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Rumen methane production, microbial population, and blood metabolites in sheep fed jengkol (*Archidendron jiringa*) peel as a substitute for native grass

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Abstract. Agro-industrial and agricultural by-products can be converted into high-quality feed by ruminants. Jengkol (*Archidendron jiringa*) peel is an agricultural by-product characterised by a high crude fibre content. The aim of this study was to evaluate whether jengkol peel could be used as a substitute for native grass as a fibre source for ruminants. The variables investigated were rumen methane prediction, microbial population, and blood metabolites in sheep. The experiment used 15 male sheep with body weights ranging from 15 to 27 kg in a randomised complete block design with three treatments (0.0%, 15.0%, and 22.5% jengkol peel substitution) and five replications. Substituting native grass with up to 22.5% jengkol peel did not adversely affect ($p > 0.05$) the total bacterial population, nor did it alter ($p > 0.05$) methane prediction, but it reduced ($p < 0.05$) the

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protozoal population by 13.95% compared with the control, likely due to the saponin content of the peel. Moreover, substitution up to 22.5% did not negatively influence ($p > 0.05$) key blood metabolites such as glucose, cholesterol, triglycerides, and high-density lipoproteins (HDL). It was concluded that jengkol peel can be substituted for native grass at levels up to 22.5% without detrimental effects on blood health or rumen bacterial populations, while reducing protozoal numbers

Keywords: *in vivo* study; jengkol by-product; native grass; rumen ecosystem; ruminants

Introduction

The utilisation of agricultural by-products, including crop residues, agro-industrial by-products, and other fibrous materials as livestock feed, represents a sustainable strategy to address the limited availability of conventional feed resources. These by-products, which are typically indigestible by non-ruminants, often retain considerable nutritional value and can be effectively digested by ruminants due to their unique rumen microbial fermentation system (Kazemi, 2025). According to E. Ungerfeld *et al.* (2023), the rumen harbours a complex community of bacteria and protozoa, which digest and ferment the different chemical fractions in agricultural by-products into volatile fatty acids as an energy source. N. Hidayah *et al.* (2019) reported that one such underutilised by-product is jengkol (*Archidendron jiringa*) peel, which contains high levels of crude fibre (3.07-35.28%) and total digestible nutrients (TDN) (15.17-19.26% and 63.87-65.82%), demonstrating its potential as ruminant feed.

The *Archidendron jiringa* (Jack) I.C. Nielsen plant, commonly known as jengkol, is a species in the *Archidendron* genus of the Fabaceae family. This plant contains biologically active secondary metabolites that can be used to treat cancer, kill bacteria and fungi, and act as antioxidants (Noviany *et al.*, 2022). The plant typically reaches a height of 25 metres with smooth, grey bark, and usually grows in communal gardens and forests. The coiled pods that hang from the tree's branches contain three to nine beans per pod. Phytochemical screening has revealed the presence of alkaloids, steroids, triterpenoids, glycosides, saponins, flavonoids, and tannins in jengkol (Noviany *et al.*, 2021). According to A. Singh *et al.* (2024), saponins improve rumen fermentation by enhancing microbial protein synthesis and nutrient digestibility. K. Loregian *et al.* (2023) stated that tannins bind to dietary proteins, reducing protein degradation in the rumen and improving protein utilisation.

The availability of native grasses, which serve as a primary fibre source for ruminants, is often inconsistent, particularly during dry seasons. This has led to an increasing need for alternative fibre sources that are locally available, cost-effective, and nutritionally suitable. A. Onyango *et al.* (2019) stated that utilising local supplementary feedstuffs can help compensate for nutritional deficiencies during dry seasons. Jengkol peel, commonly discarded as waste, holds potential as a substitute for traditional fibre sources in ruminant diets. Previous *in vitro* evaluations have shown that incorporating jengkol peel can enhance total volatile fatty acid (VFA) production and increase NH_3 concentration, both of which are important for energy supply and microbial protein synthesis, ultimately supporting livestock productivity. However, scientific data on the effects of jengkol peel substitution on rumen fermentation, methane prediction, microbial populations, and blood metabolites in *in vivo* studies remain limited. This study aimed to evaluate the potential of jengkol peel as a substitute for native grass in sheep diets by examining its effects on methane prediction, rumen microbial populations, and blood metabolites. The findings were expected to contribute to a scientific basis for the sustainable use of jengkol peel as an alternative fibre source in ruminant nutrition.

Materials and Methods

This study was conducted at the Animal Science Faculty, IPB University, Bogor farm from September to November 2019. The experiment included 15 male sheep weighing 15-27 kg. These sheep were allocated into three treatment groups (n = 5 per group) based on their live weight (LW) and arranged in a randomised complete block design. The sheep were housed in individual pens with a temperature of 25-27°C and humidity of 70-72%. Pen lamps were operated on a 12-hour light/dark cycle. The treatments were as follows: T1 = 40% concentrate (C) + 60% native grass (NG), T2 = 40% C + 45% NG + 15% jengkol peel (JP), and T3 = 40% C + 37.5% NG + 22.5% JP. The concentrate contained 10-11% crude protein and 56-60% total digestible nutrients, and was composed of rice bran, tapioca industry by-product, molasses, copra meal, NaCl, $CaCO_3$, urea, and premix (Tables 1, 2). The nutrient content of the concentrate was determined according to AOAC (2000) methods. The jengkol peel powder was prepared by sun-drying fresh jengkol peel for 3-4 days until its weight stabilised, after which it was ground into powder. Jengkol peel was collected from market waste. Based on dry matter (DM), this peel powder contained ash (3.32%), crude protein (8.16%), ether extract (0.72%), crude fibre (34.18%), nitrogen-free extract (53.64%), total digestible nutrients (52.19%), saponin (35.13%), and tannin (1.43%). Each sheep received 3.5-4.0% of its body weight as daily feed ration, provided twice daily: concentrate at 08:00 and 13:00, and chopped native grass (5-8 cm) at 10:00 and 15:00. Clean water was provided *ad libitum*. The *in vivo* experiment lasted 45 days in total, comprising 10 days for adaptation, 35 days for the treatment period, and 14 days for total collection at the end of the treatment period.

Table 1. *Feed formulation (% dry matter)*

Treatments	**T1**	**T2**	**T3**
Native grass	60	45	37.5
Jengkol peel	0	15	22.5
Rice brand	5	6	9
Cassava by-product	8	9	9
Molasses	8	8	8
Copra meal	15	13	10
NaCl	1	1	1
CaCO3	1.5	1.5	1.5
Urea	1	1	1
Premix	0.5	0.5	0.5
Total (%)	100	100	100

Note: T1 = 40% concentrate (C) + 60% native grass (NG); T2 = 40% C + 45% NG + 15% jengkol peel (JP); T3 = 40% C + 37.5% NG + 22.5% JP

Source: compiled by the authors

Table 2. *Nutrition content native grass substitution with jengkol (A. jiringa) peel (% DM)*

Ingredient (%)	**T1**	**T2**	**T3**
Ash	10.54	8.41	7.63
Ether extract	3.08	1.84	2.19
Crude protein	11.12	10.74	11.11
Crude fibre	22.93	24.32	23.58

Table 2. Continued

Ingredient (%)	T1	T2	T3
Nitrogen-free extract	52.32	54.70	55.49
Total digestible nutrient	60.24	56.81	59.07

Note: T1 = 40% concentrate (C) + 60% native grass (NG); T2 = 40% C + 45% NG + 15% jengkol peel (JP); T3 = 40% C + 37.5% NG + 22.5% JP
Source: compiled by the authors

Before morning feeding on the final days of the entire collection period, rumen fluid was collected from each sheep using a vacuum pump for volatile fatty acid and rumen microbial analysis. The molar proportion of VFA was determined using gas chromatography (GC 8A, Shimadzu Corp., Kyoto, Japan; capillary column containing 10% SP-1200, 1% H_3PO_4 on 80/100 Chromosorb WAW, with nitrogen as the carrier gas) according to the method described by A. Moss *et al.* (2000). Methane production was estimated using the following formula based on molar proportions of VFA: CH_4 = 0.45 acetate – 0.275 propionate + 0.40 butyrate. The protozoa population was quantified under a 40 × microscope using a Fuchs-Rosenthal counting chamber (4 mm × 4 mm × 0.2 mm) from a rumen liquid sample (0.5 mL) mixed with 2 mL methyl green formaldehyde saline solution. The total bacterial population was enumerated using the method described by K. Ogimoto & S. Imai (1981) with BHI media and the roller tube technique.

Blood samples were collected the day before rumen fluid sampling from the jugular vein using a Venoject needle and sterile tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant (3.00 mL, BD Vacutainer, Plymouth, Devon, UK) before morning feeding. Blood metabolite analysis included glucose (mg/dL), cholesterol (mg/dL), triglycerides (mg/dL), and high-density lipoprotein (HDL) (mg/dL) levels, determined using photometry techniques with a blood haematology analyser (Photometer Microlab 300, Vital Scientific, USA). Data were statistically analysed using analysis of variance (ANOVA), and Duncan's multiple range test was employed to examine differences between treatment means. Statistical analysis was conducted using IBM SPSS Statistics version 26. The study was approved by the Ethics Committee of the Institute of Research and Community Service, IPB University, Bogor (approval number: 155-2019 IPB). All experimental procedures were conducted in accordance with the principles outlined by FASS (2010).

Results and Discussion

The substitution of native grass with jengkol peel at levels up to 22.5% did not adversely affect the total bacterial population or predicted methane production, yet it significantly reduced ($p < 0.05$) the protozoal population by 13.95% compared with the control (Table 3). This indicates that the inclusion of jengkol peel did not impair overall rumen microbial fermentation activity, which is essential for maintaining normal rumen function and nutrient digestibility.

Table 3. *Correlation coefficients between ECM and other studied characteristics (n = 595)*

Treatments	Methane production (%)	Protozoa (log cell/mL)	Total bacteria (log cell/mL)
T1	6.06±0.24	5.95^{b}**±0.06**	7.79±1.68
T2	6.20±0.16	5.21^{a}**±0.14**	8.48±1.16
T3	6.24±0.23	5.12^{a}**±0.16**	6.85±1.18

Note: T1 = 40% concentrate (C) + 60% native grass (NG); T2= 40% C + 45% NG + 15% jengkol peel (JP); T3 = 40% C + 37.5% NG + 22.5% JP
Source: compiled by the authors

Notably, the observed 13.95% reduction in protozoal numbers is consistent with the known defaunating action of saponins. N. Hidayah *et al*. (2019) reported that jengkol peel contains 26.52% saponin, a level considerably higher than that found in the leaves (13.79%). Saponins form complexes with sterols in protozoal cell membranes, thereby increasing membrane permeability, disrupting cellular homeostasis, damaging cell walls, and ultimately causing lysis (Wallace *et al*., 2002). M. Chen *et al*. (2019) demonstrated that the steroidal saponin 1688-1 interacts with cholesterol-containing lipid membranes composed of mixed liquid-ordered (Lo) and liquid-disordered (Ld) phases, inducing membrane defects and the formation of small aggregates on the membrane surface. A. Kholif (2023) further noted that, in the rumen, saponins act as membrane-permeabilising, immunostimulating, hypocholesterolaemic, and defaunating agents that modulate ruminal fermentation. It is also well documented that saponins can provoke cell rupture, selectively eliminate ruminal protozoa, and interact with cholesterol in cell membranes, thereby improving nitrogen-use efficiency and potentially enhancing overall ruminant performance.

Protozoa are known to contribute to enteric methane production in the rumen owing to their symbiotic relationship with methanogenic archaea. Nevertheless, the observed reduction in protozoal numbers did not lead to a corresponding decrease in predicted methane emissions. This suggests that, at the inclusion levels tested, jengkol peel did not contain sufficient concentrations of active compounds to suppress methanogenesis directly, that compensatory microbial pathways maintained methane output, or that saponins are more effective against protozoa than against methanogenic archaea. Consistent with this, an in vitro study by A. Patra *et al*. (2012) showed that supplementation with Quillaja (QSP) or Yucca (YSP) saponins at 0-0.6 g/L did not alter the archaeal community. Similarly, A. Patra & Z. Yu (2014) reported that saponins (0.6 g/L), either alone or combined with nitrate (5 mM) and sulphate (5 mM), failed to reduce populations of total bacteria, *Ruminococcus albus*, or methanogenic archaea. A meta-analysis by M. Ridla *et al*. (2021) indicated that saponins generally reduce methane emissions, but their effects on methanogen populations are inconsistent across studies. Combinations of saponins with other compounds, such as nitrate, have been shown to produce substantially greater reductions in both methane production and methanogen abundance than saponins alone (Wu *et al*., 2019).

The absence of methane mitigation in the present study may also be attributable to the low tannin concentration in jengkol peel (1.43% DM), which was insufficient to inhibit methanogenic archaea or redirect metabolic pathways away from methanogenesis. Methanogenic archaea (commonly termed methanogens) are a unique group of anaerobic microorganisms that produce methane as a by-product of their energy metabolism (Wagner *et al*., 2025). A. Jayanegara *et al*. (2018) reported that tannins can directly reduce methane production by inhibiting methanogenic archaea. A. Scalbert (1991) showed that tannin monomers such as pyrogallol, gallic acid, and tannic acid are toxic to methanogens. However, G. Wischer *et al*. (2013), evaluating ten tannin sources (chestnut, mimosa, myrabolan, quebracho, sumach, tara, valonea, oak, cocoa, and grape seed), found that only extracts with very high total tannin content (chestnut ⩾ 76%, sumach ⩾ 62%, valonea ⩾ 67%) significantly lowered methane production, whereas others did not. Similarly, A. Cieslak *et al*. (2016) observed that *Sanguisorba officinalis* tannin extract (451 mg g^{-1} total tannins) reduced methane only at the highest inclusion level (100 mg per feed portion of 60% meadow hay and 40% barley meal). These findings demonstrate that the efficacy of tannins in suppressing methane varies considerably with concentration, source, chemical structure, and molecular weight.

Other rumen fermentation characteristics reported by N. Hidayah *et al*. (2020) showed that the treatments had no effect on ruminal pH (6.06 vs. 6.24) or NH_3 concentration (14.99 vs. 18.35 mM) but reduced total VFA concentration (103.23 vs. 68.90 mM) compared with the control. The lower total VFA is likely explained by the higher acid-detergent fibre (ADF) content of jengkol peel (40.84-43.78% DM) relative to native grass (30.12-41.08% DM). X. Xie *et al*. (2018) reported that diets containing corn stover with higher ADF (37.2% DM) produced lower total VFA (62.0, 61.2, and 57.0 mM at 3, 6, and 24 h post-feeding, respectively) than diets based on corn silage and alfalfa with lower ADF (19.6% DM; total VFA 89.7, 93.8, and 63.4 mM) in Hu sheep. Similarly, B. Ma *et al*. (2022) found that increasing dietary ADF from 16.65% to 29.17% DM significantly decreased total VFA from 146.61 to 130.43 mM in Chinese black Tibetan sheep. Despite the reduction in total VFA, substitution of jengkol peel up to 22.5% did not impair sheep performance (Hidayah *et al*., 2020); dry matter intake actually increased (746.74 vs. 610.42 g/head/day), whereas average daily gain (36.67 vs. 48.00 g/head/day), feed efficiency (5.06 vs. 7.62%), and final body weight (21.20 vs. 21.04 kg) remained comparable with the control.

Moreover, substitution up to 22.5% did not adversely affect the levels of key blood metabolites, including glucose, cholesterol, triglycerides, and HDL (Table 4). This indicates that jengkol peel can be used as a partial native grass replacement without disrupting the animals' metabolic health. The stability of glucose levels suggests that energy metabolism remained unaffected, whilst consistent cholesterol and triglyceride levels reflect maintained lipid metabolism. The unchanged HDL levels further support the notion that the dietary intervention did not elevate lipid transport and cardiovascular risk.

Table 4. *Blood metabolite with jengkol peel substitution*

Treatments	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)
T1	66.49 ± 4.02	38.12 ± 5.08	42.70 ± 12.53	43.29 ± 5.24
T2	58.38 ± 2.71	36.65 ± 7.04	40.64 ± 14.58	40.18 ± 6.26
T3	59.21 ± 9.50	30.18 ± 1.45	32.38 ± 16.41	38.99 ± 6.22

Note: T1 = 40% concentrate (C) + 60% native grass (NG); T2 = 40% C + 45% NG + 15% jengkol peel (JP); T3 = 40% C + 37.5% NG + 22.5% JP

Source: developed by the authors

These data are consistent with those reported by N. Hidayah *et al*. (2020), where jengkol peel substitution up to 22.5% did not disturb sheep performance. Although this treatment increased dry matter intake, average daily gain, feed efficiency, and final body weight remained comparable to the control treatment. This indicated that jengkol peel inclusion did not cause feed refusal by sheep and consequently did not decrease performance. The maintenance of normal blood metabolite profiles, similar to observations by M. Koshchavka *et al*. (2020) in cattle under comfortable conditions, suggests that sheep can tolerate the tannin content in jengkol peel without compromising palatability. S. Soares *et al*. (2020) stated that tannin compounds are widely associated with the organoleptic properties of astringency and bitterness. C. Ginane *et al*. (2011) further explained that lingual receptors in ruminants, such as sheep, cattle, and goats, detect all five basic tastes (sweet, bitter, salty, sour, and umami), with bitter taste appearing to have a negative hedonic value. Studies by J. Carulla *et al*. (2005) and R. Eckard *et al*. (2010) reported that adding tannin ⩾ 50 g/kg dry matter of feed decreased dry matter intake and weight gains in sheep and cows. In the present research, sheep tolerance to this treatment was likely due to the low tannin level in jengkol peel (1.43%). S. Ibrahim & A. Hassen (2022) reported that a higher tannin level than in the present treatment – 2% DM unencapsulated Mimosa tannin (containing 67.76% total tannin) added to total mixed ration – did not decrease dry matter intake of South African mutton Merino ram lambs. A. Pineiro-Vazquez *et al*. (2015) stated that the antinutritional effect of tannins is strongly correlated with their level of inclusion. Sheep can neutralise tannin astringency by secreting salivary proteins rich in proline (Frutos *et al*., 2004). C. Ginane *et al*. (2011) also reported that cows possess fewer genes coding for bitter receptors than other mammals, rendering them less tolerant of this taste.

The blood metabolite results in this study are similar to findings reported by A. Oni *et al*. (2017), where supplementing cassava peels with cassava leaves and cowpea haulms at different ratios (700:100:175 (T1); 500:200:275 (T2); 300:300:375 (T3); and 100:400:475 (T4) g/kg DM) did not alter blood metabolites of West African dwarf goats. This study suggests that cassava peel can be a viable feed component without adverse effects on blood health. In another study, S. Shilwant *et al*. (2023) investigated the impact of a saponin- and polyphenolic-rich composite plant extract (CPE) (seed, root, bark, and peel from different plants) at 20 g/kg diet on blood profiles in lactating Beetal goats. The results showed that CPE supplementation did not significantly affect plasma concentrations of glucose, triglycerides, cholesterol, total protein, albumin, or the albumin-to-globulin ratio. In this regard, it can be stated that the substitution of native grass with jengkol peel up to 22.5% did not negatively affect blood health or the rumen microbial community of sheep. Sheep performance also remained within safe and satisfactory parameters. This indicates that jengkol peel up to 22.5% has the potential to be used as a fibre source to substitute native grass in sheep diets. Future research should examine the same treatment in other ruminants and assess meat quality variables. Furthermore, continued research into jengkol peel as an extract product is warranted, as this material contains bioactive compounds that could potentially be used as a ruminant feed additive to decrease methane emissions and increase ruminant productivity.

Conclusions

The results obtained from this study demonstrate the effects of substituting native grass with jengkol (*Archidendron jiringa*) peel as a fibre source for ruminants. The findings showed that the substitution of native grass with jengkol peel up to 22.5% did not adversely affect ($p > 0.05$) the total bacterial population, whilst significantly decreasing ($p < 0.05$) the protozoa population by 13.95% compared with the control, although it did not alter ($p > 0.05$) methane prediction. The reduction in protozoa may be attributed to the saponin content in jengkol peel. Moreover, substitution up to 22.5% did not adversely affect ($p > 0.05$) the levels of key blood metabolites, including glucose, cholesterol, triglycerides, and high-density lipoprotein. These findings indicate that jengkol peel substitution up to 22.5% maintained blood metabolite concentrations and total bacterial populations whilst selectively reducing protozoal numbers by 13.95%, without affecting methane production. The selective antiprotozoal effect observed, combined with the maintained rumen bacterial populations and normal blood metabolite profiles, suggests that jengkol peel represents a viable alternative fibre source that does not compromise animal health or rumen function. It is concluded that jengkol peel can be used at levels up to 22.5% as a substitute for native grass in sheep diets without negatively affecting blood health or rumen bacterial communities. Future research should examine the same treatment in other ruminant species and assess meat quality parameters. Furthermore, continued investigation into jengkol peel as an extract product is warranted, as this material contains bioactive compounds that could potentially be utilised as a ruminant feed additive to mitigate methane emissions and enhance ruminant productivity.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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Вироблення метану в рубці, мікробна популяція та метаболіти крові овець за годівлі шкіркою дженголу (*Archidendron jiringa*) як замінником природної трави

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Анотація. Агропромислові та сільськогосподарські побічні продукти можуть бути перетворені жуйними тваринами на високоякісний корм. Шкірка дженголу (*Archidendron jiringa*) є сільськогосподарським побічним продуктом із високим вмістом сирої клітковини. Метою цього дослідження було оцінити, чи може шкірка дженголу використовуватися як замінник природної трави як джерело клітковини для жуйних тварин. Досліджуваними змінними були прогноз утворення метану в рубці, мікробна популяція та метаболіти крові овець. В експерименті використовували 15 баранів масою тіла від 15 до 27 кг у рандомізованому блочному дизайні з трьома варіантами годівлі (0,0 %, 15,0 % і 22,5 % заміни шкіркою дженголу) та п'ятьма повтореннями. Заміна природної трави шкіркою дженголу до рівня 22,5 % не впливала негативно ($p > 0,05$) на загальну бактеріальну популяцію та не змінювала ($p > 0,05$) прогноз утворення метану, але зменшувала ($p < 0,05$) чисельність найпростіших на 13,95 % порівняно з контролем, ймовірно, завдяки наявності сапонінів у шкірці. Крім того, заміна до 22,5 % не мала негативного впливу ($p > 0,05$) на ключові метаболіти крові, такі як глюкоза, холестерин, тригліцериди та ліпопротеїни високої щільності (HDL). Було зроблено висновок, що шкірка дженголу може замінювати природну траву до рівня 22,5 % без негативного впливу на стан крові чи бактеріальні популяції рубця, одночасно зменшуючи чисельність найпростіших

Ключові слова: дослідження *in vivo*; побічний продукт дженколу; природні трави; рубцева екосистема; жуйні тварини