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Effects of Tamanu Kernel Cake from Plantation By-product on Ruminal Digestibility and Methane Emission

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ABSTRACT

The study investigates the effects of tamanu kernel cake (TKC) as protein substitution in the dietary concentrate on ruminal digestibility and methane emission. TKC is a by-product of the plantation industry of tamanu oil. The dietary concentrate consisted of wheat pollard, rice bran, corn grain, palm kernel cake, and soybean meal. The concentrate was formulated to contain crude protein and total digestible nutrients of approximately 15% and 65%, respectively. In the present study, TKC was used to substitute protein sources at different levels, such as 0% (T0), 50% (T1), and 100% (T2). Another dietary treatment was also prepared by adding 0.5 mineral salt to T2 (T3). All dietary treatments were incubated in rumen buffer according to the method of Tilley and Terry for 48 h at 39°C. In the results, the digestibility of dry matter and organic matter from dietary T1, T2, and T3 were not different compared to T0. In ruminal fermentation, dietary treatment did not affect total VFA and ammonia. Dietary T2 and T3 resulted in lower methane emissions than dietary T0 (p<0.05). Additional mineral salt in dietary T3 did not affect methane emission compared to dietary T2. The present study concluded that the substitute of protein source with TKC at 30% reduced methane production effectively without negatively affecting ruminal digestibility and fermentation.

Keywords: Methane, protein source, rumen, substitution, tamanu kernel cake

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INTRODUCTION

Recently, the development of the ruminant industry has been considered to create a green farming model by reducing methane (CH_4) production. It occurred because high emissions of methane can contribute to the accumulation of greenhouse gases, which drive global warming and climate change

(Appuhamy et al., 2016; Johnson & Johnson, 1995). Methane results naturally during ruminal enteric fermentation by methanogen bacteria (Johnson & Johnson, 1995). Generally, 30% of total anthropogenic methane emission was contributed by the ruminant sector (Shibata & Terada, 2010). In addition, the ruminant industry in developing countries also faces the limited preference and availability of a protein source, which is relatively expensive, especially for local farmers. Thus, alternative protein sources must be studied with the consideration of local based. Interestingly, the by-product of industry plantation could be an option to supply the requirement of protein source for ruminant feed.

Tamanu (*Calophyllum inophyllum*) kernel cake (TKC) is a by-product from the plantation industry of tamanu crude oil (TCO) (Paradhipta et al., 2023a). In Indonesia, the development of TCO is increasing year by year due to the high demand for alternative biofuel following government regulation, which also increases the production of TKC. Tamanu trees are cultivated in the plantation industry in Indonesia, can be maintained easily in tropical areas, and are spread from Sumatra and Papua islands. In fact, the tamanu kernel can produce similar amounts of crude oil to palm kernel cake (Leksono et al., 2014). Therefore, the development of the TCO industry has high prospects. Kernel cake is the by-product that has high results during oil pressing. Tamanu kernel results in a cake and crude oil at a 1:1 ratio (Leksono et al., 2014). Without any utilization, tamanu cake is the only waste that can contaminate the environment. TKC is potentially used as an alternative feed for ruminants. A previous study reported that TKC contains crude protein (CP), ether extract (EE), and neutral detergent fiber (NDF) approximately at 20.1%, 15.3%, and 53.3%, respectively (Paradhipta et al., 2023a). In addition, TKC also contains total phenol 3.73%–6.47%, total flavonoid 1.13%–1.70%, saponins 0.57%–0.90%, and tannins 0.47%–0.93% (Paradhipta et al., 2023a; Umroni et al., 2024). The chemical compositions of TKC are similar to palm kernel cake (PKC), which contains a CP of around 20%–22% (Paradhipta et al., 2023a). In addition, the price of PKC and TKC is similar in the market. With the massive production of TCO in the future, the price of TKC will be cheaper. Three TCO industries have been established in Central Java Province to supply the market demand.

As a single feed, TKC has a similar ruminal digestibility to PKC and copra cake but results in a lower methane emission (Paradhipta et al., 2023a). Tamanu kernel cake contains the plant's secondary metabolites, which have a role as rumen modifiers that can inhibit the growth of *methanogens archaea* in the rumen (Bodas et al., 2012; Lee et al., 2021; Paverini et al., 2012). It could effectively decrease methane emissions in the rumen. As a by-product of oil extraction, PKC also contains secondary metabolites, but it is lower than TKC. The total phenolic content as main plant secondary metabolites in PKC is lower than 0.55% (Tsouko et al., 2019). Protein sources are commonly applied

as mixture feed as concentrate. However, the use of TKC as a mixture feed was limited in the study. Moreover, the effectiveness of TKC to reduce methane emissions was unknown. This information is important to increase the value of plantation by-products and support the development of integrated farming models among plantation and livestock industries. The present study applied TKC to substitute a protein source, such as PKC. In addition, the use of TKC as a rich source of plant secondary metabolites was combined with the supplementation of mineral salt to have beneficial effects on ruminal fermentation. Previous studies reported that supplementing mineral salt containing Se, Fe, Mn, Zn, Cu, and Co could help decrease methane emissions, especially for lactating animals (Li et al., 2017). The mineral salt could help to reduce the acetate-to-propionate ratio and increase the use of free H^+ ions for propionate production (Li et al., 2017). In addition, trace minerals such as Se and Zn potentially have an antioxidant effect, which might affect rumen methanogens diversity (Cortinhas et al., 2010; Hendawy et al., 2022; Parashuramulu et al., 2015). Therefore, the present study investigated the effect of TKC as a mixture feed on the ruminal digestibility, fermentation, and methane emission by *in vitro* technique.

MATERIALS AND METHODS

Preparation of Diet

The concentrate consisted of wheat pollard, rice bran, corn grain, soybean meal, PKC, and TKC. The TKC was collected from Purworejo Districts and was sub-sampled for laboratory analyses. TKC was used to replace the protein source in the diet. There were four dietary treatments as follows in Table 1. PKC replaced TKC at 0% (T0), 50% (T1) and 100% (T2). In dietary T3, the commercial mineral mix (Booster, PT. Agromix, Indonesia)

Table 1
Ingredients and formulation of dietary treatments (%)

Ingredients	T0	T1	T2	T3
Wheat pollard	30	30	30	30
Rice bran	20	20	20	20
Corn grain	10	10	10	10
Soybean meal	10	10	10	10
Palm kernel cake	30	15	0	0
Tamanu kernel cake	0	15	30	30
Total	100	100	100	100
Mineral salt*	-	-	-	0.5

Note. T0 = control diet; T1= the use of TKC to substitute 50% of palm kernel cake; T2 = the use of TKC to substitute 100% of palm kernel cake; T3 = dietary T2 with an additional 0.5% of trace mineral; *Fe 12.5 g/kg, Mg 1.8 g/kg, Zn 439.0 mg/kg, Se 131 µg/kg

was added as a supplement. The supplementation of commercial mineral mix was chosen and conducted based on field observation. The commercial mineral mix contained trace minerals such as Fe, Mg, Zn, Se and complex vitamins (A, D, E). All dietary treatments were formulated to contain CP approximately at 15% and total digestible nutrient (TDN) at 65%. The ingredients and formulation of dietary treatments are shown in Table 1.

Chemical Compositions of Diet

Both TKC and PKC, and all dietary treatments were sub-sampled at approximately 500 g for proximate analyses following the procedure of the Association of Official Analytical Chemists (AOAC, 2016). In the preparation, samples from TKC and dietary treatments were dried using the dry oven (Memmert UN55, Germany) at 55°C for 48 h and then ground using a Wiley mill to pass a 1 mm screen as a standard for proximate analysis. The grinding process also helped to homogenize samples. In the determination of dry matter (DM), the sample was dried at 105°C for 24 h (method 934.01). A Muffle furnace (Advantec KM-420, Japan) was used to measure the organic matter (OM), conducted at 550°C for 5 h. The CP was determined by a Nitrogen Analyzer (B-324, 412, 435 and 719 S Titrino, BUCHI, Flawil, Switzerland) following the procedure of Kjeldahl (method 984.13). The EE was analyzed according to the Soxhlet procedure (method 920.39). The fiber fractions, such as neutral detergent fiber (NDF) and acid detergent fiber (ADF), were determined by using an Ankom 200 fiber analyzer (Ankom Technology, Macedon, NY, USA) (method 2002.04 and method 973.18, respectively). The TDN was estimated based on Dahlke's model (2020) calculations.

Plant secondary metabolites from TKC and all dietary treatments were determined using Spectrophotometry UV-vis (UV-1800 Shimadzu, Japan). In this investigation, the methods of Chaovanalikit and Wrolstad (2004) and Gonzalez and Herrador (2007) were utilized for total phenol and total flavonoid, respectively. The methods of Makkar et al. (1993) and Pramono (2005) were followed for the measurements of tannin and saponins, respectively.

***In Vitro* Digestibility**

The rumen fluid was collected from two fistulated Bali cattle (*Bos indicus*) before morning feeding. In general, cattle had an average weight of 310 kg. The diet of fistulated cattle contained CP at 12.5% and metabolizable energy (ME) at 10 kcal/kg, which consisted of *Pennisetum purpureum* grass and commercial concentrate at a 7:3 ratio. Collected rumen fluid was placed in an anaerobic container at 39°C before being transferred to the laboratory. The collected rumen fluid was filtered in the laboratory using three cheesecloth layers. The rumen buffer consisted of a rumen fluid and buffer solution at a ratio of 1:4, following the procedure of McDougall (1948).

All samples were incubated in rumen buffer according to Tilley and Terry's (1963) procedure. In the incubation, a 0.5 g sample was placed in a 100 mL glass serum bottle and added with 40 mL of rumen buffer. Each treatment used quadruplicate using four incubation bottles along with four blanks. Incubation was conducted at 39°C for 48 h in the aerobic incubator. Incubation was conducted over three different periods in different weeks. After 48 h incubation, a syringe collected 10 mL of gas from each bottle. The gas was transferred into the vacuum tube for storage before methane analysis. Gas Chromatography (Shimidzu GC-2010, Japan) was used to determine methane concentration following Paradhipta et al.'s setup (2023a). In addition, helium was used as a carrier gas. The concentration of methane was expressed as ppm.

All bottles were opened and filtered using a gooch crucible to separate a sample from the rumen buffer. The rumen buffer, which consisted of pH, ammonia-N, and total volatile fatty acid (VFA), was used to evaluate ruminal fermentation. The filtered sample was used to evaluate the digestibility of a diet consisting of *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). The difference in concentrations of DM and OM, before and after ruminal incubation, were used to calculate the IVDMD and IVOMD. Collected rumen buffer was used to measure rumen pH using a digital pH meter (Ohaus AB23PH-F, China). The concentration of ammonia-N was analyzed using the colorimetric method by Chaney and Marbach (1962). The analysis of VFA using gas chromatography (GC 8A, Shimadzu Crop., Japan) is according to the description of Hidayah et al. (2023).

Ethical Approval

The animal care and *in vitro* procedures were carried out in accordance with the ethical standards of the Ethics Committee of the Integrated Laboratory for Research and Testing, Universitas Gadjah Mada (No. 00007/III/UN1/LPPT/EC/2024).

Data Analysis

Data was analyzed based on a completely randomized design using PROC GLM of Statistical Analysis Software (SAS), version 9. The model applied in the present study was $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} is the response variable, μ is the overall mean, T_i is the effect of dietary treatment, and e_{ij} is the error mean. Tukey test was applied for mean separation, and the significant differences were declared at $p \leq 0.05$.

RESULTS

Chemical Compositions

In the present study, the concentrations of DM, OM, CP, EE, NDF, ADF, and TDN from TKC were 89.5, 91.4, 22.6, 16.8, 52.6, 37.4, and 61.6%, respectively (Table 2). Generally,

the chemical compositions of TKC were similar to PKC, especially in the contents of CP, NDF, and ADF. The PKC contained DM, OM, CP, EE, NDF, ADF, and TDN approximately at 91.5, 91.8, 21.7, 10.4, 54.1, 36.4, and 62.4%, respectively. In addition, the concentration of total phenol, total flavonoid, total saponin, condensed tannin, and hydrolysable tannin from TKC were 6.23, 1.56, 0.96, 0.42, and 0.45%, respectively (Table 3).

Different substitution levels of TKC do not affect the chemical compositions of dietary treatments (Table 4). The means of DM, OM, CP, EE, NDF, ADF, and TDN from all treatments were 86.5, 88.4, 15.9, 2.53, 45.1, 27.3, and 68.7%.

The present study reported that dietary T2 and T3 had higher total phenol (p=0.001; 1.32 and 1.33 vs. 0.11 and 0.88) and total flavonoid (p=0.001; 0.35 and 0.38 vs. 0.09 and ND) compared to T0 and T1 (Table 5). In addition, saponin and tannin were not detected or in less concentration for measurement.

Table 2
Chemical compositions of tamanu kernel cake and palm kernel cake in the present study (%, DM)

Items	Feedstuffs[1]	
	TKC	PKC
Chemical compositions		
Dry matter	89.5	91.5
Organic matter	91.4	91.8
Crude protein	22.6	21.7
Ether extract	16.8	10.4
Neutral detergent fiber	52.6	54.1
Acid detergent fiber	37.4	36.4
Total digestible nutrient[2]	61.6	62.4

Note. [1]TKC = Tamanu kernel cake; PKC = Palm kernel cake; DM = Dry matter; NA = Not available

Table 3
Plant secondary metabolites of tamanu kernel cake in the present study (%, DM)

Items	Feedstuffs[1]
Total phenol	6.23
Total flavonoid	1.56
Total saponin	0.96
Total tannin	0.87
Condensed tannin	0.42
Hydrolysable tannin	0.45

Table 4
Chemical compositions of dietary treatments (%, DM)

Items	Dietary treatment[1]				SEM	p-value
	T0	T1	T2	T3		
Dry matter	86.4	86.3	86.4	86.9	0.640	0.667
Organic matter	88.5	88.7	88.1	88.4	1.174	0.952
Crude protein	15.8	16.2	16.0	15.5	1.097	0.871
Ether extract	3.04	2.38	2.30	2.41	0.719	0.587
Neutral detergent fiber	44.4	45.6	45.4	44.9	2.021	0.879
Acid detergent fiber	27.3	27.8	27.2	27.1	1.792	0.945
Total digestible nutrient[2]	68.7	68.4	68.8	68.9	1.254	0.944

Note. [1]T0 = Control diet; T1 = The use of TKC to substitute 50% of palm kernel cake; T2 = The use of TKC to substitute 100% of palm kernel cake; T3 = Dietary T2 with an additional 0.5% of trace mineral; [2]Total digestible nutrient is based on calculation; DM = Dry matter

Table 5
Secondary metabolites of dietary treatments (%, DM)

Item	Dietary treatment				SEM	*p*-value
	T0	T1	T2	T3		
Total phenol	0.11[b]	0.88[b]	1.32[a]	1.33[a]	0.044	0.001
Total flavonoid	ND[b]	0.09[b]	0.35[a]	0.38[a]	0.093	0.001
Saponin	ND	ND	ND	ND	NA	NA
Tannin	ND	ND	ND	ND	NA	NA

Note. [1]T0 = Control diet; T1= The use of TKC to substitute 50% of palm kernel cake; T2 = The use of TKC to substitute 100% of palm kernel cake; T3 = Dietary T2 with an additional 0.5% of trace mineral; DM = Dry matter; ND = Not detected (less concentration); Means with different superscript letters in a row are significantly different (p<0.05)

Digestibility

The present study reported that the application of TKC at different levels of substitution did not affect IVDMD and IVOMD during 48 h of incubation (Figure 1). T1, T2, and T3 resulted in similar results to T0. The average IVDMD was 42.9%, while the average IVNDFD was 65.7% of all treatments.

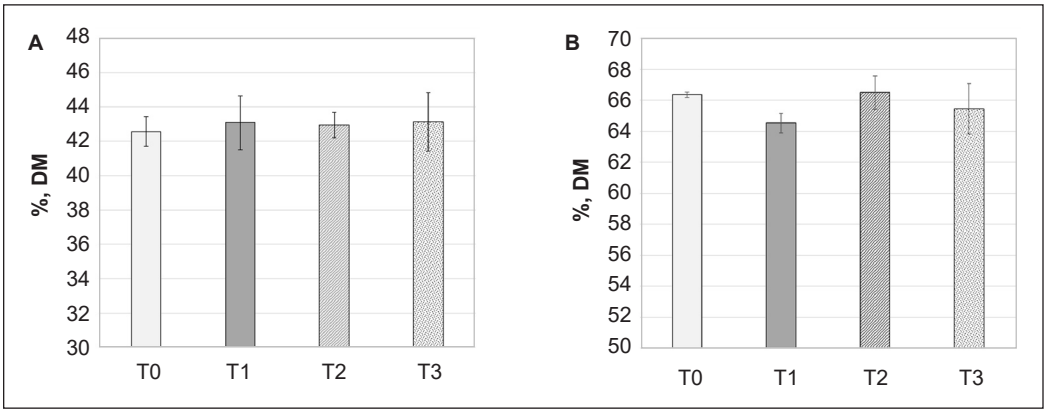

Figure 1. The *in vitro* dry matter digestibility (A) and *in vitro* organic matter digestibility (B) of dietary treatments
Note. T0 = Control diet, T1 = The use of TKC to substitute 50% of palm kernel cake, T2 = The use of TKC to substitute 100% of palm kernel cake, T3 = Dietary T2 with an additional 0.5% of trace mineral

Ruminal Fermentation

The present study reported that the application of TKC in different levels of substitution did not affect rumen pH, ammonia-N, total VFA, VFA profiles, and acetate-to-propionate ratio (Table 6). The T1, T2, and T3 had the same result compared to T0. Generally, the means of pH, ammonia-N, and total VFA from all treatments were 7.03, 7.53 mg/dL, and 1235.6

Table 6
Effects of dietary treatments on ruminal fermentation

Items	Dietary treatment[1]				SEM	*p*-value
	T0	T1	T2	T3		
pH	7.03	7.04	7.04	7.03	0.034	0.985
Ammonia-N, mg/dL	7.60	7.69	7.29	7.53	0.251	0.290
Total VFA, mg/L	1214.5	1260.1	1236.7	1231.3	136.31	0.981
Acetate, % molar	77.7	78.1	74.9	78.0	2.003	0.158
Propionate, % molar	13.5	13.6	14.3	13.0	0.728	0.176
Butyrate, % molar	8.83	8.30	10.76	9.01	1.500	0.205
Acetate: Propionate	5.75	5.79	5.24	6.01	0.441	0.159

Note. [1]T0 = Control diet, T1 = The use of TKC to substitute 50% of palm kernel cake, T2 = The use of TKC to substitute 100% of palm kernel cake, T3 = Dietary T2 with an additional 0.5% of trace mineral

mg/L. In addition, the means of acetate, propionate, and butyrate from all treatments were 77.2%, 13.6%, and 9.23%, while the mean of acetate to propionate ratio was 5.70.

Methane Emission

Dietary treatments affected ruminal methane emissions (Figure 2). The application of T2 and T3 resulted in lower methane emissions than T0 (p=0.002; 49684.6 and 50340.3 ppm vs. 55658.7 ppm). The application of T1 was not different from other dietary treatments.

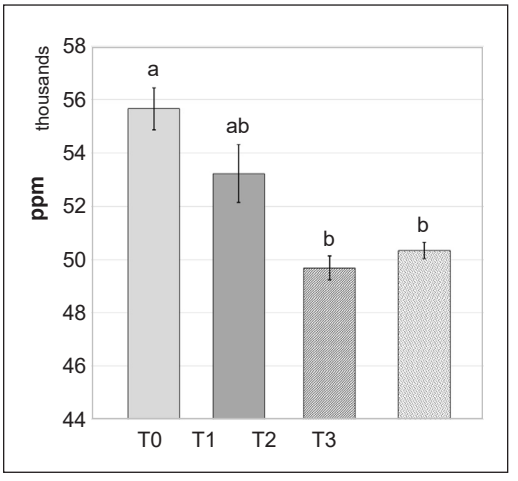

Figure 2. Ruminal methane emission by dietary treatments
Note. T0 = Control diet, T1 = The use of TKC to substitute 50% of palm kernel cake, T2 = The use of TKC to substitute 100% of palm kernel cake, T3 = Dietary T2 with an additional 0.5% of trace mineral; [a,b]Mean with different superscripts differ significantly (p<0.05)

DISCUSSION

All dietary treatments were formulated to have similar chemical compositions, such as OM, CP, EE, NDF, ADF, and TDN. However, the concentration of plant secondary metabolites was different in dietary treatments. According to our previous study, TKC is a source of total phenol and total flavonoid (Paradhipta et al., 2023a; Umroni et al., 2024), and it could be higher than PKC (Tsouko et al., 2019). TCO was produced using the mechanical pressing method (Leksono et al., 2014), where secondary metabolites might still remain in their by-product, such as TKC. Therefore, a higher proportion of TKC in dietary treatment was reported to increase the concentration of plant secondary metabolites (Table 5). Dietary treatments T2

and T3 had a similar concentration of secondary metabolites due to the same proportion of TKC in the diet. In contrast, dietary T1 had a low concentration of secondary metabolites due to a proportion of TKC in the diet.

In general, the different levels of TKC as substitution were unaffected by the rumen's digestibility and fermentation. The combination of TKC and trace minerals did not affect it. It could occur due to similar chemical compositions among dietary treatments (Table 4). However, dietary T2 and T3 could effectively decrease methane emissions. The high concentration of total phenol and total flavonoid could be a reason for the decrease in methane production by these dietaries in the present study. Previous studies reported that the use of phenol and flavonoid in proper concentration could inhibit the growth of methanogens archaea without affecting the activity of fibrinolytic microbes in the rumen (Bodas et al., 2012; Lee et al., 2021; Paverini et al., 2012). According to a previous study, plant secondary metabolites, particularly phenolic compounds, could control archaea methanogens populations. Phenolic compounds, like flavonoids and phenolic acid, interact with bacterial membrane cells due to their lipophilicity. Flavonoids inhibit cytoplasmic membrane function, cell wall synthesis, and cell wall synthesis, while phenolic acid acidifies the cytoplasm, leading to cell death (Nørskov et al., 2023). In a low dose, the effect of plant secondary metabolites has no impact on rumen fermentation, including methane emission. However, in high doses, it could present a toxic response for ruminal microbes and hoses (Bodas et al., 2012; Paverini et al., 2012). In the *in vitro* study, the decrease in digestibility is a sign of toxicity by planting secondary metabolites. This condition could inhibit the activity of fibrinolytic bacteria from degrading fiber in the diet (Bodas et al., 2012; Lee et al., 2021; Vasta et al., 2019). Results of the present study indicated that the use of TKC on dietary T2 and T3 did not affect a toxic condition in the ruminal ecosystem. This confirmed that TKC is a viable protein replacement source, such as PKC while reducing methane emissions without negatively affecting the rumen ecosystem. However, the use of TKC at 50% for substitution is still in low doses of plant secondary metabolites, which could not yet have a big impact on methane emission.

A combination of TKC and mineral salt had no effects on methane emissions. This can be seen from the results of methane production by dietary T2 and T3. Mineral salt was expected to reduce the ratio of acetate to propionate, which could decrease methanogenesis (Li et al., 2017). However, in the present study, dietary treatments did not affect the acetate-to-propionate ratio. The previous study also reported that using mineral salt could help increase feed efficiency and animal performance, but it does not always promise the reduction of methane emissions (Grešáková et al., 2021; Son et al., 2023). The dose of trace minerals in the present study was in range according to many previous studies (Grešáková et al., 2021; Hendawy et al., 2022; Son et al., 2023; Li et al., 2017). The difference in physiological status, animal breed, nutrients in the diet, feeding strategies, and utilization of mineral salt might be the factors that affect the variety of results in methane production

(Grešáková et al., 2021; Li et al., 2017; Son et al., 2023). Especially the ingredient and nutrient content of the diet in the present study were different compared to previous studies (Grešáková et al., 2021; Hendawy et al., 2022; Son et al., 2023; Li et al., 2017), which could be a main reason for none effect of dietary T3 on methane reduction. In general, the reduction of methane emission in the present study was mainly affected by using TKC as a protein substitution in the concentrate diet.

CONCLUSION

The present study concluded that TKC could be used to substitute a protein source in the concentrated diet without presenting any negative effects on ruminal digestibility and fermentation through *an in vitro* technique. Substituting protein sources in the concentrate with TKC at 30% could reduce ruminal methane emission. However, combining TKC and mineral salt did not effectively reduce methane emissions.

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