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Impact of dietary supplementation with corn distillers dried grains with soluble and extruded soybeans on fermentation parameters, digestibility, and lipid metabolism in ewes fed hay-based forage

Edwaldo Villanueva Pedraza[a], Pompeyo Ferro[a], Jeiner Alexander Villanueva Guerrero[a], Johnny Cueva Valdivia[b], Guido Ayay-Arista[a], Euclides Ticona Chayña[a], Jose Alberto Carlos Ramos[a], Papa Pio Ascona Garcia[a], Anthonny Smith Guevara Flores[a] and Rafael Alejandro Palladino[c]

[a]National Intercultural University Fabiola Salazar Leguía of Bagua, Bagua, Amazonas, Peru; [b]National University of Barranca, Barranca, Lima, Peru; [c]Nacional University de Lomas de Zamora, Buenos Aires, Argentina

ABSTRACT

The expansion of the biofuels industry has increased the production of co-products such as distillers dried grains (DDGS) and extruded soybeans (ESB), known for their low cost and ability to improve animal production. This study evaluated the effects of DDGS and ESB on ruminal fermentation, digestibility and lipid metabolism in sheep fed hay forage (FH). SA 4x4 Latin square experimental design was used with four rumen-cannulated ewes (62.0 ± 7.13 kg live weight) assigned to four treatments: control (FH 73% + sunflower pellet 26% + urea 1%), DDGS (FH 74% + DDGS 25% + urea 1%), ESB (FH 75% + ESB 25%) and soybean oil (SO, FH 71% + sunflower pellet 26% + SO 2% + urea 1%). Dry matter digestibility (DMD) was highest with extruded soybeans (ESB), while DDGS showed intermediate values. Both supplements improved ruminal pH stability, indicating enhanced fermentation conditions. ESB also increased the concentrations of linoleic acid (C18:2 *n*-6) and *α*-linolenic acid (C18:3 *n*-3) in the rumen. In conclusion, the inclusion of DDGS and ESB in hay-based diets improved ruminal pH regulation and the fatty acid profile, contributing to better overall fermentation dynamics.

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Long-chain fatty acids; α-linolenic acid; biohydrogenation; ruminal fermentation; ruminal metabolism

Introduction

The growth of the biofuel industry, driven by the need to diversify energy sources and reduce environmental impact, has increased the production of agricultural and industrial by-products (Melendez et al. 2022). In regions such as North America, Europe, and Latin America, biofuel production increased by 41% over the last decade, reaching a total volume of 112 million cubic metres in 2021 (Torroba et al. 2021). Among the most relevant by-products are dried distillers' grains with solubles (DDGS), a by-product of corn fermentation for ethanol production (Tittor and López 2020; Gurbuz, Y., 2007). Extruded soy, obtained through extrusion and pressing in the oilseed processing industry (Cheng and Rosentrater 2019). The high nutritional quality of these by-products makes them strategic resources for formulating diets for ruminants, promoting both environmental sustainability and productive efficiency in livestock systems (Obeidat 2018; Marcos et al. 2020). From a nutritional perspective, dried distillers' grains with solubles (DDGS) have a high concentration of fibre and protein, with increases of 2.2 to 3 times compared to the original corn grain (Böttger and Südekum 2018a). Additionally, its lipid profile is enriched with polyunsaturated fatty acids (PUFA), predominantly linoleic acid (C18:2), which represents approximately 59% of its total lipid content (Mohammadi Shad et al. 2021). Similarly, extruded soy is characterised by its high protein value and significant linoleic acid content, with proportions ranging from 49.03% to 53.02% of the total fatty acids (Aussanasuwannakul et al. 2023; Giallongo et al. 2015a).

However, the inclusion of diets with high concentrations of polyunsaturated fatty acids (PUFA) in ruminants can modify biochemical processes in the rumen, particularly biohydrogenation (BH), a

CONTACT Edwaldo Villanueva Pedraza evillanueva@unibagua.edu.pe edduvp@gmail.com National Intercultural University Fabiola Salazar Leguía of Bagua, Jr. Ancash 520, Bagua, 01721, Amazonas, Peru

Article highlights

- The inclusion of extruded soybeans in sheep diets improves dry matter digestibility.
- The inclusion of DDGS in sheep diets enhances the stability of ruminal Ph, promoting overall rumen health.
- Significant increases in linoleic acid and α-linolenic acid levels were recorded in sheep fed extruded soybeans.
- Supplementation with soybean oil markedly elevates cis-9, trans-11 CLA concentrations in rumen fluid.
- This study highlights the potential of DDGS and extruded soybeans as sustainable alternatives in sheep nutrition.

fundamental adaptive mechanism of ruminal bacteria that detoxifies PUFA (Lock and Bauman 2004). During BH, these lipids undergo a series of enzymatic transformations, including the isomerization of the cis-12 double bond to trans-11, leading to the formation of conjugated linoleic acid (cis-9, trans-11 C18:2, CLA). This isomer can also originate in the mammary gland from vaccenic acid (trans-11 C18:1) through the action of the Δ9-desaturase enzyme, which improves the lipid profile of milk (Bauman and Griinari 2001). Conjugated linoleic acid (cis-9, trans-11 C18:2, CLA) has been associated with various physiological benefits, such as anticancer properties and its ability to reduce the risk of metabolic and cardiovascular diseases in humans (Kim et al. 2016 ; Basak and Duttaroy 2020).

The inclusion of oilseeds in the diet of ruminants has been identified as an effective strategy to modify the lipid profile of milk fat, promoting an increase in the concentrations of conjugated linoleic acid (CLA cis-9, trans-11 C18:2) in milk (Shingfield et al. 2003; Chilliard et al. 2007). According to studies by Alipanahi et al. (2019), feeding extruded soy to goats improved milk production and composition, increasing CLA (cis-9, trans-11 C18:2) and vaccenic acid (trans-11 C18:1) levels, enhancing the milk's nutraceutical quality. Similarly, the study by Fatahnia et al. (2018) on dairy cows fed a diet of extruded soy also showed improvements in milk yield and quality, highlighting that oilseeds increase CLA and PUFA levels. On the other hand, it has been reported that feeding DDGS to dairy cows (Clark et al. 2024) and sheep (Neofytou et al. 2021) increases the concentration of unsaturated fatty acids in milk, particularly conjugated linoleic acid (CLA cis-9, trans-11 C18:2).

Studies on the supplementation with DDGS and extruded soy have demonstrated that these by-products have the ability to increase the levels of polyunsaturated fatty acids (PUFA) in ruminant milk (AbuGhazaleh et al. 2002; Neofytou et al. 2021; Clark et al. 2024). However, despite their high nutritional value and importance as a source of high-quality lipids, the impact of these by-products on small ruminants, such as sheep, remains understudied, particularly regarding their influence on ruminal fermentation, nutrient digestibility, and lipid metabolism. Therefore, the present study aims to evaluate whether the inclusion of DDGS and ESB, as protein and energy co-products, can influence ruminal fermentation patterns, particularly pH stability, total nutrient digestibility, and lipid metabolism in sheep fed hay-based forage.

Materials and methods

Ethics and compliance approval

All procedures involving animal handling were approved by the Ethics and Animal Welfare Committee (Approval No. 5229/2017) of the Faculty of Agronomy and the Animal Care and Use Committee of the Department of Animal Production, University of Buenos Aires. The experiment was conducted in accordance with the Peruvian Animal Protection and Welfare Law (Law No. 30407) and the guidelines established by the World Organisation for Animal Health (OIE) to ensure the welfare of animals used for scientific purposes.

Animals, diets and experimental design

The study was conducted at the facilities of the Animal Nutrition Chair, Department of Animal Production, Faculty of Agronomy, University of Buenos Aires (FAUBA), located at 34°59'13"S and 58°48'55"W. Four ewes with an average live weight of 62.0 ± 7.13 kg, each fitted with permanent ruminal cannulae, were used in the experiment. The animals were housed in individual metabolic cages with free access to water and were fed once daily at 8:00 am with a ration equivalent to 4% of their live weight on a dry matter basis. The animals were not under production, and body weight gain was not recorded, as the study focused on evaluating nutrient digestibility rather than performance. Intake was monitored to ensure uniform consumption across treatments and avoid confounding effects on digestion. The experimental design followed a 4 × 4 Latin square, consisting of four dietary treatments and four experimental periods of 21 days each. Each period included 14 days for diet adaptation and 7 days for sampling and data collection. The diets, formulated with Festuca hay (*Festuca arundinacea*) as the base ingredient, were balanced to be isonitrogenous and isoenergetic, including DDGS, extruded soybean, sunflower expeller, urea and soybean oil. All diets, designed according to NRC (NRC, 2007) (Pu lina and Nudda (2004) had similar concentrations of crude protein (15%) and ether extract (4.4) on a dry matter (DM) basis. The DM dietary treatments were (T1) control diet (CONT) with sunflower expeller + urea, (T2) DDGS (DDGS + urea), (T3) ES (extruded soybean) and (4) AS (sunflower expeller + urea + soybean oil). The total ration was offered daily at 4% of live weight in DM in individual feeders, maintaining a forage:concentrate ratio of 75:25 in DM (Table 1).

Sample collection and chemical analysis

The ration components, both concentrates and forages, were sampled daily and then a set of samples was established for each period. These combined samples were dried at 55 °C in a forced air oven for 48 hours, which were then ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to a 1 mm sieve, and stored at −20 °C for further analysis. Total nitrogen (TN) content was determined by the Kjeldahl method and multiplied by 6.25 to calculate crude protein content. Ethereal extract and ash were determined according to AOAC guidelines (INTERNATIONAL & INTERNATIONAL, 2019). The neutral detergent (NDF), acid detergent (FDA), and acid detergent lignin (ADL) fractions were determined according to the methods of Van Soest et al. (1991), using the ANKOM® 220 (ANKOM Technology, Macedonia NY-USA), and the results were expressed including residual ash. Fatty acids from the concentrates were analysed as methyl esters in hexane by gas chromatography according to Campione et al. (2020) with GC-2010 SHIMADZU, using an Rtx2330 capillary column (105 m length × 0.32 mm × 0.2 *μ*); injection volume 1.0 μl; temperature

Table 1. Ingredient composition, nutritional profile and fatty acids of experimental diets (CONT, DDGS, ESB, AS).

	Diets			
Ingredients	CONT	DDGS	ESB	AS
Ingredients, g/kg fed Pasture hay	745.00	740.00	750.00	716.00
DDGS	0.00	250.00	0.00	0.00
Extruded soybeans	0.00	0.00	250.00	0.00
Sunflower Expeller	250.00	0.00	0.00	264.00
Soybean oil	0.00	0.00	0.00	15.00
Urea	5.00	10.00	0.00	5.00
Chemical composition (g/kg DM)				
Dry matter	964.00	962.00	966.00	964.00
Organic matter	914.00	922.00	918.00	915.00
Crude protein	157.00	156.00	153.00	154.00
Neutral detergent fibre	597.00	633.00	574.00	581.00
Acid detergent fibre	316.00	297.00	288.00	308.00
Ethereal extract	40.00	40.20	42.00	41.50
Total ash	86.00	78.00	82.00	85.00
[5] *Fatty acid profile (g/100g TFA)*				
Lipids (g/100 g DM)	3.00	4.50	5.60	4.50
C14:0	0.80	0.80	0.80	0.80
C16:0	15.00	16.70	16.70	14.70
C18:0	2.90	2.70	3.30	2.90
C18:1 cis-9	23.90	12.60	10.30	24.90
C18:1 cis-11	1.00	1.00	1.10	1.00
C18:2 *n*-6	7.10	16.60	17.20	15.00
C18:3 *n*-3	3.70	4.00	3.50	3.60
C20:0	0.00	0.00	2.40	0.10
C22:0	0.00	0.00	0.00	0.00
C24:0	1.50	1.50	1.50	1.50

Abbreviations: CONT, control diet; DDGS, DDGS diet; ESB, extruded soybean diet; AS, soybean oil diet; AGT, total fatty acids; EM, metabolizable energy.

programme 60–240 °C; injector temperature 250 °C; detector temperature 250 °C and helium as carrier gas, according to standard. Food fatty acids were extracted from freeze-dried samples and converted to fatty acid methyl esters (FAMEs) by a one-step procedure using chloroform and 2% (v/v) sulphuric acid in methanol (Shingfield et al. 2003), with nonadecanoic acid (Sigma-Aldrich, St. Louis, MO, U.S.A.) as internal standard. Fatty acid concentration was expressed as g/100 g fat.

Dry matter intake, in vivo digestibility and diet analysis

Daily feed intake was determined as the difference between supply and remainder by calculating the dry matter intake (DM) over the 21 days of each period. In vivo digestibility was assessed using faecal collection harnesses according to Kitessa et al. (1999). Total faeces per animal were weighed at the end of a 24-hour period, and a subsample of 10% of the faeces collected daily during the 7 experimental days of each period was selected. All samples were ground to a 1 mm sieve and analysed for dry matter digestibility (DMD), organic matter (DMO), detergent neutral fibre (DFDN) and detergent acid fibre (DFDA), using the methodologies of DeVries (2004).

Sampling of rumen fluid and analysis of fermentation characteristics

Approximately 50 ml of rumen fluid, including both solid and liquid phases, were collected to analyse the fatty acid profile and characterise the rumen environment. Samples were taken before feeding (8:00 h) and every 4 hours during a 24-hour period (8:00, 12:00, 16:00, 20:00, 0:00, and 4:00 h) on day 14 of each experimental period. Immediately after collection, rumen pH was measured using a portable pH metre (Orion Model 720, Thermo Electron Corporation, Beverly, MA, U.S.A.), and samples were subsequently stored at –20 °C until analysis. An aliquot of rumen fluid stored at –20 °C was thawed overnight at 4 °C and used for the determination of volatile fatty acids (VFA) and ammonia nitrogen (NH_3-*N*). For the VFA analysis, 1.6 ml of rumen fluid was mixed with 400 µl of methanoic acid (98–100% HCO_2H), centrifuged at 7000 × g for 15 minutes at 4 °C, and the supernatant was frozen for further analysis. The concentration of VFA was determined following the method described by Erwin et al. (Erwin et al. 1961), using a gas chromatograph (GC-2014, Shimadzu Corporation) equipped with a polar capillary column (Stabilwax, Restek, Bellefonte, PA). For the NH_3-*N* analysis, 2 ml of rumen fluid was mixed with 1 ml of sulphuric acid (0.5 M) and the samples were stored frozen until analysis. The concentration of NH_3-*N* was determined using the phenol-hypochlorite colorimetric method described by Broderick and Kang (1980).

Analysis of ruminal fatty acid profile

The lipid content of the rumen was extracted in duplicate, following the method described by Vasta et al. (2008). For fatty acid extraction, 0.5 g of freeze-dried rumen contents were weighed and mixed with 2 ml of sodium methoxide in methanol (0.5 M; Sigma) in a polypropylene tube (16 ml). The mixture was incubated in a water bath at 50 °C for 10 min. Subsequently, it was cooled for 5 min and 3 ml of methanolic hydrochloric acid (**5%**) was added. After stirring the mixture, the samples were again incubated in a water bath at 78 °C for 12 min. Subsequently, the samples were cooled for 7 min, treated with 3 ml of hexane and 5 ml of K2CO3 (6%), vortexed and centrifuged at 2500 g for 5 min. The hexane clean phase was extracted with a microfilter (17-0.45µm) and transferred to a chromatography vial. The samples were analysed on a gas chromatograph, model CP 3800 equipped with a CP-Sil 88 capillary column (100 m × 0.25 mm × 0.2 µm, Varian CP7484). Fatty acids were identified by comparing relative retention times with a commercial standard mixture (FAME mix, sigma-Aldrich, U.S.A.).

Statistical analysis

Data for CMS, CMO, DMD, DMO, DMO, DFDN, DFDA, ruminal GA profile, VFA and CH4 were subjected to analysis using a 4 × 4 Latin square design replicated four times. Statistical analyses were performed by analysis of variance (ANOVA) with the MIXED procedure of SAS (version 9.4, 2004, SAS Institute Inc., Cary, NC), according to the following model:

$$Yijkl = \mu + Si + Cj\,(Si) + Pk + Dl + Esijkl$$

where *Yijkl* is the dependent variable, observed in the i-th square, j-th ewe, k-th period and l-th diet; ?: is the overall mean.

Si is the fixed effect of the square (ewe group).

C(*Si*) is the random effect of the sheep within the square; *Pk*:is the period fixed effect.

D: is the diet fixed effect; and *Esijkl*. is the residual error.

Ruminal fermentation data were analysed as repeated measures, summing sampling time points (08:00, 12:00, 16:00, 20:00, 00:00 and 04:00 hours with respect to morning feeding) and their interaction (diet by time) as fixed effects to the above statistical model. Effects were considered significant if $P < 0.05$, and P values between 0.05 and 0.10 were considered a trend.

Results

Consumption and digestibility of nutrients

Consumption and digestibility data are summarised in Table 2. Dry matter (DM) and organic matter (OM) intake, both in absolute terms and relative to metabolic body weight, did not differ significantly among treatments ($P > 0.05$). However, the inclusion of extruded soybeans (ESB) significantly improved nutrient digestibility compared to the control diet, with dry matter digestibility (DMD) and organic matter digestibility (OMD) increasing by 16.3% and 14.3%, respectively ($P < 0.05$). In contrast, no significant differences were observed in the digestibility of neutral detergent fibre (NDF) and acid detergent fibre (ADF) across treatments ($P > 0.05$).

Ruminal fermentation parameters

Table 3 shows that ruminal pH was significantly higher in the DDGS group compared to the control ($P < 0.05$). In contrast, no significant differences were observed among treatments for ammonia nitrogen (NH_3-N), total volatile fatty acids (VFAs), or individual VFA concentrations, including acetate, propionate, butyrate, valerate, isobutyrate, and isovalerate ($P > 0.05$).

All treatments (CONT, DDGS, ESB, and AS) exhibited a notable decline in ruminal pH during the first 12 hours post-feeding, reaching their minimum values within this timeframe. Among the diets, DDGS maintained the highest average pH, followed in order by ESB, AS, and CONT ($P = 0.021$; Figure 1).

Ruminal fatty acid profile

The ruminal fatty acid (FA) profile of sheep fed CONT, DDGS, ESB, and AS diets showed no significant differences for most individual fatty acids ($P > 0.05$; Table 4). However, linoleic acid (C18:2 *n*-6) concentration was significantly higher in the ESB group compared to DDGS, AS, and CONT ($P = 0.010$). Similarly, α-linolenic acid (C18:3 *n*-3) was more abundant in the ESB group (2.0 vs. 1.6 g/100 g TFA; $P = 0.003$). In

Table 2. Effects of different diets on intake and digestibility in ruminants.

	Diets					
Variable	CONT	DDGS	ESB	AS	SEM	*p*-value
Consumption						
DM, g day^{-1}	1216.00	1262.00	1244.00	1217.00	21,70	0.535
MS, g MS kg^{-1} PV^{0,75}	50.30	52.30	51.30	50.80	0.90	0.574
MO, g MO day^{-1}	1111.00	1166.00	1142.00	1112.00	20.10	0.368
MO, g MO kg^{-1} PV^{0,75}	46.00	48.30	47.10	46.40	0.80	0.404
Digestibility, %						
Dry matter	43.00^{b}	47.00^{ab}	50.00^{a}	46.00^{ab}	0.90	0.017
Organic matter	47.00^{b}	50.90^{ab}	53.70^{a}	49.60^{ab}	0.80	0.017
Neutral detergent fibre	42.00	46.70	47.10	41.00	2.00	0.235
Acid detergent fibre	31.00	32.00	34.10	36.70	3.20	0.718

Abbreviations: CONT, control diet; DDGS, DDGS diet; ESB, extruded soybean diet; AS, soybean oil diet; AGT, total fatty acids; EM, metabolizable energy; SEM: standard error of the mean.

Note: Standard error of the mean.^{ab} Means within the same row with different letters in the superscript differ ($P < 0.05$) by Tukey's test.

Table 3. Impact of different diets on rumen fermentation and metabolites in ruminants.

Variable	Diets				SEM	*p*-value
	CONT	DDGS	ESB	PG		
pH	6.40[b]	6.60[a]	6.5[ab]	6.5[ab]	0.02	0.021
N-NH_3 (g/dl)	24.00	24.50	34.60	27.10	3.04	0.239
AGV total (mmol/L)	69.50	65.10	73.70	72.40	1.83	0.872
Acetate (mmol/L)	71.30	70.10	69.90	71.10	0.32	0.728
Propionate (mmol/L)	19.10	20.10	19.50	19.10	0.20	0.660
Butyrate (mmol/L)	6.40	7.30	7.00	6.30	0.15	0.248
Valerate (mmol/L)	0.80	0.80	0.80	0.80	0.04	0.991
Isovalerate	1.20	1.00	1.30	1.20	0.05	0.658
Isobutyrate	1.50	1.40	1.60	1.70	0.07	0.469
Relation Acet/Prop	3.80	3.50	3.60	3.80	0.05	0.688

Abbreviations: CONT, control diet; DDGS, DDGS diet; ESB, extruded soybean diet; AS, soybean oil diet; AGT, total fatty acids; EM, metabolizable energy; SEM: standard error of the mean. [ab] Means within the same row with different letter in the superscript differ statistically (P < 0.05) with Tukey's test.

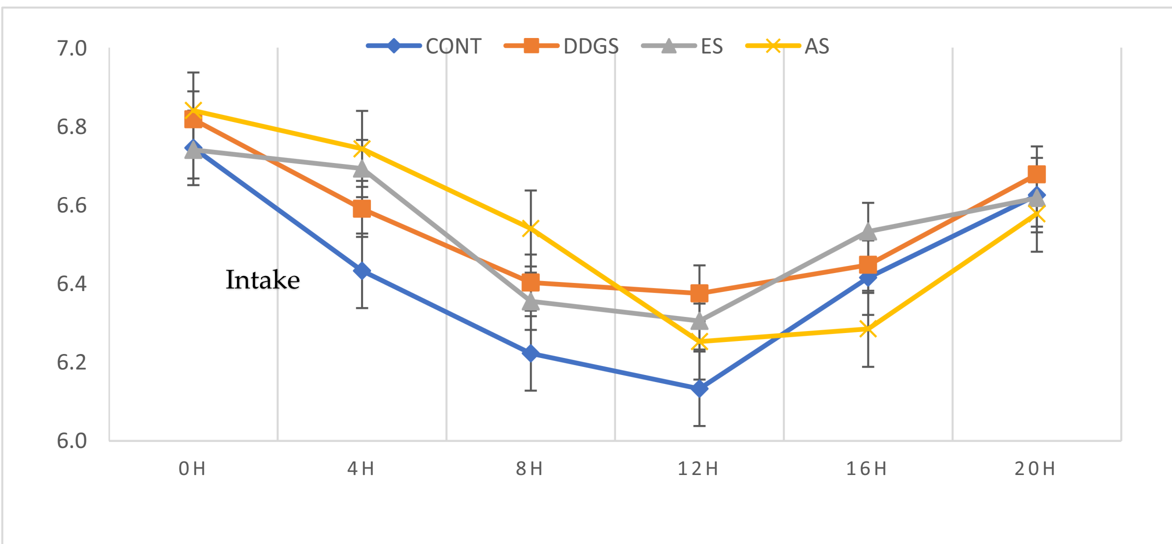

Figure 1. Effects of diets on ruminal pH throughout the day. All treatments (CONT, DDGS, ESB and AS) showed a significant decrease in pH during the first 12 hours, reaching the lowest values in this period. In particular, it was observed that the DDGS diet had the highest pH, followed by the ES, AS and CONT diets ($P = 0.021$; Figure 1).

contrast, soybean oil (AS) supplementation resulted in a ~2.75-fold increase in the concentration of conjugated linoleic acid (CLA; C18:2 cis-9, trans-11) compared to the other treatments, which did not differ significantly among themselves ($P = 0.015$).

Discussion

DMI did not differ significantly among treatments, indicating that the inclusion of DDGS or ESB did not negatively affect feed intake or palatability in non-productive ewes. Average intake ranged from 1216 to 1262 g/day, which aligns with values reported by Abudabos et al. (2021) in lambs fed DDGS. Similarly, Sorensen et al. (2021) found no significant changes in DMI when DDGS replaced soybean meal at various inclusion levels in Boer crossbred goats. These findings are also consistent with Cheraghi-Kamalan et al. (2019), who observed stable DMI in steers fed diets containing lipid-rich soybean products such as extruded and roasted soybeans. Collectively, these results support the inclusion of DDGS and ESB as acceptable ingredients in forage-based diets for small ruminants without compromising voluntary intake.

Feeding sheep with forage-based diets supplemented with ESB resulted in improved digestibility of DM and OM compared to the control and DDGS treatments. The higher digestibility observed in the ESB group is likely due to the physicochemical modifications induced by the extrusion process, which enhances protein solubility and facilitates enzymatic hydrolysis in the small intestine (Giallongo et al. 2015; Hossein

Table 4. Effect of inclusion of DDGS, ESB, AS and CONT in sheep diets on rumen fatty acid content (g/kg DM).

Variable	Treatments				SEM	*p*-value
	CONT	DDGS	ESB	AS		
Total lipids (g/100 g DM)	5.30	4.60	5.30	6.40	0.36	0.093
C12:0	0.30	0.30	0.40	0.30	0.07	0.907
C14:0	1.40	1.50	1.20	1.10	0.18	0.561
C14:1	0.30	0.20	0.30	0.30	0.04	0.870.
C15:0	1.40	1.20	1.20	1.30	0.16	0.850
C16:0	12.80[ab]	14.20[a]	13.40[ab]	10.70[b]	0.47	0.020
C17:0	0.60	0.50	0.60	0.60	0.07	0.722
C18:0	35.40	36.60	36.60	38.00	0.96	0.503
C18:1 *trans9*	0.60	0.70	0.60	0.80	0.12	0.673
C18:1 trans11	6.10	9.40	8.70	6.50	0.83	0.122
C18:1 *cis9*	9.40	5.40	5.10	8.90	0.78	0.136
C18:1 *cis11*	0.30	0.30	0.50	0.40	0.03	0.140
C18:2 *n*-6	3.00[b]	4.50[b]	5.30[a]	3.60[b]	0.47	0.010
C18:3 *n*-3	1.60[b]	1.60[b]	2.00[a]	1.60[b]	0.04	0.003
C18:2 cis9, trans11	0.04[b]	0.03[b]	0.05[b]	0.11[a]	0.01	0.015
C18:2 trans10, cis12	0.09	0.09	0.09	0.10	0.01	0.527
C20:4 n6	0.70	0.59	0.70	0.79	0.04	0.141
C22:1 cis13	0.11	0.05	0.05	0.06	0.02	0.184
C20:5 n3	0.23	0.16	0.18	0.10	0.02	0.073
C24:0	0.69	0.68	0.55	0.66	0.04	0.268
C22:5 n3	0.17	0.12	0.07	0.14	0.04	0.513
C22:6 n3	0.14	0.10	0.10	0.08	0.02	0.517

Abbreviations: CONT, control diet; DDGS, DDGS diet; ESB, extruded soybean diet; AS, soybean oil diet; AGT, total fatty acids; EM, metabolizable energy; SEM: standard error of the mean. D: effect of the diet factor; T: effect of the time factor; D×T: interaction.
[ab]Means within the same row with different letters in the superscript differ statistically ($P < 0.05$) with Tukey's test.

Abadi et al. 2023) These results are consistent with previous findings in ruminants fed processed soybean products under low-quality forage conditions (Albro et al. 1993). Although no significant differences were detected in the digestibility of NDF and ADF, the improved OM digestibility with ESB suggests a greater contribution from non-fibre fractions, particularly CP and EE. The effectiveness of ESB in enhancing CP and EE digestibility has been attributed to heat-induced denaturation and increased lipid availability, which collectively improve nutrient accessibility for ruminal and post-ruminal digestion (Machado et al. 2017; Alipanahi et al. 2019). While CP and EE digestibility were not quantified in the present study, the chemical composition of the ESB diet and supportive evidence from the literature provide a robust explanation for the observed improvements in nutrient utilisation.

The diurnal pattern of ruminal pH observed in this study showed the expected postprandial decline between 4 and 5 hours after feeding, followed by a gradual recovery. Average ruminal pH ranged from 6.40 in the control group to 6.60 and 6.50 in the DDGS and ESB groups, respectively. These values remained within the physiological range considered optimal for microbial activity and fibre fermentation in the rumen (Kolver and De Veth 2002). The higher pH values observed in the DDGS and ESB treatments compared to the control suggest a stabilising effect on the ruminal environment, which is critical for maintaining fermentation efficiency and microbial function. This pH stabilisation is likely the result of multiple interacting factors. DDGS, characterised by low starch and moderate fibre content, slows down fermentation kinetics and limits acid accumulation in the rumen (Böttger and Südekum 2018). Moreover, its fibrous composition promotes mastication and salivation, thereby enhancing ruminal buffering capacity through bicarbonate influx (Liu et al. 2020). In the case of ESB, the elevated lipid content may suppress the activity of fermentative bacteria, particularly those involved in amylolysis and cellulolysis, resulting in lower VFA production and a reduced acidogenic load (Machado et al. 2017; Frutos et al. 2020). Additionally, lipid supplementation has been associated with decreased lactic acid production and a lower risk of subacute ruminal acidosis (Enemark 2008). These mechanisms likely contributed to the greater pH stability observed in the ESB and DDGS groups.

Dietary treatments did not influence ruminal NNH3 levels; all treatments exhibited normal average values of 3.43 mg/dl, considered optimal for fermentation and microbial growth (Oh et al. 2008). Diets with lower rumen degradable protein content, particularly those containing DDGS and hay, may limit ammonium availability in the rumen (Pancini et al. 2019), which could explain the minimal differences observed between treatments. Additionally, the high fermentability of ES/DDGS products likelypromotes nitrogen capture by ruminal microbes (Oh et al. 2008), contributing to similar ruminal ammonium levels across treatments.

The volatile fatty acids (VFA) content in the rumen is influenced by several factors, including feed type, feeding frequency, digestion rate and microbial activity (Gumilar et al. 2018 ; Wang et al. 2020). In this study, the ratio of concentrate (25%) to hay forage (70%) in sheep did not significantly impact total VFA concentration or molar concentrations of acetic, propionic and butyric acid. While increased forage intake typically reduces VFA concentration due to a decrease in degradable carbohydrates and starch (Ranathunga et al. 2019; Krogstad et al. 2021), recent research suggests that some feeds, such as DDGS, do not significantly alter VFA levels in dairy cows on high roughage diets, underscoring the role of microbial activity (Krogstad et al. 2021). Furthermore, high forage diets in lactating dairy cows, did not show a significant difference on total VFA production, highlighting the complexity of these factors in ruminal VFA regulation (Gurbuz et al. 2008; Ranathunga et al. 2019).

The relationship between diet and ruminal fatty acid composition is critical for the quality of dairy and meat products, as these are important sources of fatty acids in the human diet. Excess saturated fatty acids (SFA) have been linked to cardiovascular risks due to their impact on LDL cholesterol (Abughazaleh and Buckles 2007; Yanai and Tada 2018; Parodi 2020). Therefore, modifying fatty acid composition in the rumen through dietary supplementation can increase polyunsaturated fatty acids (PUFA) and reduce SFA (Frutos et al. 2020; Hervás et al. 2021). In this study, vaccenic acid (C18:1 trans11) was the predominant fatty acid, with concentrations ranging from 35% to 38%, and no significant variations among the different diets. However, the cis-9, trans-11 CLA isomer concentration was higher in the rumen of sheep supplemented with soybean oil (AS). Previous research has shown that soybean oils inclusion in the diets of dairy sheep significantly increases PUFA and cis-9, trans-11 CLA isomer levels (Bauman and Griinari 2001; Rodriguez et al. 2020).

Studies on the effects of diet on ruminal fatty acid composition and milk fat have revealed a relationship between C18:1 trans10 and C18:2 trans10, cis12, and diet-induced inhibition of milk fat production, particularly in dairy cows fed starch or vegetable oils rich diets (Machado et al. 2017; Bougouin et al. 2019). This phenomenon has also been observed in dairy sheep, although the specific mechanisms may differ across species (Chilliard et al. 2007; Nudda et al. 2020 ; Renes et al. 2020). Contrary to expectations, this study found minimal concentrations of C18:1 trans10 and C18:2 trans10, cis12 across all dietary treatments, including DDGS, ESB and AS. Recent research suggests that dairy sheep may not be as susceptible to diet-induced milk fat depression from high starch supplementation, though remain sensitive to C18:2 trans10, cis12 regulation in lipogenesis gene expression (Toral et al. 2018).

Conclusions

Supplementing hay-based diets with DDGS and extruded soybeans improved key ruminal parameters in non-productive ewes. ESB significantly enhanced dry matter digestibility and increased the concentration of beneficial polyunsaturated fatty acids in the rumen, particularly linoleic (C18:2 *n*-6) and α-linolenic (C18:3 *n*-3) acids. Both DDGS and ESB also contributed to greater ruminal pH stability, reflecting more favourable fermentation conditions. These results support the strategic use of DDGS and ESB as effective dietary components to optimise rumen function and nutrient utilisation in forage-based feeding systems for small ruminants.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Notes on contributor

I am a Zootechnical Engineer graduated from the Universidad Nacional de Pedro Ruiz Gallo, in Lambayeque, and I am currently pursuing a PhD in Sciences for Sustainable Development at the Universidad Nacional Toribio Rodríguez de Mendoza. I hold a Master's Degree in Business Administration (MBA) from Universidad César Vallejo (2019-2021) and a Master's Degree in Animal Production from Universidad de Buenos Aires, Argentina (2013-2015).My professional experience covers both the private and public sectors, excelling in university teaching and technical assistance for development at local, regional and national levels. I have led comprehensive projects in productive and social areas, working closely with technical actors in rural communities. Currently, I work at the Universidad Nacional Fabiola Salazar

Leguía in Bagua, where I am the Director of Innovation and Technology Transfer, and I participate in scientific events as a speaker and organiser. Currently, my research focus is on microbiology and animal nutrition.

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