Original Research

Journal of Advanced Veterinary Research
(2026) Volume 16, Issue 2, 239-244

Effects of vegetable oil coating on soybean meal: Ruminal digestibility and fermentation characteristics through *In Vitro* study

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ARTICLE INFO

Received: 16 October 2025

Accepted: 24 December 2025

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Keywords:

In Vitro Fermentation, Soybean Meal, Undegraded Dietary Protein (UDP), Vegetable Oil

ABSTRACT

This study aimed to evaluate the efficacy of sunflower (SFW), corn (CRN), soybean (SOY), and canola (CNL) oils as natural protein protectants for soybean meal (SBM) in ruminant diets. The primary objectives were to assess their ability to reduce ruminal protein degradation, alter nutrient composition, as well as influence ruminal fermentation, digestibility, and methane mitigation potential. SBM was coated with 5% of each vegetable oil, air-dried, and oven-stabilized. Rumen buffer was prepared by mixing rumen fluid and McDougal solution at a ratio of 1:4. Uncoated SBM (CON) and all dietary treatments were incubated with 50 mL rumen buffer for 48 h at 39 °C. During incubation, the gas pressure was collected at 3, 6, 12, 24, and 48 h. The results showed that oil coating significantly increased dry matter (DM) ($p=0.020$) from 92.61% to 95.16–95.42% and ether extract ($p=0.003$) from 0.44% to 0.77–2.41%. Meanwhile, it reduced organic matter ($p=0.012$) from 65.34% to 54.45–63.05%, compared to dietary CON. In the ruminal digestibility, all dietary treatments reduced ($p=0.002$) DM digestibility from 69.84% to 51.35–55.97% and total degradable fraction from 25.57 to 19.71–22.05 ($p<0.001$). Crude protein digestibility varied among oils ($p=0.008$), with SFW (47.21%) and CRN (32.16%) showing the highest protection, followed by CNL (54.98%) and SOY (72.63%). In fermentation characteristics, all dietary treatments had no effect on rumen pH, ammonia-N, and total VFA production. These results suggest that sunflower and corn oils can serve as effective natural protectants for soybean meal protein, preserving its post-ruminal value without compromising rumen fermentation characteristics.

Introduction

Protein is an essential nutrient in ruminant feed, playing a key role in growth, milk production, tissue repair, and other physiological functions (Grechkina, 2023). A significant portion of dietary protein is degraded in the rumen before it can be used by the animal, leading to impaired growth and reproduction (Schwab and Broderick, 2017). To address this issue, the use of Undegraded Dietary Protein (UDP) has become an important solution. Several studies have shown that UDP is resistant to rumen microbial degradation, allowing more protein to be absorbed in the small intestine and used by the animal's body (Widyobroto *et al*., 2018). UDP is often directly absorbed in the small intestine rather than serving merely as a nitrogen source for microbial protein synthesis. Consequently, there is a need to develop strategies for protecting high-quality protein to ensure its availability for absorption in the small intestine (Bongartz *et al*., 2018).

The concept of UDP has become an important solution for improving protein utilization efficiency in ruminants (Astuti *et al*., 2020). The mechanism for inhibiting degradation in the rumen focuses on protecting the protein from microbial activity. Several methods can be used, including the application of formaldehyde (Wulandari *et al*., 2017) or more natural alternatives, such as vegetable oils (González *et al*., 2020). Previous studies have shown that vegetable oils not only protect protein in feed ingredients but also offer additional benefits. These include antioxidant content, polyunsaturated fatty acids (PUFAs), and monounsaturated fatty acids (MUFAs), which serve as important energy sources in body metabolism (Fabjanowska *et al*., 2023; Van Tran *et al*., 2017). In addition, vegetable oils can inhibit the process of methanogenesis in the rumen, and their unsaturated fat content helps reduce methane gas production, which is a major environmental pollutant resulting from fermentation processes in ruminants (da Silva and Rial, 2025).

Soybean meals (SBM) are the main source of protein in ruminant diet due to its crude protein (CP) content and amino acid profile rich in lysine. However, most of the nitrogen in SBM is rapidly soluble and undergoes deamination, increasing NH_3 concentrations (Mezzomo *et al*., 2018). Protecting SBM means shifting some of its nitrogen from rumen-degradable protein (RDP) to rumen-undegradable protein (RUP) that remains digestible in the small intestine, ensuring a greater flow of essential amino acids (specifically lysine) to the duodenum and supporting growth and milk synthesis (Ayyat *et al*., 2021).

The use of oils for protein protection holds potential as a more environmentally friendly feed option (Ibrahim *et al*., 2021). Traditionally, protein protection methods have relied heavily on fats derived from animal sources, such as tallow or other animal fats (Behan *et al*., 2019). The use of animal fats often raises concerns regarding safety and environmental sustainability (Renna *et al*., 2022). To address the concerns, recent innovations have focused on using plant-based fats, such as tallow oil substitutes or other vegetable oils rich in unsaturated fatty acids (Wulandari *et al*., 2024). Linoleic acid–rich oils act via 2 complementary routes as a physical barrier coating feed particle, thereby reducing the access of ruminal proteolytic enzymes (suppressing protein solubilization and early degradation). The oils also act through the biotic effects of PUFAs on rumen microbes, particularly Gram-positive bacteria and protozoa were in proteolysis and deamination (Jafari *et al*., 2018). These mechanisms generally lower NH3–N concentrations and channel more nitrogen toward microbial protein synthesis, an indicator of improved protection (Fiorentini *et al*., 2015).

According to previous studies, soybean oil is dominated by PUFAs. Approximately 65% of its total fat is PUFAs, with the main composition being 50% linoleic acid (omega-6) and 15% α-linolenic acid (omega-3). The MUFA content is dominated by oleic acid at 26%. Meanwhile, this oil has a very low saturated fatty acid (SFA) content of only 8%, which mainly consists of palmitic acid (C16:0) and stearic acid (C18:0). The profile makes the oil a good source of omega-3 and omega-6 but it is less stable to heating compared to oils with higher MUFA content (Ayorinde *et al*., 2000). Coconut oil has a composition that is almost the opposite of soybean oil. Approximately 80% of its fat content is SFAs, with lauric

ISSN: 2090-6277/2090-6269/

acid (C12:0) being dominant at 47-56%, followed by myristate (C14:0) and caprylate (C8:0). The content of unsaturated fatty acids is very minimal, with 6% MUFA (oleic acid) and 2% PUFA (linoleic acid). The dominance of medium-chain SFAs makes coconut oil very stable against oxidation and quickly metabolized to produce energy (Alves *et al*., 2019). Canola oil is often considered to have the most balanced profile. Over 60% of its fat content is MUFA, with oleic acid (C18:1) as the main component. In addition, its PUFA content is 27%, consisting of linoleic acid (omega-6) and α-linolenic acid (omega-3) in a good ratio. The total SFA is very low, namely less than 7%. This combination of high MUFA and low SFA provides good thermal stability while also offering the desired nutritional benefits (Farahmandfar *et al*., 2015).

The sunflower oil profile varies greatly depending on the type. Conventional varieties have a very dominant PUFA content (up to 75%), specifically linoleic acid (omega-6), with MUFA around 14% and SFA 15%. However, selectively bred high-oleic varieties have a very different profile, where the MUFA (oleic acid) content can significantly increase to 43%, similar to canola oil (Akkaya, 2018). The increase in MUFA makes high-oleic varieties more heat-resistant. These compositional divergences influence oxidative stability, essential-fat balance, and physiological effects. Soybean oil provides a mix of ω-6 and ω-3, coconut oil supplies readily metabolised saturated energy, canola oil offers the most balanced spectrum, and sunflower oil can be tailored to specific applications. Despite their promising chemical characteristics, empirical evidence regarding the ruminal digestion of these oils and their capacity to attenuate methane emissions remains limited. Therefore, this study aims to evaluate the efficacy of sunflower (SFW), corn (CRN), soybean (SOY), and canola (CNL) oils as natural protein protectants for soybean meal (SBM) in ruminant diets. The primary objectives were to assess their ability to reduce ruminal protein degradation, alter nutrient composition, as well as influence ruminal fermentation, digestibility, and methane mitigation potential.

Materials and methods

Preparation and treatment

The experimental materials comprised SBM purchased from an animal feed store and several vegetable oils consisting of sunflower oil, corn oil, soybean oil, and canola oil obtained from the market. Those vegetable oils had a different profile of fatty acids (Table 1), which could show effects on rumen digestibility. This study used a completely randomized design with 5 treatments and 4 replicates per treatment. Each experimental unit consisted of a soybean meal (SBM)-based formulation, with treatments differing in the type of vegetable oil added at 5% (w/w) relative to SBM. The treatments consisted of SBM without vegetable oil (CON), those protected with sunflower oil (SFW), corn oil (CRN), soybean oil (SOY), and canola oil (CNL). In addition, the coating process was conducted by spraying each vegetable oil into the soybean meal and mixing manually until homogeneous. The purpose of this spraying was to form a protective layer on the surface of the soybean meal. After coating, a post-coating stabilization stage was carried out, where the coated soybean meal was left to dry in open air at room temperature for a period of 2 to 3 days. The drying process was then continued in a more controlled manner by placing the samples in an oven set at 55°C for 6 hours using a dry oven (Maskot-OV 45, China). This oven drying removed any remaining moisture more thoroughly and uniformly, ensuring the samples were completely dry and stable before testing. After completing all drying stages, 200 g of soybean meal from each treatment group was carefully weighed. These samples represented each treatment variation and were ready for further testing, such as analyses of chemical compositions and ruminal digestibility.

Nutrient compositions

A sample of 200 g was prepared for each treatment and then used for dry matter (DM) analysis (AOAC No. 934.01; AOAC, 2005) and organic matter (OM) analysis (AOAC No. 942.05; AOAC, 2005). The measurement of DM was conducted using a dry oven (Sanyo MOV-112, Japan) at 105oC for 24 hours. The measurement of OM was conducted using a furnace (Electric Muffle Furnace KM-420, Suhatherm Manufacturing Indonesia). CP content was determined according to the Kjeldahl method (AOAC Method 984.13; AOAC, 2005) using an N analyzer (PYREX Kjeldahl Nitrogen Distilling Apparatus STJoints S030792357, United States of America). Ether extract (EE) content was analyzed by applying the Soxhlet method (AOAC Method 920.39; AOAC, 2005) using a thermostatic water bath (SMWBL-2003, California, United States). Crude fiber (CF) content was determined according to the analysis of Wendee (AOAC Method 962.09-1971; AOAC, 2010).

Ruminal incubation

The rumen fluid was collected from cannulated Bali heifer cattle (Bos sundaicus) in the early morning before the animals were fed. Cattle were fed Pennistum purpureum and commercial concentrate at a ratio of 7:3, which was formulated for isoenergy and isoprotein. In this study, the body weight of the cattle was 300 kg. The Animal care for canulated cattle was approved by the Integrated Laboratory for Study Testing, Universitas Gadjah Mada (Approval No. 20/PPT/XI/2024). Before sampling of rumen fluid, a thermos flask was pre-warmed with 39– 40°C water to match the physiological temperature of the rumen fluid. Subsequently, the water was discarded and replaced with the freshly collected fluid, and the flask was sealed immediately. The rumen fluid was filtered through a double layer of roll gauze or hydrophilic cheesecloth (30 yd × 16 cm) to remove feed particles (Amanullah *et al*., 2022).

McDougall's buffer solution, which acted as artificial rumen saliva, was prepared at a 4:1 ratio (buffer: rumen fluid), heated to 39 to 40°C, and flushed with CO_2 for 5-10 sec to maintain anaerobic fermentation conditions following the procedure of Tilley and Terry (1963). Each 100-mL glass fermenter received 0.50 g of the dietary samples, followed by 50 mL of the rumen buffer mixture. The headspace was flushed with CO_2 for

Table 1. Fatty acid profiles from several vegetable oils

Oil types	Major saturated fatty acids	Total SFA (%)	Principal MUFA(s)	Total MUFA (%)	Principal PUFA(s)	Total PUFA (%)	Refereces
Soybean Oil	Palmitat C16:0 ≈ 6% Stearat C18:0 ≈ 2%	8.0	Oleat C18:1 ≈ 26 %	26.0	Linoleat C18:2 ≈ 50 %; α-Linolenat C18:3 ≈ 15 %	65.0	(Ayorinde, Garvin, and Saeed 2000)
Corn Oil	Palmitic acid (C16:0) Stearic Acid (18:0)	10.72 1.85	Oleic acid (C18:1)	27.65	linoleic acid (C18:2) Linolenic Acid (18:3)	57.26 1.22	(Moreau *et al*., 2009)
Canola Oil	Palmitat C16:0 4% – 5%	7.0	Oleat C18:1 57–65 %	60.0	Linoleat C18:2 17–21 %; α-Linolenat C18:3 7–10 %	27	(Farahmandfar, Asnaashari, and Sayyad 2015)
Sunflower Oil	Palmitat C16:0 ≈ 5% Stearat C18:0 ≈ 3%	15.0	Oleat C18:1 14–43 %	variable	Linoleat C18:2 44–75 %	dominan	(Akkaya 2018)

5–10 seconds to establish anaerobiosis, and the bottles were incubated at 39°C for 48 hours with manual shaking every 8 hours. Gas production was measured manually at 0, 3, 6, 12, 24, and 48 hours, following (Lavrenčič *et al*., 2015). After 48 hours, the total gas was collected, and a 3-mL aliquot was transferred to a 3-mL vacuum blood tube for methane analysis. The contents of each fermenter were then filtered through crucibles lined with glass wool. Fermentation residues were collected for digestibility analysis. Dry matter digestibility (DMD) and organic matter digestibility (OMD) were determined through a drying and combustion process. Residues were filtered using a vacuum pump, then dried in an oven at 105°C for 24 hours and weighed to calculate DMD. Subsequently, the dried samples were incinerated in a furnace at 550°C for 2 hours, then the furnace was turned off and the samples were left in the furnace for ±24 hours. The samples were weighed again to calculate the OMD (Andueza and Martin-Rosset, 2025). Meanwhile, crude protein digestibility (CPD) was determined using a different method. The residue was manually filtered using Whatman No. 41 filter paper and a funnel until dry. In addition, the residue retained on the filter paper was further analyzed to calculate the CPD (Chrenková *et al*., 2019). The supernatant was analyzed for pH using a pH meter (Ohaus AB23PH-B Benchtop pH Meter, United States). Determination of the concentration of ammonia-N was carried out using the Kjeldahl method, in which ammonia released during distillation was captured in a boric acid solution and measured by titration using a standard acid.

The concentration of volatile fatty acids (VFAs) and lactic acid in the samples was determined using Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) equipped with a UV-Vis or Refractive Index (RI) detector. Before analysis, the aqueous samples were prepared through a critical cleanup process, which included first centrifuging at 12,000 g for 15 minutes to precipitate and remove coarse suspended solids and proteins that could clog the HPLC column. The resulting supernatant was then meticulously diluted with milli-Q water to bring the analyte concentrations within the optimal detection range of the instrument and reduce matrix interference. Following dilution, the samples were centrifuged again to ensure the removal of any fine particulates. The final clarification step included filtering the supernatant through a 0.22 µm syringe filter directly into an HPLC vial, which was essential to protect the expensive chromatographic column from blockage and damage. For the mobile phase, a binary solvent system was used, consisting of an acidified phosphate buffer (adjusted to pH 2.0 using ortho-phosphoric acid) and an organic modifier, a mixture of acetonitrile and methanol. The low pH of the buffer served to suppress the ionization of fatty acids, making it more hydrophobic and thereby improving their retention and separation on a reversed-phase C18 column. This method allowed for the precise identification and quantification of individual VFA and lactic acid peaks based on their distinct retention times compared to authenticated external standards (Zhou *et al*., 2021).

In this study, the feed samples were subjected to proximate analysis to determine their basic nutrient composition, including DM, OM, CP, EE, and CF, according to AOAC (2005) standard procedures, before these data were incorporated into the in-vitro digestibility calculations. Following the *In Vitro* process, digestibility analysis was conducted on the post-fermentation residue to calculate the coefficients of DMD, OMD, and CPD. The fermentation parameters analyzed included fluid pH, VFA, and ammonia (NH3) concentrations. Gas productions were recorded at 0, 3, 6, 12, 24, and 48 hours and modeled using the equation by McDonald (1981) :

$$p=a+b\ (1-e^{-c(t-L)}) \qquad \text{for } t>L.$$

Where a signified the immediately fermentable fraction; b was the potentially fermentable fraction; a+b denoted total fermentable fraction; c was the fractional fermentation rate; L was the lag phase; and t was time of incubation (hours).

Statistical analysis

The collected data were analyzed using one-way analysis of variance (ANOVA). Statistical significance was declared at $p < 0.05$. When significant differences were found among treatments, the analysis was followed by Duncan's Multiple Range Test (Steel and Torrie, 1993). Calculations were performed using SPSS Studio software version 27. The statistical model was $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} = response variable, μ = overall mean, T_i = the effect of vegetable oil i, and e_{ij} = error term.

Results

Nutrient compositions of soybean meal

Several types of oil were tested to evaluate their effects on the contents of DM, OM, CP, CF, and EE in the nutritional composition of soybean meal (SBM) as an effort to enhance its nutritional value for ruminant feed (Table 2). The additive effect of all types of vegetable oil significantly increased the DM content of soybean meals (p=0.020). In addition, the CON without oil showed the lowest value (92.61%), while all oil treatments, such as SFW (95.42%), CRN (95.32%), SOY (95.21%), and canola (CNL: 95.16%), were not different. OM content decreased significantly with oil addition (p=0.012). The CON (65.34%) exhibited the highest OM. Among oil treatments, CNL (63.05%) was not different from CON but was higher than SFW (57.67%), CRN (57.49%), and SOY (54.45%). The concentration of CP did not differ significantly between treatments (p=0.571). No significant difference was observed in the concentration of CF between treatments (p=0.133). This showed that the addition of 5% vegetable oil did not alter the fiber fraction of the soybean meal in a biologically meaningful way. Crude fat content increased significantly (p=0.003) with the addition of oil, which was a direct consequence of the 5% inclusion. The CNL treatment (2.41%) provided the highest increase, followed by SOY (1.64%), CRN (0.89%), and SFW (0.77%). Based on the result of this study, the control (CON: 0.44%) remained the lowest.

Table 2. Effects of Different Vegetable Oil Coatings on the Chemical Composition of Soybean Meal (%, DM).

Item[1]	Vegetable oils[2]					SEM	*P-value*
	CON	SFW	CRN	SOY	CNL		
DM	92.61^{b}	95.42^{a}	95.32^{a}	95.21^{a}	95.16^{a}	0.34	0.02
OM	65.34^{a}	57.67^{b}	57.49^{bc}	54.45^{c}	63.05^{ab}	1.23	0.01
CP	26.52	21.43	14.08	16.47	18.66	2.29	0.57
EE	0.44^{c}	0.77^{c}	0.89^{ab}	1.64^{bc}	2.41^{a}	0.22	0.00
CF	3.80^{a}	2.77^{ab}	2.41^{b}	2.67^{ab}	2.49^{b}	0.2	0.13

[1]DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fiber; [2]Different superscript letters abc within the same row show statistically significant differences ($p < 0.05$). CON=SBM without vegetable oil; SFW= SBM + 5% sunflower oil; CRN= SBM + 5% corn oil; SOY= SBM + 5% soybean oil; CNL= SBM + 5% canola oil.

In Vitro digestibility

The results of this study were presented in Table 3. The value of DMD showed a highly significant difference between treatments (p=0.002). Dietary CON achieved the highest value (69.84%), while all oil groups showed a significant decrease, namely sunflower oil (SFW: 54.49%), corn oil (CRN: 53.90%), soybean oil (SOY: 51.35%), and canola oil (CNL: 55.97%). No significant differences were observed between oil types in this study. A significant difference was observed in the OMD (p=0.041). The control (CON: 93.85%) and sunflower oil (SFW: 90.50%) exhibited higher values than corn oil (CRN: 86.04%), soybean oil (SOY: 84.87%), and canola oil (CNL: 84.13%), while CNL showed the lowest OMD. CPD differed significantly between treatments (p=0.008). This soybean oil treatment (SOY: 72.63%) showed the highest value, followed by the control (CON: 52.35%)

and canola oil (CNL: 54.98%), with sunflower oil (SFW: 47.21%) and corn oil (CRN: 32.16%) being the lowest.

Table 3. Effects of Different Vegetable Oil Coatings on *In Vitro* Digestibility of soybean meal (%, DM)

Item[1]	Vegetable oils[2]					SEM	*P-value*
	CON	SFW	CRN	SOY	CNL		
DMD	69.84[a]	54.49[b]	53.90[b]	51.35[b]	55.97[b]	1.84	0.00
OMD	93.85[a]	90.50[ab]	86.04[b]	84.87[b]	84.13[b]	1.25	0.04
CPD	52.35[b]	47.21[b]	32.16[c]	72.63[a]	54.98[b]	4.56	0.01

[1]DMD, dry matter digestibility; OMD, organic matter digestibility; CPD, crude protein digestibility;
[2]Different superscript letters abc within the same row show statistically significant differences ($p < 0.05$). CON=SBM without vegetable oil; SFW= SBM + 5% sunflower oil; CRN= SBM + 5% corn oil; SOY= SBM + 5% soybean oil; CNL= SBM + 5% canola oil.

Gas production and fermentation kinetics

In Vitro fermentation kinetics were modeled using the equation by Paradhipta *et al.* (2023) to capture the dynamics of gas production from the immediately fermentable fraction (a) and the potentially fermentable fraction (b). The resulting total gas production potential (a+b) showed how the 5% vegetable oil coating influenced the rumen microbial fermentation process, as presented in Table 2. A highly significant difference was observed in the potentially fermentable fraction (b) and the total gas production potential (a+b) between treatments ($p<0.001$). The control group without oil (CON) exhibited the highest values for both b (24.77 mL/g DM) and a+b (25.57 mL/g DM), which were statistically higher than all oil treatments. Among the oil treatments, corn oil (CRN: 21.95 mL/g DM) led to a higher b value than canola oil (CNL: 19.35 mL/g DM), while sunflower (SFW: 19.62 mL/g DM) and soybean oil (SOY: 19.79 mL/g DM) were intermediate. A similar pattern was observed for a+b, with CRN (22.05 mL/g DM) being higher compared to SFW (19.71 mL/g DM), SOY (20.29 mL/g DM), and CNL (20.42 mL/g DM).

Rumen pH and Ammonia-N concentration

The values of pH and ammonia-N concentration, presented in Table 5, reflected the rumen microbial fermentation response to vegetable oil coating, providing context to the results on digestibility and gas production. These indicators represented the balance between acid production from fermentation, the system's buffering capacity, the rate of protein deamination, and nitrogen utilization by microbes. The pH value did not show a significant difference between treatments ($p=0.210$). In addition, the values across all treatments remained within a normal physiological range for rumen fermentation (6.31 to 6.99). This stability showed that the addition of 5% vegetable oil did not disrupt the acid-base balance of the *In Vitro* system. Similarly, the NH_3 concentration did not reach a level of statistical significance ($p=0.065$), although a numerical trend was observed. The concentration ranged from 37.45 mg/dL (CRN) to 47.25 mg/dL (CNL).

Composition of volatile fatty acid

The VFA profile, as shown in Table 5, showed the main fermentation pathways in the rumen and served as an indicator of metabolic balance between hydrogen producers (acetate, butyrate) and hydrogen utilizers (propionate, fat biohydrogenation). In addition, the addition of 5% vegetable oil to SBM modified the VFA composition, although most changes only showed a tendency ($0.05 < p < 0.10$) and did not reach conventional statistical significance. Acetic acid concentration showed a downward trend in all oil treatments (42.75 to 45.844 mmol/L) compared to the control (47.38 mmol/L), which was not statistically significant ($p=0.078$). Sunflower oil (SFW: 42.75 mmol/L) showed the lowest value, while canola oil (CNL: 45.84 mmol/L) was closest to the control value. Propionic acid showed a similar downward trend (15.84 to 17.38 mmol/L vs.

Table 4. Effects of Different Vegetable Oil Coatings on Gas Production and Fermentation Kinetics of soybean meal.

Item[1]	Vegetable oils[2]					SEM	*P-value*
	CON	SFW	CRN	SOY	CNL		
a, mL/g DM	0.79	0.09	0.11	0.5	1.08	0.19	0.39
b, mL/g DM	24.77[a]	19.62[bc]	21.95[b]	19.79[bc]	19.35[c]	0.56	0.00
a+b, mL/g DM	25.57[a]	19.71[c]	22.05[b]	20.29[bc]	20.42[bc]	0.55	0.00
c, mL/h	0.11	0.14	0.11	0.19	0.18	0.01	0.15
L, h	1.36	0.9	0.68	0.96	0.37	0.54	0.65

1 a is the immediately fermentable fraction; b is the potentially fermentable fraction; a+b is total fermentable fraction; c is the fractional fermentation rate; L is the lag phase.
2Different superscript letters abc within the same row show statistically significant differences ($p < 0.05$). CON=SBM without vegetable oil; SFW= SBM + 5% sunflower oil; CRN= SBM + 5% corn oil; SOY= SBM + 5% soybean oil; CNL= SBM + 5% canola oil.

Tabel 5. Effects of Different Vegetable Oil Coatings on pH and Ammonia-N and VFA of soybean meal.

Item	Vegetable oils[1]					SEM	*P-value*
	CON	SFW	CRN	SOY	CNL		
pH	6.48	6.62	6.99	6.31	6.84	0.10	0.21
Ammonia-N, mg/dL	44.45[ab]	40.78[ab]	37.45[b]	40.60[ab]	47.25[a]	1.19	0.07
Total VFA, mmol/L	75.07	67.28	68.73	70.3	72.8	1.00	0.08
Acetate, mmol/L	47.38[a]	42.76[b]	43.63[ab]	43.88[ab]	45.84[ab]	0.60	0.08
Propionate, mmol/L	17.77[a]	15.84[b]	15.91[b]	16.79[ab]	17.38[ab]	0.29	0.10
Butyrate, mmol/L	6.62	5.85	6.17	6.41	6.53	0.14	0.41
Isovalerate, mmol/L	3.29	2.84	3.02	3.22	3.04	0.07	0.23
acetate : propionate	2.68	2.7	2.74	2.61	2.64	0.03	0.55

[1]Different superscript letters abc within the same row show statistically significant differences ($p < 0.05$). CON=SBM without vegetable oil; SFW= SBM + 5% sunflower oil; CRN= SBM + 5% corn oil; SOY= SBM + 5% soybean oil; CNL= SBM + 5% canola oil.

control 17.77 mmol/L; p=0.102). Sunflower oil (SFW: 15.83 mmol/L) and corn oil (CRN: 15.91 mmol/L) exhibited the lowest values. Butyrate did not differ significantly (p=0.411) and ranged from 5.84 to 6.62. CON was the highest, while the oil treatments clustered slightly below it without a clear pattern between oil types. Mechanistically, some butyrate producers (Butyrivibrio group) were among the most sensitive to PUFA. As a result, high PUFA fat had the potential to suppress butyrate formation. However, at moderate supplement levels and substrates such as SBM, the effects were often weak or variable and not statistically significant Click or tap here to enter text.. Isovaleric acid results showed a moderate downward trend in the oil groups (2.84 to 3.22 mmol/L vs. control 3.29 mmol/L; p=0.234). SFW (2.84 mmol/L) was the lowest, while SOY (3.22 mmol/L) was close to control. Small numerical variations between treatments showed that adding 5% oil was insufficient to alter iso-VFA formation under these test conditions. Total VFA concentration showed a decreasing trend in oil treatments (67.28 to 72.80 mmol/L) compared to the control (75.07 mmol/L), with p=0.078. In addition, it represented the overall production of VFAs from microbial fermentation and was closely related to feed digestibility. The acetate to propionate ratio did not show significant differences among treatments (p=0.549), with values ranging from 2.61 to 2.74. This ratio was an important indicator of fermentation efficiency in the rumen.

Discussion

The increase in DM was consistent with the hydrophobic nature of lipid layers, which acted as a moisture barrier during the drying process (Milani and Nemati 2022). According to previous studies, the absence of differences between oil types suggested that all vegetable oils, regardless of their fatty acid profile, functioned as effective physical barriers against water retention (Akkaya 2018). This was consistent with a study showing that lipid-based coatings reduced water migration or retention and moisture content of materials (Rux *et al*. 2023), thereby increasing the solid fraction (DM).

The decrease in OM could be influenced by the oxidation of organic compounds during drying, particularly in treatments with oils rich in PUFA. Soybean oil had a high PUFA content, while sunflower oil was also high in linoleic acid, making both more susceptible to thermal oxidation compared to oils richer in MUFA (Akkaya 2018). However, canola oil, which was dominated by MUFA (oleic acid ~60–62%), had better oxidative stability, which explained its tendency to maintain a relatively higher OM content compared to the high-PUFA oils (Ayyildiz *et al*. 2015).

This expected outcome confirmed that the addition of vegetable oils, which were composed primarily of triglycerides, did not chemically alter the protein fraction of the soybean meal. The oil acted solely as a physical coating on the surface of the meal particles, forming a protective lipid layer without interacting with or modifying the intrinsic nitrogenous compounds (Bionaz *et al*., 2020). In this study, the stability of the CP value was fundamental, as the nutritional intervention (oil coating) was targeted at modulating fat content and ruminal fiber digestion, not protein quality or availability. Therefore, the lack of significant change validated that the treatment effectively enhanced other nutritional aspects while preserving the primary protein value of the soybean meal, which was its key nutritional attribute (Giallongo *et al*. 2015).

The lack of a significant effect suggested that the fiber matrix of the soybean meal itself remained unchanged by the physical addition of oil (Bionaz, *et al*., 2020). In nutritional terms, this was a relevant result as it confirmed that the primary strategy of oil addition to increase energy density did not come at the expense of altering the dietary fiber composition of the ration (Xin *et al*. 2025). The minor numerical variations observed between treatments were considered normal experimental variability and were not attributed to the effect of the oil treatments.

The differences between oil types could be attributed to 2 main factors, consisting of (1) Oxidative stability: Oils high in PUFA (e.g., soybean, sunflower, corn) were more prone to oxidation during heating and drying, resulting in the loss of volatile compounds and a potential decrease in measurable EE (Kozłowska and Gruczyńska 2018). (2) Viscosity and adhesiveness: Oil viscosity varied between types and affected how well the oil adhered to and was retained by the SBM matrix. Oils with favorable viscosity and surface tension, such as canola oil, could form a more homogeneous and stable film, leading to higher measured fat retention (Sahasrabudhe *et al*. 2017).

This reduction in DMD was consistent with 2 well-known mechanisms that the lipid coating effect, which reduced wetting, colonization, and microbial enzyme access to feed particles. The toxic effect of unsaturated fatty acids on rumen microbes, particularly cellulolytic bacteria, which inhibited cell wall and DM digestion (Bionaz *et al*., 2020). The decrease in OMD was associated with changes in rumen microbial metabolism when lipids were added, leading to suppression of methanogenesis and shifts in bacterial populations. The significantly higher CPD observed in the SOY treatment suggested that soybean oil was less effective in protecting SBM protein from ruminal degradation compared to other oils, particularly corn oil. However, the significant suppression of CPD by corn oil (CRN) showed that it could strongly inhibit proteolytic microbes or bind to dietary protein, rendering it less accessible (Anam *et al*. 2020).

This significant decrease in the fermentable fraction (b) and total gas potential (a+b) in all oil treatments was primarily due to the inhibition of rumen microbial fibrolytic activity. Free fatty acids released from oil hydrolysis, particularly PUFAs, were toxic to key fiber-digesting bacteria such as Ruminococcus albus and Fibrobacter succinogenes (Sears *et al*., 2024). This inhibition reduced the breakdown of the fermentable fraction of the substrate, leading to lower total gas production. The variation in the degree of inhibition between oil types could be attributed to their distinct fatty acid profiles and bioavailability within the rumen environment. However, the immediately fermentable fraction (a), the fractional fermentation rate (c), and the lag phase (L) did not differ significantly between treatments (p>0.05). The non-significant effect on these parameters showed that the initial solubilization of readily available nutrients and the subsequent onset of fermentation were not substantially altered by the oil coatings. This suggested that the primary inhibitory effect of the oils was on the microbial consortium responsible for degrading the structural, potentially fermentable fraction (b) of the soybean meal, rather than on the overall initiation of the fermentation process (Bionaz *et al*., 2020).

The maintenance of a stable pH suggested that McDougall's buffer was effective and that the production of fermentation acids (VFA) was not drastically altered in a way that overwhelmed the system's buffering capacity (Kumar and Tariq 2024). This variation in NH_3 reflected the dynamic balance between proteolysis (protein degradation releasing ammonia) and ammonia assimilation by microbes for protein synthesis (Giallongo *et al*. 2015). The numerical trend suggested that corn oil (CRN) had slightly suppressed proteolytic activity or enhanced microbial uptake of ammonia, while canola oil (CNL) resulted in relatively less efficient microbial capture of available nitrogen in this *In Vitro* setting. However, the lack of statistical significance suggested that under these specific conditions, the addition of 5% oil did not consistently alter nitrogen metabolism.

The decreasing trend in acetic acid was caused by the selective inhibition of the oil on acetogenic bacteria such as Fibrobacter succinogenes and Ruminococcus flavefaciens. PUFA-rich oils (sunflower, corn) specifically inhibited the acetogenic fermentation pathway through destabilization of microbial cell membranes and disruption of metabolism (Vargas *et al*., 2020). The decrease in propionate was associated with a reduction in substrates for propionogenic bacteria such as Selenomonas ruminantium (Sawanon *et al*., 2011). Iso-VFA (isovaleric/isobutyric acid) primarily originated from the deamination of branched chain amino acids (BCAA; leucine, valine, isoleucine) and acted as a growth factor for cellulolytic bacteria. Since the basic protein composition (SBM) was relatively similar and oil did not contribute to the carbon skeleton of BCAAs, iso-VFA production tended to be stable (Mitchell *et al*. 2023). The decline in

total VFA across oil treatments suggested a slight suppression of overall microbial fermentation activity, which correlated with reduced OMD. This could be attributed to the antimicrobial effects of unsaturated fatty acids on certain rumen microbial populations, potentially limiting the complete degradation of feed components (Varghese *et al*. 2022). A higher ratio showed greater acetate production relative to propionate, which was associated with increased hydrogen availability for methanogenesis and consequently higher methane production (Wang *et al*. 2016). The relatively stable acetate: propionate ratios across treatments suggested that 5% vegetable oil supplementation did not substantially alter the fundamental pattern of rumen fermentation efficiency. However, the numerical variations observed (with SOY showing the lowest ratio at 2.61) showed subtle shifts in microbial population dynamics that could potentially affect methane emission patterns, though these changes were not statistically significant under the current experimental conditions (Ibrahim *et al*. 2021).

Conclusion

Applying a 5% vegetable oil coating to soybean meal alters its physicochemical properties and rumen fermentation without affecting CP or fiber content. The coating increases DM and extract values, specifically with canola oil, while polyunsaturated oils reduce OM due to oxidation. All oils reduce gas production and DMD, showing inhibition of fiber-degrading microbes. Soybean oil enhances ruminal protein digestibility, while corn oil suppresses it. These results suggest that oil coating is a promising strategy to modulate nutrient availability in ruminants, with effects varying by oil type, and warrant further in vivo validation.

Acknowledgements

The authors express sincere gratitude to the Academic Excellence Enhancement Program Scheme A 2024 for the financial support provided. This support has been extremely helpful in ensuring the smooth and continuous progress of this research.

Conflict of interest

The authors have no conflict of interest to declare.

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