Journal of Animal and Feed Sciences, 34, 4, 2025, xxx–xxx, https://doi.org/10.22358/jafs/203389/0000
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

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ORIGINAL PAPER

Effects of soybean lecithin supplementation on ruminal fermentation, polysaccharide hydrolase activity, and nutrient digestion in sheep

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KEY WORDS: digestion, enzyme activity, rumen fermentation, sheep, soybean lecithin

Received: 20 January 2025
Revised: 12 March 2025
Accepted: 26 March 2025

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ABSTRACT. Soybean lecithin (SL) is commonly used as a nutritional supplement in animal diets. This study investigated the effects of SL on feed intake, ruminal fermentation, rumen microbial populations, digestive enzyme activities, and apparent nutrient digestibility in sheep. The feeding trial involved four Chinese Merino rams fitted with permanent rumen fistulae in a 4 × 4 Latin square design, fed a basal diet supplemented with 0, 10, 20, and 40 g/day of SL for 22 days. SL addition significantly increased the intake of ether extract (EE), phosphorus, and the apparent digestibility of EE and acid detergent fibre (ADF) ($P < 0.05$). It also influenced the total counts of bacteria and fungi ($P < 0.05$), while protozoal populations remained unaffected ($P > 0.05$). The concentrations of total volatile fatty acids and ammonia nitrogen decreased significantly ($P < 0.05$), whereas the activities of carboxymethyl cellulase and filter paper cellulase increased ($P < 0.05$) with rising levels of SL supplementation in sheep rumen fluid. These findings suggest that dietary SL can improve rumen fermentation parameters, stimulate digestive enzyme activity, and increase the apparent digestibility of EE and ADF. The results of this study indicate that 20 g/day is the optimal SL supplementation level in sheep diets.

Introduction

Soybean lecithin (SL), a by-product of soybean oil processing, is composed of phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, essential fatty acids, and certain vitamins (Zhu et al., 2020). Its chemical structure includes both hydrophilic (fatty acid chains) and lipophilic (glycerol, phosphorus, and the functional moiety) components, conferring emulsifying properties that enhance the rapid and efficient utilisation of dietary energy, particularly from fat sources (Viñado et al., 2019). The functional groups in SL such as choline, ethanolamine, and inositol exhibit diverse biological activities, including growth promotion, antioxidant effects, lipid metabolism regulation, and immune support. Moreover, they contribute to the structural integrity of cellular membranes (Shi et al., 2019; Shen et al., 2021; Tan et al., 2022). Therefore, SL is a cost-effective and readily available functional feed additive, providing crude energy, phosphorus, choline, linoleic, and linolenic acids in animal nutrition.

Dietary SL supplementation has been shown to improve feed efficiency and growth performance in various animal species. In poultry, SL containing a high level of free fatty acids can partially replace soybean oil or be combined with acidic oils to improve the digestibility of fatty acids, dry matter,

protein, and fat (Viñado et al., 2020). SL addition to piglet diets has been shown to improve average daily gains and feed digestibility (Daněk et al., 2005). SL is also widely used as a nutritional additive in aquaculture, where it has been reported to improve growth and feed conversion efficiency (Yang et al., 2023). In ruminants, the presence of diverse microorganisms in rumen suggests that SL supplementation may alter the microbial community and thus affect the nutrient digestion and absorption. Studies in Simmental steers demonstrate that SL improves growth and increases ruminal volatile fatty acid (VFA) production without negatively affecting the dominant ruminal bacterial populations (Chen et al., 2020). Feeding de-oiled SL to Holstein cows has been shown to increase endogenous phospholipid synthesis and alter rumen digestion, reducing dry matter intake and changing milk composition without affecting fatty acid decomposition or absorption. Similar effects have also been observed in Angus steers (Fontoura et al., 2021). However, limited data are available regarding the effects of SL on rumen fermentation parameters, nutrient digestion, and metabolism in sheep.

Therefore, the present study aimed to evaluate the effects of dietary SL supplementation on rumen digestion and metabolism, microbial counts in rumen fluid, digestive enzyme activity, feed intake, and apparent nutrient digestibility. The findings will help determine the potential of SL as a functional dietary supplement in sheep production systems.

Material and methods

SL was purchased in the form of a yellow or brownish-yellow powder from Beijing Meryas Phospholipid Technology (Beijing, China). The product contained ≥97% total phospholipids, ≥95% acetone-insoluble matter, ≤2.0% moisture loss (drying reduction), ≤0.3% n-hexane insoluble matter, an acid value of ≤36 mg KOH/g, and a peroxide value of ≤10 mEq/kg.

Animals, diets, and experimental design

All experimental procedures involving animals were approved by the Animal Care Committee of Xinjiang Agricultural University (approval no. 2020021). Four 2-year-old healthy Chinese Merino (average body weight 35 ± 5 kg), each fitted with a permanent rumen fistula, were assigned to four dietary treatments in a 4 × 4 Latin square design. The sheep were fed twice daily at 9:00 and 21:00. Each feeding consisted of 200 g of concentrate supplemented with 0, 5, 10, or 20 g of SL was given to the animals, followed by *ad libitum* access to wheat straw and clean drinking water. The ingredient and nutrient compositions of the concentrate and wheat straw are detailed in Table 1. Each experimental period lasted 22 days, including a 12-day adaptation period, a 7-day faeces collection period, and a 3-day ruminal fluid sampling period.

Table 1. Ingredient and nutrient composition of the concentrate and wheat straw (air-dry basis)

Ingredients of concentrate	Composition, %	Nutrients, %	Concentrate	Wheat straw
Yellow maize, ground	64.0	OM	94.1	86.9
Cottonseed meal	33.6	CP	27.7	2.32
NaCl	1.68	Ca	0.30	0.47
Urea	0.72	P	0.44	0.07
Premix[1]	0.05	Cellulose	8.60	41.3
		Haemicellulose	10.9	21.9
		Lignin	3.64	9.66

[1] provided per kg of diet: g: 0.75 I, 0.45 Se, 0.030 Co, 1.27 Cu, 23.0 S; KIU: vit. A 4000, vit. D 800; OM – organic matter, CP – crude protein, Ca – calcium, P – phosphorus; nutrient compositions were based on direct measurements

Nutrient intake and apparent digestibility

During the trial, small samples of mixed concentrate and straw were collected daily, thoroughly mixed, and stored in sealed containers. Daily feed intake was precisely recorded for each group, with the average intake calculated for the entire experimental period. During the faeces collection period, samples were taken twice daily at 9:00 and 21:00. All faeces excreted by each animal over the 7-day period were collected separately. A 10% subsample of the total weight was taken, air-dried, ground, and stored at 4 °C for further analysis.

Dry matter (DM), crude protein (CP), ether extract (EE), organic matter (OM), calcium (Ca), and total phosphorus (P) contents in the feed and faecal samples were determined using AOAC International (1999) standard methods. Acid detergent fibre (ADF), neutral detergent fibre (NDF), and acid detergent lignin were determined according to the method described by Van Soest et al. (1991), with cellulose and hemicellulose contents calculated as described by Chen et al. (2011). Nutrient apparent digestibility was calculated as follows:

$$\text{Apparent digestibility (\%)} = \frac{\text{nutrients in feed (g)} - \text{nutrients in faeces (g)}}{\text{nutrients in feed (g)}} \times 100\%$$

Ruminal fermentation analysis

Ruminal fluid samples (60 ml per sheep) were collected through permanent fistulas before feeding (0 h) and at 1.5, 4, 8, and 12 h after morning feeding. The pH was measured directly after sampling using an ISO720 pH meter (Orion, Espoo, Finland). For VFA and ammonia nitrogen analysis, 30 ml of ruminal fluid was collected and immediately stored at −20 °C for ruminal VFA and ammonia nitrogen (NH_3-N) determinations. For microbial population and enzyme activity analysis, 10 ml of ruminal fluid was centrifuged at 1000 *g* for 10 min to remove feed particles, and the supernatant was flash-frozen in liquid nitrogen before storage at −80 °C.

Rumen fluid samples were thoroughly mixed before analysis, and crotonic acid was used as the internal standard. Quantitative analysis was conducted using a GC-2010 gas chromatograph (Shimadzu, Kioto, Japan) equipped with a capillary column and a flame ionisation detector. The gas chromatography conditions were maintained with injector and detector temperatures of 230 ℃ and 240 ℃, respectively. The column oven temperature was programmed to increase from 55 °C to 200 ℃ at a rate of 13 °C/min, held for 30 s and then terminated. Nitrogen served as the carrier gas at a flow rate of 5.0 ml/min. Concentrations of acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate in the rumen fluid were quantified. Ammonia nitrogen concentrations were determined colorimetrically using ammonium chloride as the standard and an Infinite M200 microplate reader (Tecan, Männedorf, Switzerland) (Wei et al., 2024).

Enzyme activity in ruminal fluid

The enzymatic activities of β-xylanase, carboxymethylcellulase (CMCase), filter paper cellulase (FPase), and β-glycosidase in ruminal fluid were determined using a colorimetric method involving 3,5-dinitrosalicylic acid (DNS) (Yu et al., 2020). Following centrifugation at 15000 *g* for 15 min at 4 ℃, the supernatant was collected for analysis. The assay procedure involved incubating 0.5 ml of rumen fluid supernatant (0.2 ml for CMCase) with 1.0 ml of specific substrate solution (0.5% oat xylan for β-xylanase, 0.5% sodium carboxymethylcellulose for CMCase, 0.5% Whatman no. 1 filter paper for FPase, or 0.5% salicin for β-glycosidase, all prepared in 0.2 mol/l phosphate buffer, pH 6.0) and adjusting the final volume to 3.0 ml with additional buffer. Incubation conditions varied by enzyme: 39 °C for 1 h (β-xylanase and FPase), 0.5 h (CMCase), or 2 h (β-glycosidase). Reactions were terminated by adding 2.0 ml DNS solution, followed by boiling for 5 min and centrifugation at 15000 *g* for 15 min at 4 °C. The absorbance of the resulting supernatant was measured at 540 nm using an Infinite M200 microplate reader (Tecan). Enzyme activity is expressed as units (U) per ml of rumen fluid, where one unit corresponds to the amount of enzyme required to release 1 μmol of glucose or xylose per minute under the specified assay conditions.

Microbial counts

After thawing, 1 ml of rumen fluid was thoroughly mixed, and total rumen microbial DNA was extracted directly using the CTAB method (Minas et al., 2011). DNA quality was assessed by electrophoresis on a 1% agarose gel. Conventional PCR was performed using an iCycler thermal cycler (Bio-Rad, Hercules, CA, USA) in a 20 μl reaction volume containing 2.0 μl 10 × PCR buffer, 2.0 μl of dNTP mix (2.5 mM each), 1.0 μl of TaqDNA polymerase (5.0 U/μl; Takara Bio Inc, Otsu, Shiga, Japan), 0.2 μl of each primer (10 μM each), 1.0 μl of microbial DNA (50 ng/μl), and 13.6 μl of ddH_2O. The PCR cycling conditions for total bacteria were as follows: initial denaturation at 94 ℃ for 5 min, then 35 cycles of 94 ℃ for 30 s; 64.6 ℃ for 30 s; and 72 ℃ for 30 s, followed by a final extension at 72 ℃ for 5 min. The corresponding PCR programme for fungi consisted of initial denaturation at 95 ℃ for 5 min, followed by 35 cycles of 95 ℃ for 30 s; 59 ℃ for 20 s; and 72 ℃ for 30 s, and a final extension at 72 ℃ for 5 min. The amplicons were separated by electrophoresis on 2% agarose gel, purified using a DNA purification kit (TIANgel; Tiangen, Beijing, China), then subcloned into pGM-T vector, and propagated in *Escherichia coli* DH5α (Tiangen). Recombinant plasmids underwent DNA sequencing, following sequence alignments. The quantified plasmid standards were serially diluted (10-fold) to concentrations ranging from 10^3 to 10^7 copies. Quantitative real-time PCR was performed using a LightCycler® 2.0 System (Roche, Basel, Switzerland). Each quantitative PCR mixtures contained 10.0 μl SYBR Premix Dimer Eraser™ (Takara Bio Inc, Otsu, Japan), 0.3 μl of each primer (10 μM each), 50 ng of microbial DNA, and ddH_2O to a final volume of 20 μl. Standard curves were generated by plotting the threshold cycle (C_t) values against the logarithm of DNA copy numbers. The absolute quantification of target DNA copies was calculated based on the standard curve according to previous report (Singh et al., 2014).

The primer sequences for quantitative PCR analysis of total bacteria and fungi are listed in Table 2 (Denman and McSweeney, 2006). Protozoal counts in rumen fluid were determined using optical microscopy (MB1000; Keyence, Shanghai, China) as described previously (Wei et al., 2024).

Table 2. PCR primers for quantitative PCR analysis of ruminal bacteria and fungi

Microorganisms	Sequence (5'→3')	Product size, bp	Reference
Total bacteria	1114F: CGGCAACGAGCGCAACCC	130	Denman et al (2006)
	1275R: CCATTGTAGCACGTGTGTAGCC		
Fungi	Fungi-F: GAGGAAGTAAAAGTCGTAACAAGGTTTC	120	
	Fungi-R: CAAATTCACAAAGGGTAGGATGATT		

Statistical analysis

Data are expressed as means ± SEM and analysed using general linear models (GLMs) in SPSS software (version 20.0; IBM, Armonk, NY, USA) according to the equation:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk},$$

where: y_{ijk} – observation; μ – overall mean; α – fixed effect of SL supplementation (j = 1 to 4); β – random effect of animals (i = 1 to 4); γ – fixed effect of treatment periods (k = 1 to 4); and ε_{ijk} – residual error. Mean comparisons were performed using Tukey's test. Differences were considered significant at $P < 0.05$.

Results

Effect of SL supplementation on feed intake

Dietary SL supplementation significantly influenced nutrient intake patterns (Table 3). The inclusion of SL up to 20 g/day increased intake of wheat straw, OM, DM, CP, cellulose, and Ca. A slight decline was noted with further SL addition, but the differences were not statistically significant ($P > 0.05$). In contrast, EE and P intake demonstrated a linear dose-dependent response ($P < 0.05$), with the highest SL dose (40 g/day) increasing EE intake by 159.92% (62.9 vs 24.2) and P intake by 44.55% (3.18 vs 2.20) relative to the control. Intermediate doses (10 g/day and 20 g/day) elevated EE intake by 40.91% (34.1 vs 24.2) and 83.88% (44.5 vs 24.2), and P intake by 11.82% (2.46 vs 2.20) and 24.55% (2.74 vs 2.20), respectively ($P < 0.05$).

Effect of SL supplementation on ruminal fermentation characteristics

Dietary SL supplementation significantly altered ruminal fermentation parameters (Table 4). Rumen pH decreased ($P < 0.05$), with the most pronounced reduction observed at 10 g/day ($P < 0.05$). In addition, while acetate and valerate concentrations remained unaffected by SL supplementation ($P > 0.05$), total VFA levels showed a linear decrease. Butyrate, isobutyrate, and isovalerate concentrations generally exhibited quadratic decreases with increasing SL levels. On the other hand, propionate concentrations increased both linearly and quadratically ($P < 0.05$), exceeding the control group by approx. 6.35% (18.4 vs 17.3) at 40 g/day SL ($P < 0.05$). The NH_3-N concentration also increased significantly with SL supplementation compared to the control group ($P < 0.05$), and followed a quadratic trend with increasing SL levels.

Table 3. Effects of soybean lecithin (SL) supplementation on feed intake in sheep (g/day sheep, n = 4)

Items	SL, g/day				SEM	*P*-value		
	0	10	20	40		M	L	Q
Wheat straw	705.1	711.4	752.3	681.0	31.95	0.511	0.834	0.271
DM	1018.5	1034.1	1081.9	1035.3	29.68	0.514	0.488	0.335
OM	914.6	928.6	970.8	930.8	25.95	0.508	0.464	0.338
CP	130.06	130.89	133.16	131.35	1.329	0.463	0.340	0.357
EE	24.18^{d}	34.05^{c}	44.47^{b}	62.88^{a}	0.505	<0.001	<0.001	<0.001
NDF	591.0	595.4	624.7	573.7	22.87	0.511	0.834	0.271
ADF	400.6	403.7	424.5	388.4	16.21	0.511	0.834	0.271
Ca	4.89	4.92	5.14	4.76	0.173	0.519	0.173	0.277
P	2.20^{d}	2.46^{c}	2.74^{b}	3.18^{a}	0.026	<0.001	<0.001	0.012

DM – dry matter, OM – organic matter, CP – crude protein, EE – ether extract, NDF – neutral detergent fibre, ADF – acid detergent fibre, Ca – calcium, P – phosphorus, SEM – standard error of the mean, M – main effect, L – linear effect, Q – quadratic effect; ^{abc} – means with different superscripts are significantly different at $P < 0.05$

Table 4. Effect of soybean lecithin (SL) supplementation on ruminal fermentation parameters in sheep

Items	SL, g/day				SEM	*P*-value		
	0	10	20	40		M	L	Q
pH	6.46^{a}	6.34^{c}	6.42^{ab}	6.40^{bc}	0.020	0.006	0.184	0.064
Total VFA, mM	82.55^{ab}	84.01^{a}	80.53^{ab}	79.76^{b}	1.281	0.088	0.043	0.387
Acetate, %	70.60	70.35	70.27	70.22	0.243	0.701	0.267	0.696
Propionate, %	17.27^{b}	17.08^{b}	17.23^{b}	18.41^{a}	0.153	<0.001	<0.001	<0.001
Butyrate, %	10.02^{a}	10.37^{a}	10.30^{a}	9.41^{b}	0.175	0.001	0.018	0.001
Isobutyrate, %	0.75^{ab}	0.79^{a}	0.79^{a}	0.70^{b}	0.022	0.015	0.126	0.005
Isovalerate, %	0.81^{a}	0.85^{a}	0.82^{a}	0.73^{b}	0.022	0.001	0.005	0.003
Valerate, %	0.56	0.55	0.58	0.53	0.017	0.279	0.510	0.216
NH_3-N, mM	10.27^{c}	13.10^{a}	11.35^{b}	10.98^{bc}	0.354	<0.001	0.810	<0.001

VFA – volatile fatty acids, NH_3-N – ammonia nitrogen, SEM – standard error of the mean, M – main effect, L – linear effect, Q – quadratic effect; ^{abc} – means with different superscripts are significantly different at $P < 0.05$

Effect of SL supplementation on ruminal digestive enzyme activity

SL supplementation significantly influenced fibrolytic enzyme activities (Table 5). While xylanase and β-glycosidase activities were not significantly affected, CMCase and FPase activities increased linearly with rising SL levels ($P < 0.05$). Maximum CMCase activity was recorded at 20 g/day SL (73.9 vs 65.4, 13.00% above control). FPase activity increased progressively, reaching 47.65% (3.78 vs 2.56, 20 g/day) and 60.55% (4.11 vs 2.56, 40 g/day) above control levels ($P < 0.05$).

Effect of SL supplementation on rumen microbial counts

SL supplementation significantly altered rumen microbial counts (Table 6). Total bacterial counts increased quadratically with increasing SL levels ($P < 0.05$). Supplementing SL with the feed significantly reduced the fungal population in the rumen when compared to the control group ($P < 0.05$), while the number of protozoa was not significantly affected ($P > 0.05$).

Effect of SL supplementation on apparent digestibility

SL supplementation improved apparent nutrient digestibility in sheep (Table 7). The apparent digestibility of DM, OM, CP, NDF, Ca, and P in sheep increased after SL supplementation compared to the control group. These nutrients showed a tendency to initially increase and then decline with higher SL inclusion levels, though the differences were not statistically significant ($P > 0.05$). In contrast, ADF digestibility increased linearly, reaching its highest value at the 20 g/day SL dose, i.e., 19.02% (43.8 vs 36.8) higher than in the control ($P < 0.05$). The apparent digestibility of NDF increased with SL supplementation, but the differences were not significant compared to the control group ($P > 0.05$).

Table 5. Effect of soybean lecithin (SL) supplementation on ruminal digestive enzyme activity in sheep

Enzyme, IU/ml	SL, g/day				SEM	*P*-value		
	0	10	20	40		M	L	Q
β-xylanase	47.33	45.98	53.87	49.65	3.241	0.406	0.345	0.673
CMCase	65.44^{b}	64.99^{b}	73.08^{a}	70.49^{ab}	2.054	0.082	0.021	0.486
FPase	2.56^{b}	3.08^{ab}	3.78^{a}	4.11^{a}	0.326	0.056	0.011	0.785
β-glycosidase	3.79	3.67	4.11	4.80	0.422	0.317	0.113	0.376

CMCase – carboxymethylcellulase, FPase – filter paper cellulose, SEM – standard error of the mean, M – main effect, L – linear effect, Q – quadratic effect; ^{ab} – means with different superscripts are significantly different at $P < 0.05$

Table 6. Effect of soybean lecithin (SL) supplementation on rumen microbial counts in sheep

Microorganisms	SL, g/day				SEM	*P*-value		
	0	10	20	40		M	L	Q
Total bacteria, log(copies/ml)	10.64^{ab}	10.62^{ab}	10.58^{b}	10.71^{a}	0.038	0.109	0.159	0.048
Fungi, log(copies/ml)	6.98^{a}	6.76^{b}	6.76^{b}	6.68^{b}	0.057	0.004	0.001	0.222
Protozoa, log(counts/ml)	5.82	5.84	5.81	5.81	0.012	0.308	0.222	0.397

SEM – standard error of the means, M – main effect, L – linear effect, Q – quadratic effect; ^{ab} – means with different superscripts are significantly different at $P < 0.05$

Table 7. Effect of soybean lecithin (SL) supplementation on apparent digestibility in sheep

Items, %	SL, g/day				SEM	*P*-value		
	0	10	20	40		M	L	Q
DM	51.75	52.36	53.99	55.94	1.335	0.218	0.055	0.633
OM	54.61	55.23	57.00	59.41	1.337	0.149	0.035	0.527
CP	64.80	65.71	64.63	67.03	1.974	0.817	0.548	0.718
EE	63.24^{c}	73.96^{b}	78.38^{b}	88.03^{a}	2.577	0.003	<0.001	0.842
NDF	47.29	48.15	50.53	50.39	1.292	0.291	0.090	0.714
ADF	36.79^{b}	37.18^{b}	43.83^{a}	42.63^{ab}	1.666	0.050	0.018	0.649
Ca	28.88	23.64	20.33	17.42	8.960	0.824	0.383	0.901
P	21.37	21.81	13.13	27.64	4.489	0.253	0.631	0.168

DM – dry matter, OM – organic matter, CP – crude protein, EE – ether extract, NDF – neutral detergent fibre, ADF – acid detergent fibre, Ca – calcium, P – phosphorus, SEM – standard error of the mean, M – main effect, L – linear effect, Q – quadratic effect; ^{abc} – means with different superscripts are significantly different at $P < 0.05$

EE digestibility demonstrated a linear dose-response, increasing significantly by 17.09% (74.0 vs 63.2), 24.05% (78.4 vs 63.2), and 39.24% (88.0 vs 63.2) at successive SL levels compared to the control ($P < 0.05$).

Discussion

SL is a natural surfactant that can be used not only as both a nutritional feed additive and a fat replacer in animal diets. It provides essential nutrients including phosphorus, choline, and essential fatty acids (linoleic and linolenic acids) while improving feed palatability and utilization efficiency. Previous studies have demonstrated that SL improves nutrient digestion and absorption in animals (Chen et al., 2020; Viñado et al., 2020; Yang et al., 2023). Furthermore, Hill et al. (2009) reported that dietary SL increased daily weight gain and feed intake in calves during the first week of life. In the present study, SL addition significantly increased EE and P intake in sheep, likely due to its composition, which includes lipids, phospholipids, choline, and unsaturated fatty acids. As a result, SL has a higher EE and P contents, leading to proportional increases in EE and P intake with higher SL supplementation. In addition, the distinctive flavour of SL improves feed palatability while providing energy and multiple nutrients that improve feed nutritional value. Additionally, SL facilitates the absorption of lipids and fat-soluble vitamins (Polycarpo et al., 2016). These properties collectively contribute to improved nutrient intake when SL is incorporated into ruminant diets.

Rumen pH is affected not only by the interaction between rumen VFAs and salivary buffer salts, but also by diet composition, feeding schedule, and rumination time. It reflects the combined regulation of VFA and NH_3-N production, absorption, and utilization in the rumen. Chen et al. (2020) reported a linear decrease in ruminal pH value and a quadratic increase in acetate and total VFA concentrations with increasing SL supplementation in Simmental steers. In contrast, Abel-Caines et al. (1998) observed only marginal pH fluctuations (6.7–6.9) within normal physiological ranges in dairy cows receiving SL supplementation, with stable total VFA concentrations and molar proportions. This aligns with the present findings, where no significant change in pH was observed. The lack of a significant effect on rumen pH in sheep may be related to changes in the composition and abundance of rumen microorganisms introduced with SL, as well as potential SL degradation, which may alter its effect on rumen fermentation. The current study demonstrated that SL supplementation significantly influenced VFA concentration in sheep rumen fluid, initially increasing to subsequently decrease at higher SL inclusion levels. This pattern may be attributed to changes in diet composition caused by varying SL amounts, which in turn influenced the composition, abundance, and enzymatic activity of rumen microorganisms. Notably, excessive SL supplementation appeared to partially inhibit rumen fermentation processes in sheep. The presence of NH_3-N in rumen fluid is essential for the development of various rumen cellulolytic bacteria, and its elevated levels promote microbial activity, thereby facilitating the digestion of fibrous material. This interpretation is consistent with earlier findings suggesting that NH_3-N was more effectively utilised for microbial protein synthesis due to the greater availability of carbon and energy sources provided by elevated VFA levels, thereby improving nitrogen assimilation.

Rumen digestion is fundamentally mediated by microbial activity, with ruminants relying on enzymes produced by rumen microorganisms to break down feed components. The cellulolytic pro-

cess in the rumen is primarily driven by three key microbial groups: bacteria, fungi, and protozoa. Surfactants, such as SL, can interact with microbial cell membranes due to their high lipid content. These interactions may alter membrane hydrophobicity and adhesion properties, which could explain the observed shifts in rumen microbial diversity and abundance following surfactant supplementation in ruminant diets. Lee et al. (2003) showed that 0.05% Tween 80 significantly stimulated the growth of rumen bacteria and fungi, whereas a 0.10% concentration inhibited their development. Similarly, Hristov et al. (2003) found that 0.05% Tween 80 had no significant effect on the number of rumen protozoa, but higher concentrations (0.1 and 0.2%) increased the proportion of bacterial nitrogen utilised by protozoa. As both SL and Tween 80 are surfactants, they have similar modes of action on rumen microorganisms. According to Chen et al. (2020), the five most abundant bacterial phyla in the rumen (*Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Spirochaetae*, and *Actinobacteria*) and five genera (*Prevotella*, *Bacteroidales_BS11_gut*, *Prevotellaceae_UGG-033*, *Rikenellacese_RC*, and *Coprostanoligenes*) were not significantly affected by SL supplementation ($P > 0.05$), while less abundant bacterial groups showed a linear decline with increasing SL supplementation. The results of this experiment indicated that SL significantly influenced bacterial and fungal populations in sheep rumen ($P < 0.05$), while protozoal numbers remained unaffected ($P > 0.05$). These observations are consistent with previous findings of Lee et al. (2003) and Hristov et al. (2003), and suggest that surfactants may stimulate rumen bacterial growth within optimal concentration ranges. On the other hand, excessive supplementation may exert toxic effects on microbial growth.

Cellulose is a glucose polymer linked by 1,4-β-glucosidic bonds and constitutes the primary structural component of plant cell walls. In ruminants, cellulose digestion occurs through microbial adhesion to fibrous material and enzymatic hydrolysis (Hua et al., 2022). Cellulases secreted by these microorganisms mainly include three enzymes: β-1,4 endocellulases, β-1,4 exocellulases, and β-glucosidases, which act synergistically to degrade cellulose, thereby directly affecting feed conversion efficiency (Pérez et al., 2002). Complementary xylanase is mainly responsible for the breakdown and digestion of hemicellulose (Taguchi et al., 2004). SL, as a surfactant, can reduce the surface tension of cellulose and the reaction medium, improve cellulase adsorption and facilitate a more uniform distribution of the enzyme throughout the reaction system. This promotes enzyme-substrate interactions and improves the hydrophilicity of cellulose, making it more accessible to enzymatic degradation, thus improving cellulase efficiency. CMCase functions as a β-1,4 endocellulase, while FPase exhibits activity comparable to β-1,4 exocellulases. Kim et al. (2004) reported a 24.4% increase in CMCase activity in beef cattle supplemented with 10 g/day of Tween 80 compared to the control group. In the present study, SL supplementation in sheep diets significantly increased CMCase and FPase activities in rumen fluid, which was consistent with the findings of Kim et al. (2004). The specific activity of xylanase also increased, but the difference was not statistically significant ($P > 0.05$). Enzyme activities exhibited a biphasic response to SL concentration, initially increasing before declining at higher doses. This pattern likely reflects surfactant-mediated effects on enzyme-substrate interactions, where optimal concentrations enhance activity by reducing surface tension and improving accessibility, while excessive concentrations may lead to micelle formation that inhibits enzymatic function. The differential responses among enzymes suggest varying sensitivity to surfactant effects, potentially related to structural or functional differences in their catalytic mechanisms. These findings demonstrate the concentration-dependent modulation of rumen cellulolytic enzymes by surfactants, though further research is needed to fully elucidate the underlying molecular interactions.

Previous studies have demonstrated that surfactants can enhance enzymatic degradation by preventing cellulase inactivation, increasing cellulose hydrolysis, and promoting rumen bacterial activity, thereby increasing nutrient digestion and absorption (Helle et al., 1993). Specifically, Wang et al. (2020) observed that adding Tween to sheep diets increased the apparent digestibility of cellulose and haemicellulose by 4.46 and 4.69%, respectively, although without significantly affecting dietary DM, OM, or CP digestion. Kamande et al. (2000) similarly reported that 0.5% Tween 80 increased total tract DM digestion by 9.1% in sheep. As a non-polar surfactant, SL can stimulate the activity of both internal and external ruminal enzymes by improving their stability, thereby increasing the apparent feed digestibility. The current results showed that SL supplementation significantly improved ADF digestibility, particularly in the 20 g/day treatment group, where cellulose digestibility was 19.02% higher than in

the control group ($P < 0.05$). Although the apparent digestibility of NDF also increased with SL supplementation, the difference was not statistically significant ($P > 0.05$), possibly due to enhanced cellulase activity and increased enzyme adsorption to the substrate. A significant improvement in EE digestibility was observed with increasing SL levels, suggesting a potential role in modulating lipid transport, accumulation, and metabolism in sheep. Meanwhile, the apparent digestibility of DM, OM, CP, Ca, and hemicellulose was also higher following SL supplementation, although the changes were not significant, which was broadly consistent with the results reported by Liu et al. (2020).

Conclusions

Our findings demonstrate that dietary supplementation with soybean lecithin (SL) effectively improves rumen fermentation parameters, digestive enzyme activity, and acid detergent fibre digestibility in sheep. Therefore, SL can be considered a natural modulator of rumen fermentation capable of optimising roughage utilisation in ruminant nutrition. Considering the dose-dependent responses observed in this study, the recommended level of SL supplementation is 20 g/day for optimal improvement of fibre digestion in sheep production systems. This dosage represents the most effective balance between improved performance and economic feasibility under the present experimental conditions.

Acknowledgments

This work was supported by the Program for Science and Technology Innovation Talents (2022TSYCLJ0014) and the Special Project of the Central Government Guidance on Local Science and Technology Development (ZYYD2023B09).

Conflict of interests

The Authors declare that there is no conflict of interest.

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