated as the ratio of weight gain to DMI.

Statistical Analyses

Statistical analyses were performed with SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Preliminary data examination revealed that all dependent variables except for plasma uric acid were normally distributed. The plasma uric acid concentrations were reciprocally transformed to improve normality. Data for nutrient digestion, N balance, and BW were analyzed using the MIXED procedure of SAS. The model included protein source, nisin, and protein source $\times$ nisin interaction as fixed effects, with block, and lamb within protein

source $\times$ nisin as random effects. Data for rumen fermentation characteristics, plasma metabolites, DMI, ADG, and G:F were analyzed with week as repeated measures using covariance type of autoregressive (1), which provides the best fit according to Akaike's information criterion. Fixed effects within the model included protein source, nisin, week, interactions of protein source $\times$ nisin, protein source $\times$ week, nisin $\times$ week, and protein source $\times$ nisin $\times$ week, with block, and lamb within a block as random effects. Degrees of freedom were calculated using the Kenward-Roger option. Differences were considered to be statistically significant when the P -values were ≤ 0.05 , and trends were declared at $0.05 < P \leq 0.10$. All reported values are least squares means unless otherwise stated.

RESULTS AND DISCUSSION

Dietary Nutrient Composition

Ingredients and chemical composition of the experimental diets are shown in Table 1. The corn DDGS used in the current study was received from a single batch, and the nutrient content was within the variation range reported by Spiehs et al. (2002). The content of CP, NDF, ADF, and ether extract (EE) for SBM vs. that of DDGS are 50.1% vs. 30.9%, 17.3% vs. 37.5%, 9.0% vs. 18.3%, and 3.1% vs. 12.7%, respectively. Because the two diets had the same combined content of DDGS/SBM and ground corn (12% SBM plus 24% ground corn vs. 20% DDGS plus 16% ground corn), the higher concentration of EE, NDF, and ADF in the DDGS than in the SBM might have contributed to the greater concentrations of EE, NDF, and ADF of the DDGS-based than the SBM-based diets.

Rumen Fermentation Characteristics

No interaction ($P \geq 0.10$) of week $\times$ protein $\times$ nisin, week $\times$ protein, week $\times$ nisin, or protein $\times$ nisin was detected on any of the analyzed rumen fermentation parameters except for the isobutyrate concentration that was affected ($P = 0.03$) by the interaction of week $\times$ protein. Lambs receiving DDGS had lower ($P \leq 0.04$) ruminal acetate and butyrate concentrations than those fed SBM, but ruminal propionate concentrations were not different ($P = 0.39$), leading to a trend of reduced total VFA ($P = 0.07$) for DDGS-fed lambs. Few studies have examined the effects of feeding DDGS-containing diets on rumen fermentation in growing lambs. The only study found in the

literature (Avila-Stagno et al., 2013) also found reduced total ruminal VFA concentration in growing lambs receiving increased concentrations of wheat DDGS that replaced SBM, alfalfa hay, and soybean hulls. Similar to the current results, several previous studies using dairy cows also showed a decrease in total ruminal VFA by corn DDGS substitution for SBM (Nichols et al., 1998; Kleinschmit et al., 2006; Benchaar et al., 2013). Those authors attributed the VFA decrease to mainly the lack of nonstructural carbohydrates in DDGS (Nichols et al., 1998; Kleinschmit et al., 2006). In the present study, the DDGS-based diet also resulted in a lower ($P < 0.01$) acetate to propionate ratio than the SBM-based diet due to reduced acetate but similar propionate concentration, a finding in agreement with that of Benchaar et al. (2013) in dairy cows.

Ruminal $\text{NH}_3\text{-N}$ concentration was lower ($P < 0.01$) in the lambs fed DDGS than in those fed SBM (Table 2), which was probably attributable to the greater digestibility of the SBM protein than the protein in DDGS (Kleinschmit et al., 2007). The lower RDP content in the DDGS diet likely reduced peptide degradation and AA deamination, thereby reducing NH_3 production. This premise is substantiated by the lower concentrations of total branched-chain VFA ($P = 0.01$), which primarily arise from deamination of AAs (Patra and Yu, 2014). Similar to the current results in lambs, reduced concentrations of $\text{NH}_3\text{-N}$ and branched-chain VFA have been previously reported in dairy cows fed DDGS compared to cows fed SBM (Nichols et al., 1998; Anderson et al., 2006; Kleinschmit et al., 2006; Benchaar et al., 2013). In contrast, unchanged $\text{NH}_3\text{-N}$ concentrations were also found in previous studies on beef cattle fed increasing corn DDGS (Leupp et al., 2009; Walter et al., 2012; Hünerberg et al., 2013), which is probably due to the increased CP level in DDGS diets compared with control in these experiments.

Nisin, a bacteriocin, is a potential alternative to antibiotics in modulating rumen fermentation. Previous *in vitro* studies have demonstrated that nisin can be an alternative to monensin in decreasing acetate:propionate ratio and inhibiting AA degradation (Callaway et al., 1997; Shen et al., 2016). Therefore, decreased acetate:propionate ratio and reduced NH_3 production by nisin addition are expected. However, nisin addition did not affect ($P \geq 0.20$) any of the rumen fermentation parameters in the growing lambs (Table 2). The dose of nisin used in the present *in vivo* study (30.5 mg/kg of DM) was based on that (2 $\mu\text{mol/L}$) used in previous *in vitro* studies (Callaway et al., 1997; Shen

Table 2. Effects of dietary protein sources and nisin on rumen fermentation characteristics in growing lambs¹

Item	Protein source			Nisin			<i>P</i> -value ²		
	SBM	DDGS	SEM	Control	Nisin	SEM	Protein	Nisin	P × N
pH	6.58	6.66	0.036	6.61	6.63	0.036	0.12	0.57	0.39
NH ₃ -N, mg/dL	7.18	5.56	0.268	6.27	6.48	0.268	<0.01	0.58	0.56
Total VFA, mM	91.94	86.57	2.020	89.38	89.13	2.020	0.07	0.93	0.69
Acetate, mM	60.21	56.31	1.321	58.64	57.88	1.321	0.04	0.69	0.67
Propionate, mM	17.05	17.75	0.567	17.08	17.71	0.567	0.39	0.44	0.33
A:P	3.60	3.20	0.074	3.48	3.33	0.074	<0.01	0.16	0.32
Butyrate, mM	11.60	9.71	0.340	10.71	10.60	0.340	<0.01	0.81	0.34
Valerate, mM	1.03	1.05	0.052	1.06	1.03	0.052	0.79	0.59	0.76
Isobutyrate, mM	0.79	0.67	0.022	0.73	0.74	0.022	<0.01	0.86	0.36
Isovalerate, mM	1.25	1.08	0.057	1.15	1.18	0.057	0.06	0.72	0.73
Total BCVFA, mM	2.04	1.76	0.074	1.88	1.92	0.074	0.01	0.75	0.99

A:P, acetate:propionate; BCVFA, branched-chain VFA.

¹Ruminal fluid was collected using an oral stomach tube approximately 3 h after morning feeding on the seventh day of weeks 6 and 8.

²No interaction ($P \geq 0.10$) of week × protein × nisin, week × protein, or week × nisin was detected on all measurements. P × N is the interaction of protein × nisin.

et al., 2016). Previous studies have demonstrated that artificial rumen has much lower solid content and total microbial biomass (including bacteria, fungi, and protozoa) than the rumen (Soto et al., 2012), and thus the results of in vitro studies should not be simply extrapolated to in vivo conditions (Patra and Yu, 2015). Therefore, the lack of nisin treatment effects on rumen fermentation is likely attributable to the dosage being too low. In future studies, different doses of nisin should be tested to further evaluate its efficacy in modifying rumen fermentation.

Nutrients Intake and Digestibility

No interaction ($P \geq 0.96$) of protein source with nisin was detected with respect to any of the nutrient intake measurements (Table 3). The inclusion of DDGS did not affect ($P \geq 0.48$) DM, OM, or CP intake. However, the EE intake was greater ($P < 0.01$), NDF intake tended to be greater ($P = 0.07$), and ADF was numerically greater ($P = 0.21$) for lambs receiving DDGS than for SBM-fed lambs, which could be explained by the higher concentrations of EE, NDF, and ADF of the DDGS-based than the SBM-based diets. Similar to the current results, Felix et al. (2012) also reported unchanged DM intake but increased EE, NDF, and ADF intake in lambs fed increasing corn DDGS. However, because of the increased CP level in most DDGS-based diets, CP intake also increased (Felix et al., 2012). In another trial using dairy cattle, Zhang et al. (2010) found that substitution of rolled barley and canola meal with 20% DDGS from corn

and wheat did not affect DM or OM but increased NDF and EE intake, which agrees with the current findings in lambs.

There were interactions ($P < 0.05$) between protein source and nisin for apparent digestibility of DM, OM, NDF, and ADF (Table 3). The SBM-control diet had the greatest, while the DDGS-control diet had the least (interaction; $P < 0.01$) DM and OM apparent digestibility. In contrast, the SBM-nisin treatment has the least, while the DDGS-nisin treatment has the greatest (interaction; $P \leq 0.02$) apparent digestibility of NDF and ADF. These interactions were not expected and might have resulted from the differences in chemical composition between the DDGS and SBM diets. No interaction was detected with respect to apparent digestibility of CP and EE ($P \geq 0.23$). None of the measurements of nutrient digestibility was affected ($P \geq 0.13$) by nisin addition. The DDGS-fed lambs had less ($P < 0.01$) CP apparent digestibility, but a greater ($P < 0.01$) EE apparent digestibility than those fed SBM. Consistent with the current results, Willms et al. (1991) also found reduced CP apparent digestibility in lambs fed DDGS, which is substantiated by the reduced ruminal NH₃ and BUN concentrations. In contrast, Felix et al. (2012) reported a linear increase in CP apparent digestibility in lambs fed increasing dietary DDGS. The disparity in CP digestibility between different studies may be due to different experimental conditions but have also been attributed to differences in the drying process of DDGS at bioethanol plants (Spiehs et al., 2002). The greater apparent digestibility of EE in the DDGS-fed lambs was in agreement with

Table 3. Effects of dietary protein sources and nisin on feed intake and apparent total tract digestibility of nutrients in growing lambs¹

Item	Protein source			Nisin			<i>P</i> -value ²		
	SBM	DDGS	SEM	Control	Nisin	SEM	Protein	Nisin	P × N
Intake, g/d									
DM	1,280.9	1,250.6	36.67	1,254.1	1,277.3	36.67	0.50	0.65	0.96
OM	1,178.1	1,146.5	33.32	1,151.6	1,173.0	33.32	0.51	0.66	0.96
CP	197.5	192.0	5.64	193.0	196.5	5.64	0.48	0.65	0.96
NDF	503.8	543.9	15.03	519.0	528.6	15.03	0.07	0.65	0.98
ADF	273.5	288.4	8.08	278.4	283.5	8.08	0.20	0.65	0.98
EE	67.1	87.7	2.20	76.7	78.1	2.20	<0.01	0.66	0.98
Digestibility, %									
DM ³	66.9	64.6	0.43	66.0	65.5	0.43	<0.01	0.41	<0.01
OM ³	69.4	67.0	0.46	68.6	68.1	0.46	<0.01	0.43	<0.01
CP	68.6	65.9	0.55	67.5	67.0	0.55	<0.01	0.45	0.33
NDF ³	50.2	52.2	0.80	51.8	50.6	0.80	0.09	0.32	<0.01
ADF ³	51.5	54.3	0.90	53.4	52.4	0.90	0.03	0.44	0.02
EE ²	68.9	71.6	0.69	71.0	69.5	0.69	<0.01	0.13	0.23

¹Diet, ort, and fecal samples were collected from each lamb on the first to third day of week 9.

²P × N is the interaction of protein × nisin.

³Least squares means of apparent digestibility of DM, OM, NDF, and ADF (%) for SBM-control, SBM-nisin, DDGS-control, DDGS-nisin were 68.1^a, 65.7^b, 63.9^b, and 65.3^b (DM); 70.7^a, 68.2^{ab}, 66.5^b, and 68.0^b (OM); 52.5^a, 47.9^b, 51.0^{ab}, and 53.4^a (NDF); 53.6^{ab}, 49.3^b, 53.2^{ab}, and 55.5^a (ADF), respectively. ^{a,b}Means within an indicator without a common superscript differ ($P < 0.05$).

the report of [Zhang et al. \(2010\)](#) who reported that EE digestibility increased (62.6% vs. 80.0%) when cows were fed 20.1% DDGS instead of rolled barley, canola meal, and corn gluten meal. Similarly, [Walter et al. \(2012\)](#) found a linear increase of apparent digestibility of EE when heifers were fed increasing amount of corn DDGS. The greater EE digestibility may be attributed to the higher dietary EE content ([Palmquist and Conrad, 1978](#)) and the unsaturated nature of the fat of corn DDGS ([Zinn et al., 2000](#); [Walter et al., 2012](#)).

Plasma Metabolites

Plasma concentrations of blood metabolites could be used as indicators of nutritional status ([Pambu-Gollach et al., 2000](#)). In the present study, no interaction ($P \geq 0.06$) of week × protein × nisin, week × protein, week × nisin, or protein × nisin was detected on any of the plasma metabolites except for the interaction of week × protein on glucose ($P = 0.04$; [Table 4](#)). The DDGS-fed lambs had lower ($P < 0.01$) BUN and albumin concentrations than those fed SBM, but total plasma protein concentration was similar ($P = 0.94$). The reduced BUN and albumin concentrations in lambs receiving DDGS is probably attributed to the lesser ruminal protein degradability and total tract CP digestibility. The reduced BUN in lambs receiving DDGS was also substantiated by the decreased ruminal NH₃, which is an important precursor of BUN ([Benchaar et al., 2013](#)). The plasma

concentrations of globulin, creatinine, uric acid, total cholesterol, triglyceride, and alkaline phosphatase were not influenced ($P \geq 0.30$) by DDGS, which indicates that DDGS can be included at rates up to 20% without causing any health problems that can affect their performance. Uric acid is a purine metabolite that can be degraded by hepatic enzyme uricase to allantoin in most mammals. In the present study, plasma uric acid concentration was reciprocally transformed to improve normality. Nisin addition decreased uric acid reciprocal transformation value ($P = 0.04$), which means increased uric acid concentration in the nisin treatment. Further study is still needed to determine the reason why nisin increased plasma uric acid concentration. Except for uric acid, nisin did not affect ($P \geq 0.32$) any of the other plasma metabolites, which might have been a result of the lack of influence on rumen fermentation.

Nitrogen Balance

Nitrogen metabolism measurements were not influenced ($P \geq 0.33$) by nisin or nisin × protein source interaction ([Table 5](#)). The N intake (g/d), total N excretion, and N retention, expressed either as g/d or a proportion of N intake (%), did not differ ($P \geq 0.23$) between the two protein sources. However, the N excretion pathway changed when DDGS replaced SBM. When expressed as a proportion of N intake (%), the fecal N excretion was greater ($P < 0.01$), while the urinary N excretion

Table 4. Effects of dietary protein sources and nisin on plasma metabolites in growing lambs¹

Item	Protein source			Nisin			P-value ²		
	SBM	DDGS	SEM	Control	Nisin	SEM	Protein	Nisin	P × N
Glucose, mg/dL	75.0	71.5	1.34	73.0	73.5	1.34	0.08	0.78	0.81
BUN, mg/dL	18.6	16.1	0.35	17.1	17.6	0.35	<0.01	0.32	0.72
Globulin, g/dL	3.48	3.58	0.067	3.48	3.57	0.067	0.30	0.37	0.99
Albumin, g/dL	2.39	2.28	0.034	2.34	2.33	0.034	<0.01	0.72	0.16
Total protein, g/dL	5.86	5.85	0.062	5.82	5.89	0.062	0.94	0.41	0.57
Creatinine, mg/dL	0.55	0.55	0.017	0.55	0.56	0.017	0.92	0.66	0.85
Uric acid ³ , mg/dL	6.23	5.97	0.342	6.62	5.59	0.342	0.59	0.04	0.77
Total cholesterol, mg/dL	53.0	54.6	1.84	54.2	53.4	1.84	0.56	0.75	0.63
Triglyceride, mg/dL	33.7	35.1	2.36	33.6	35.1	2.36	0.45	0.44	0.06
Alkaline phosphatase, IU/L	337.6	306.6	23.36	309.0	335.2	23.36	0.36	0.43	0.59

¹Blood samples were collected each lamb at approximately 3 h after morning feeding on the sixth day of weeks 6 and 8.

²No interaction ($P \geq 0.07$) of week × protein × nisin, week × protein, or week × nisin was detected on all measurements except for interaction of wk and protein on glucose ($P = 0.04$). P × N is the interaction of protein × nisin.

³Reciprocal transformation value.

tended to be less ($P = 0.06$) for lambs receiving DDGS than those fed SBM. Such increase of fecal N and decrease in urinary N was likely caused by reduced CP digestibility of the DDGS. Nitrogen excretion by livestock and N₂O emission from manure contribute to air and ground water pollution. Whereas the shift of N excretion from urine to feces observed in the current study may have the potential to alleviate the impact to the environment. Urinary N is rapidly converted to NH₃, whereas fecal N is converted to NH₃ at a much slower rate (Bussink and Oenema, 1998). Besides, reducing urine N output from ruminants is also critical to reducing N₂O emissions (Dijkstra et al., 2013). Therefore, shifting N excretion from urine to feces is recognized as a means to increase the environmental stability of manure N (Varel et al., 1999).

The decreased urinary N and increased fecal N excretion observed in the DDGS-fed lambs would likely reduce N loss in the form of NH₃ as well as direct and indirect N₂O emissions.

To our knowledge, few published studies have investigated in detail the effects of including DDGS on N utilization in lambs. In sheep and beef cattle diets, DDGS was mainly added as an energy source, which results in increased dietary CP concentration. The only study in lambs (Felix et al. (2012) and those in beef cattle (Salim et al., 2012; Walter et al., 2012; Hünerberg et al., 2013) found increased total N excretion (g/d) probably because of increased dietary CP level. Under isonitrogenous dietary conditions in beef steers, Salim et al. (2012) observed increased fecal N excretion (% of N intake) but decreased urinary N excretion (% of N intake)

Table 5. Effects of dietary protein sources and nisin on nitrogen balance in growing lambs¹

Item	Protein source			Nisin			P-value ²		
	SBM	DDGS	SEM	Control	Nisin	SEM	Protein	Nisin	P × N
N intake, g/d	31.6	30.7	0.90	30.9	31.4	0.90	0.48	0.65	0.96
Fecal N excretion									
g/d	9.93	10.49	0.37	10.02	10.40	0.37	0.28	0.46	0.59
% of N intake	31.4	34.1	0.55	32.5	33.1	0.55	<0.01	0.45	0.33
Urinary N excretion									
g/d	13.1	11.5	0.51	12.3	12.3	0.51	0.01	0.88	0.64
% of N intake	42.0	37.6	1.58	40.0	39.6	1.58	0.06	0.87	0.69
Total N excretion									
g/d	23.0	22.0	0.82	22.4	22.7	0.82	0.23	0.75	0.55
% of N intake	73.5	71.6	1.73	72.4	72.7	1.73	0.48	0.92	0.52
N retention									
g/d	8.55	8.74	0.65	8.50	8.79	0.65	0.83	0.76	0.61
% of N intake	26.5	28.3	1.73	27.6	27.3	1.73	0.46	0.93	0.50

¹Fecal and urine samples were collected twice daily before each feeding on the first day to third day of week 9.

²P × N is the interaction of protein × nisin.

Table 6. Effects of dietary protein sources and nisin on DMI, ADG, and G:F in growing lambs

Item	Protein source			Nisin			<i>P</i> -value ¹		
	SBM	DDGS	SEM	Control	Nisin	SEM	Protein	Nisin	<i>P</i> × <i>N</i>
DMI, g/d									
Weeks 1–4	1,046.0	952.4	31.32	1,015.5	982.9	31.32	0.03	0.44	0.81
Weeks 5–8	1,266.5	1,235.5	36.09	1,234.5	1,267.6	36.09	0.53	0.50	0.83
Overall	1,156.3	1,093.0	26.20	1,125.0	1,125.3	26.20	0.07	0.92	0.91
ADG, g/d									
Weeks 1–4	236.6	199.8	10.73	219.7	216.7	10.73	0.03	0.84	0.80
Weeks 5–8	234.9	251.3	12.32	242.1	244.1	12.32	0.23	0.88	0.94
Overall	235.9	225.6	8.89	231.3	230.2	8.89	0.35	0.92	0.85
G:F									
Weeks 1–4	0.226	0.210	0.008	0.214	0.217	0.008	0.13	0.83	0.56
Weeks 5–8	0.183	0.204	0.007	0.195	0.192	0.007	0.04	0.73	0.88
Overall	0.204	0.206	0.006	0.206	0.205	0.006	0.99	0.95	0.63

¹No interaction ($P \geq 0.09$) of week × protein × nisin, week × protein, or week × nisin was detected on all measurements except for interaction of wk and protein on overall G:F ($P = 0.04$). *P* × *N* is the interaction of protein × nisin.

when DDGS was added at 16.7% of DM, which agrees with the findings of the current experiment in lambs. On the contrary, Hünnerberg et al. (2013) found increased urinary N excretion (% of N intake) but decreased fecal N excretion (% of N intake) for beef cattle fed 40% DDGS in place of 35% barley grain and 5% canola meal. Benchaar et al. (2013) observed a linear decline in fecal N output (% of N intake), but unaffected urinary N excretion (% of N intake) when DDGS was fed to dairy cattle at increasing levels. Discrepancies between studies in N excretion in response to feeding DDGS could be related to differences in DDGS source and addition levels, animal species, and the type of feed ingredients of the basal diet being replaced by DDGS.

Growth Performance

No interaction ($P \geq 0.09$) of week × protein × nisin, week × protein, week × nisin, or protein × nisin was detected on lambs growth performance except for the interaction of week × protein on overall G:F ($P = 0.04$; Table 6). The overall DMI tended to be less ($P = 0.07$) in the lambs fed DDGS than in those fed SBM, but the overall ADG were not influenced ($P = 0.35$) by protein source. In a previous study, Huls et al. (2006) reported unchanged DMI and ADG in lambs fed isonitrogenous diets containing 20% of DDGS, resulting in similar G:F between DDGS- and SBM-base diets, which are in agreement with the current findings. However, a reduced DMI with decreased ADG but similar G:F was observed in growing lambs receiving 40% DDGS-based diet compared with those receiving a cottonseed meal-based diet (Whitney et al., 2014). Felix et al. (2012) found that at 20%

of DM, the same inclusion level with the current experiment, DDGS did not affect lamb growth, but at 40% and 60%, DDGS resulted in decreased lamb feedlot performance. Differences in experimental conditions, such as DDGS supplement level, experimental period, animal species, and the type of feed ingredients of the basal diet, may contribute to the discrepancies in DMI and ADG among studies.

One interesting finding of the present study is that the two different protein sources affected growth performance in an age/time-dependent manner. From weeks 1 to 4, DDGS resulted in lesser ($P = 0.03$) DMI and ADG than SBM, which contributed to the trend for decreased BW ($P = 0.07$; Figure 1), but unaffected G:F ($P = 0.13$). In contrast,

Figure 1. Effects of dietary protein sources and nisin on BW in growing lambs. In week 4, the SBM-fed lambs had a trend to increase BW compared to those fed DDGS ($P = 0.07$), while the BW did not differ between SBM and DDGS ($P \geq 0.13$) in other week. The BW was not influenced by nisin or nisin × protein source interaction ($P \geq 0.33$) in each week. Error bars indicate the SE.

from weeks 5 to 8, DDGS did not affect ($P \geq 0.23$) DMI or ADG but resulted in a greater ($P = 0.04$) G:F than SBM. The numerically increased ADG and higher G:F observed in DDGS-fed lambs might be because of compensation for lower BW gain during weeks 1 to 4, resulting in no difference in final BW between the two diets. These results indicate that lambs may need longer time to adapt to the DDGS. Many trials, especially in dairy and beef cattle, are of relatively short duration, such as 4- or 5-wk periods in Latin square-style experiments. The present study confirmed that short-term research experiments probably do not accurately reflect the growth performance response expected when feeding DDGS continuously for long periods. In the present study, nisin addition did not affect ($P \geq 0.33$) DMI, ADG, G:F or BW, which might have resulted from the lack of influence on rumen fermentation, nutrient digestion, or N utilization.

CONCLUSIONS

Replacing SBM in an isonitrogenous lamb diet with DDGS reduced ruminal $\text{NH}_3\text{-N}$, BUN, and urinary N excretion (% of N intake), but increased fecal N excretion (% of N intake). The shift of N excretion from urine to feces should reduce N loss in the form of NH_3 as well as direct and indirect N_2O emissions. The growth performance (DMI, ADG, G:F, and BW) of lambs fed with the two different protein sources responded differently with time. However, the final BW was not influenced. Nisin supplementation at the tested dose did not affect rumen fermentation, nutrient digestion, plasma metabolites (except for uric acid), N utilization, or growth performance. Results of the present study indicate that DDGS can substitute SBM to grow Hu lambs to reduce N excretion via urine without adverse effects on animal performance, but nisin may need to be fed at different concentrations to evaluate its efficacy further.

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