

Research Article



Fermentation Characteristics and In Vitro Nutrient Digestibility of Fermented Corn Cob-Based Feed Supplemented With Soybean Oil Calcium Soap Using Rumen Fluid of Etawa Crossbreed Goats

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Abstract | This study aimed to evaluate the fermentation characteristics and in vitro nutrient digestibility of feed based on the fermented corn cob supplemented with soybean oil calcium soap (CaS-soybean oil) using microbial rumen fluid of Etawa crossbreed goats. The experimental trial consisted of five treatment rations with four replicates arranged in a randomized block design. The treatment rations were a mix of 60% concentrate (25% fermented corn cobs and 75% agricultural by-products) and 40% forage supplemented with different levels of CaS-soybean oil (0%, 1.5%, 2.5%, 3.5% and 4.5%). The measured research parameters were (i) fermentation characteristics (pH, NH₃-N and total volatile fatty acid (VFA)), (ii) nutrient digestibility (dry matter digestibility (DMD), organic matter digestibility (OMD), crude fiber digestibility (CFD) and crude protein digestibility (CPD)). The results showed that fermented corn cob-based rations supplemented with CaS-soybean oil at different levels significantly ($P < 0.01$) affected fermentation characteristics and ration nutrient digestibility. The different levels of CaS-soybean oil (1.5%–4.5%) produced optimal fermentation characteristics (pH, NH₃-N and total VFA) to support the growth and activity of microorganisms to optimally digest nutrients during in vitro incubation. Supplementation of 4.5% CaS-soybean oil in the fermented corn cob-based rations resulted the best NH₃-N levels and total VFA production, followed by the highest nutrient digestibility (DMD, OMD, CPD and CFD) compared to the other treatments. The study concluded that rations based on fermented corn cobs and other agricultural by-products supplemented with 4.5% CaS-soybean oil produced ideal fermentation characteristics to support optimal growth and activity of microorganisms digesting feed nutrients.

Keywords | Corn cob, CaS-soybean oil, Fermentation, Digestibility, In vitro

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INTRODUCTION

Energy source ingredients are the most expensive nutrient component in livestock feed, considering that the ingredients cost around 80-90% of the total ration cost

(McDonald et al., 2010). The high cost of providing energy feed-stuffs has been one of the reasons why the livestock industries and research institutions are exploring locally sourced, inexpensive, and abundantly available energy-source feed ingredients. Corn cob is one of the agricul-

tural by-products of corn plantations (Seran et al., 2020) that could serve the purposes because it is easy to obtain, has adequate nutritional content and is abundantly available (Fairus et al., 2013). Corn cobs are leftover from the corn after the shelling off the maize (Seran et al., 2020). Corn cobs have the potential as an energy source feed ingredient due to their biomass and price. However, its use is limited by the high crude fiber content and low crude protein and their digestibilities.

The nutritional content of corn cobs consists of 90.0% dry matter, 88.5% organic matter, 2.8% crude protein, 0.7% crude fat, 1.5% ash, 32.7% crude fiber, 80% cell wall, 6.0% lignin, and 32% acid detergent fiber (ADF) (Widaningsih et al., 2018). Fortunately, low nutrient content in feed ingredients can be solved using feed processing technology or combination with high-density energy ingredients. One of the high-energy-density feed ingredients widely used in the broiler industry is vegetable oils. Generally, feed ingredients from vegetable oils have high energy density and contain highly beneficial polyunsaturated fatty acids (PUFA). The use of fat in the ration can increase the energy density of the ration, improves consumption and energy use efficiency, and boosts PUFA content in livestock meat products (Fiorentini et al., 2015). Garcia and Casal (2013) reported that vegetable oil supplementation containing high PUFA was shown to reduce saturated fatty acid levels in the body tissues of monogastric animals.

Various vegetable oils frequently used in ruminant and monogastric rations include soybean, palm, and sunflower. The levels of unsaturated fatty acid in vegetable oils vary depending on the types of the source, such as soybean oil (23.5% oleic, 54.6% linoleic and 8.3% linolenic), palm oil (39.2% oleic, 10.1% linoleic and 0.4% linolenic) and sunflower oil (12.0% oleic, 77.5% linoleic and 0.4% linolenic) (O'Brien, 2004). Despite their advantages as a source of energy and PUFA, vegetable oils in ruminants must be controlled because PUFA in feed can hurt the rumen fermentation process, reducing crude fiber digestibility and dry matter consumption (Manso et al., 2006). In addition, PUFA in the rumen will be hydrogenated into saturated fatty acids. The process of PUFA bio-hydrogenation by rumen microbes will reduce the effectiveness of PUFA utilization to improve the quality of ruminant fat products. PUFA bio-hydrogenation in the rumen cannot be avoided because it is a defensive mechanism for rumen microbes against the bacteriostatic effects of PUFA, which can damage cell membrane integrity and reduce microbial growth (Maia et al., 2010). If the adverse impact and bio-hydrogenation process of PUFAs can be controlled, vegetable oils can improve the quality of ruminant meat and dairy products (Maia et al., 2010). One of the efforts to suppress the harmful effects of PUFA fatty acids in interfering with

microbial growth and activity and the fermentation process in the rumen is fat technology containing high PUFA in the form of fatty acid calcium soap (Block et al., 2005).

PUFA protection in calcium soap can improve energy digestibility, allow feed ingredients to mix, contribute Ca minerals and increase PUFA content in meat and milk ruminants (Lounglawan et al., 2008). However, the quality and nutritional properties of fatty acid calcium soap products are greatly influenced by the manufacturing method and how they are stored before use (Block et al., 2005). The use of soy calcium soap (SCa) has been shown to improve fermentation characteristics (pH, VFA and $\text{NH}_3\text{-N}$) and nutrient digestibility of rations in Bali cattle (Bain et al., 2016).

Regarding the above-mentioned potential advantages and drawbacks, it is surprising that related research and industrial applications for using these specific feed ingredients for livestock are few and far between. Therefore, this study was carried out to evaluate the fermentation characteristics and in vitro nutrient digestibility of fermented corn cob-based rations supplemented with soybean oil calcium soap using rumen fluid inoculum of Etawa crossbreed goats. The research hypothesis was that supplementation of CaS-soybean oil at different levels in rations based on fermented corn cobs and other agricultural by-products can affect fermentation characteristics and nutrient digestibility in vitro

MATERIALS AND METHODS

The feed ingredients used in the study consisted of forage (dwarf elephant grass), corn cobs, rice bran, pollard, soybean meal, coconut meal, molasses, CaCO_3 , CaS-soybean oil and urea. Dwarf elephant grass was freshly obtained from local pasture land, while the corn cobs were sourced from smallholder corn plantations in Muna Regency, Southeast Sulawesi, Indonesia. The materials for analysis used consisted of Whatman filter paper No. 41. A total of 6 liters of McDoughal solution made from ingredients were NaHCO_3 (58.8 g), $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (42 g), KCl (3.42 g), NaCl (2.82 g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.72 g) dan CaCl_2 (0.24 g). HgCl_2 , H_2SO_4 , Boric Acid (H_3BO_3 crystal), Brom Cresol Green (BCG), Methyl Red (MR), Aquadest, Pepsin and N-Hexane. Effective microorganism-4 (EM-4), molasses and water were used in the corn cob fermentation. Materials needed to produce soybean oil calcium soap consisted of mazola product soybean oil, NaOH, 50 ml KOH, HCl 0.5N, distilled water and technical CaCl_2 crystals.

The source of microbial inoculum for the in vitro digestibility test came from the rumen fluid of adult male Etawa crossbreed goats. The equipment used in corn cob fermentation consisted of digital scales, tarpaulins, machetes,

bucket thermometers, drums or plastic bags, digital scales, fermenter tubes, ventilated rubber caps, water bath shakers, CO₂ gas cylinders, porcelain cups, 105°C ovens, 600°C electric furnaces, Whatman No. 41 filter paper, Conway cups, Erlenmeyer flasks, distillation equipment, and NH₃-N titration equipment. In vitro research methods were used to observe fermentation characteristics and measure nutrient digestibility following the optimization procedure (Tilley and Terry, 1963). The materials for the in vitro method consisted of pepsin HCl solution 0.2%, aquadest, saturated HgCl₂ solution, saturated NaCO₃ solution, H₂SO₄ 0.005 N solution, Boric acid indicator, 0.5 N HCl solution, 15% H₂SO₄ solution, NaOH 0.5N solution and PP indicator solution (phenol phtalein 0.1%).

RESEARCH DESIGN AND TREATMENTS

The experimental units comprised five treatments with four replications arranged in a randomized block design (based on the rumen fluid collection period). The ration composition consisted of 60% concentrate and 40% forage. The concentrates were formulated by mixing fermented corn cob meal as the main ingredient (25%) with other agricultural by-product feed ingredients (tapioca waste, pollard, fine rice bran, sago dregs, coconut meal and molasses). The treatment ration consisted of 5 types, namely R1 (ration without CaS-soybean oil), R2 (ration supplemented with 1.5% CaS-soybean oil), R3 (ration supplemented with 2.5% CaS-soybean oil), R4 (ration supplemented with 3.5% CaS-soybean oil) and R5 (ration supplemented with 4.5% CaS-soybean oil). The composition of feed ingredients and nutritional content of each treatment ration are presented in Table 1.

The composition of feed ingredients in the treatment ration was formulated to meet the nutrient requirements of the male Etawa crossbred goat based on the recommendations of Kearn (1982) with nutritional composition (% dry matter) of total digestible nutrient (TDN) (75.60-79.12%), crude protein (13.00-14.66%), crude fat (12.05-13.21%) and 45.58%-48.10% nitrogen free extract (NFE) (Table 2).

RESEARCH PROCEDURE

Corn Cob Fermentation: Corn cob fermentation was carried out following the modified procedure of Seran et al. (2020). Corn cobs from smallholder farmers were finely chopped until the same size and air-dried. A mixed solution consisting of molasses, Effective Microorganism (EM-4) and distilled water (mixing ratio 1:1:10) was sprayed onto the surface of the chopped corn cobs using a sprayer, stirred until the molasses-EM4 solution was evenly distributed on the chopped corn cobs. The treated corn cobs were then placed into a silo, compacted, tightly closed (airtight) and incubated aerobically for 21 days. The fer-

mented corn cobs were removed from the silo and then aerated and finely ground. The finely ground fermented corn cobs were packed in plastic clips and stored in the freezer to avoid sample damage before being mixed with other feed ingredients for subsequent nutrient content and in vitro analyses.

Calcium Soap Making: Soybean oil calcium soap was prepared according to the method of Kumar et al. (2006) using ingredients: Mazola soybean oil, distilled water, NaOH and technical CaCl₂ crystals. The procedure for making calcium soap begins with determining the saponification number according to the amount of NaOH required (Apriyantono et al., 1989). The production of soybean oil calcium soap was carried out using the optimized procedure developed by Bain et al. (2017) by dissolving 964 g of technical CaCl₂ crystals in 1.5 liters of distilled water and 566 g of NaOH material dissolved in distilled water until the volume of solution in a plastic bucket is 5.5 liters and 4.1 kg of soybean oil is mixed and heated in a reactor with milk at 238°C, then stirred at 1500 rpm for 30 minutes until the mixture of NaOH and soybean oil is completely dissolved. The CaCl₂ solution was dripped slowly until the oil, NaOH solution, and CaCl₂ mixture formed a solid soybean oil calcium soap. Excess water during the solidification process, water was removed. Then, the soybean oil calcium soap solid was stored in a stainless steel container, ground and crushed until the soybean oil calcium soap was ready to be used as an ingredient in making research rations.

In vitro Fermentation and Measurement: In vitro fermentation was carried out following Tilley and Terry's method. Treatment feed samples (500 mg) and 40 ml of McDougall's solution were put into 100 ml fermenter tubes. Into each tube containing the experimental ration, 10 ml of rumen fluid of Etawa crossbred goat was added, stirred gently, supplied with CO₂ gas, and incubated on a shaker bath at 39°C. The goat rumen fluid as a source of microbial inoculum was collected in the morning 4 hours after feeding through the mouth using a stomach tube connected to a vacuum pump. Ethical Approval from Animal Care and Use Committee (AUAC), Bogor Agricultural University No. 08-2015 IPB approved the fluid collection procedure. Rumen fermentation characteristics variables were pH (measured with a pH meter), Concentration of NH₃-N (N-Ammonia) analyzed with Conway micro diffusion method (Conway, 1962), and total VFA concentration analyzed using steam distillation method (General Laboratory Procedures, 1966). The 48 hours of incubated fermentation liquids were measured for dry and organic matter digestibility.

The process started with the in vitro liquid in the ferment

Table 1: Composition of raw materials for treatment ratios (%dry matter)

Feed ingredient	Treatment				
	R1	R2	R3	R4	R5
	-----%-----				
Dwarf elephant Grass	40.00	40.00	40.00	40.00	40.00
Concentrate	60.00	60.00	60.00	60.00	60.00
Tapioca waste	9.00	15.70	16.20	7.20	8.00
Pollard	26.00	20.02	20.40	23.50	20.00
Fine rice bran	6.00	7.01	8.00	9.60	6.20
Sago dregs	8.00	6.00	6.00	10.50	14.00
Coconut meal	22.80	21.00	18.10	16.50	18.10
Corn cob fermentation	25.00	25.00	25.00	25.00	25.00
Molasses	1.00	1.00	1.20	1.30	1.30
CaCO ₃	1.00	1.00	1.00	1.20	1.20
Urea	1.20	1.50	1.60	1.70	1.70
Soybean oil Calcium soap	0.00	1.50	2.50	3.50	4.50

Description: R1 (60% concentrate + 40% forage grass without CaS-soybean oil), R2 (60% concentrate + 40% forage grass containing 1.5% CaS-soybean oil), R3 = ration (60% concentrate + 40% forage grass containing 2, 5% CaS-soybean oil), R4 (ration (60% concentrate + 40% forage grass containing 3.5% CaS-soybean oil), R5 (60% concentrate + 40% forage grass containing 4.5% CaS-soybean oil).

Table 2: Nutrient composition of treatment ratios (% dry matter)

Nutrient	Treatment (%)				
	R1	R2	R3	R4	R5
Dry matter	88.86	88.38	88.85	88.43	88.74
Ash	7.87	7.85	8.08	8.02	8.04
Crude protein	13.00	13.42	13.41	13.83	14.66
Ether extract	12.05	12.25	12.80	13.00	13.21
Crude fiber	20.02	19.97	20.13	17.05	17.60
NFE	47.08	46.51	45.58	48.10	46.49
TDN	75.60	75.88	76.22	79.12	78.97

er tube containing ration in a shaker bath at 39°C for 48 hours. The 48-hour incubated fermentation liquids were measured for their dry and organic matter digestibility. The process started with the in vitro liquid in the fermenter tube was taken out from the shaker bath and added with 2-3 drops of HgCl₂ solution to stop the microorganism fermentation process, then centrifuged at 1500 rpm to separate the supernatant and substrate. The substrate was then filtered using *Whatman* paper No.41 (distributed by Voight Global, PO Box 1130, Lawrence, Kansas 660044 USA), placed in a porcelain cup, and dried in an oven set at 105°C for 8 hours, determining its dry weight. It was then followed by an incineration set at 550°C for 4 hours to measure the ash weight.

DATA ANALYSIS

The data were analyzed using one-way ANOVA followed by Duncan's Multiple Range Test using the IBM SPSS Statistics for Windows, version 21.0 Armonk, 2012, New

York. Differences were considered significant at P<0.05 and P<0.01.

RESULTS AND DISCUSSION

FERMENTATION CHARACTERISTICS

The fermentation characteristics and in vitro digestibility of rations based on fermented corn cobs and agricultural by-product feedstuffs supplemented with CaS-soybean oil with male Etawa crossbreed goat inoculum source are presented in Table 3.

pH CONTENT

The results of the analysis of variance showed that supplementation of calcium soap soybean oil (CaS-soybean oil) at different levels in fermented corn cob-based rations and local feed ingredients did not have a significant effect (P>0.05) on pH levels. Nevertheless, they significantly impacted (P<0.01) NH₃-N levels and total VFA levels of

in vitro fermentation. The resulting pH level is a neutral pH category (6.78-6.80), considered an ideal pH range for in vitro fermentation ecosystems to support the growth and activity of microorganisms in digesting feed nutrients during incubation. In addition, different levels of CaS-soybean oil usage resulted in varying $\text{NH}_3\text{-N}$ concentrations. The highest $\text{NH}_3\text{-N}$ levels were obtained in treatment R5 but were not significantly different ($P>0.05$) from treatments R3 and R4. The lowest $\text{NH}_3\text{-N}$ levels were produced in the ration treatment R1 (control treatment). $\text{NH}_3\text{-N}$ levels in treatments R3, R4 and R5 were higher ($P<0.05$) compared to R1 and R2. Furthermore, differences in supplementation levels of calcium soap in rations based on fermented corn cobs combined with local ingredients significantly ($P<0.01$) affected the production of total VFA in vitro fermentation.

The highest total VFA content was obtained in the ration supplemented with 4.5% CaS-soybean oil (R5), and the lowest total VFA production was produced in the control treatment (R1) or without calcium soap. Statistically, the total VFA production in the R2, R3, R4 and R5 treatments were not significantly ($P>0.05$) different (relatively the same). However, the total VFA production produced in all treatments with the range (150.86 mM-174.49 mM) is still classified as optimal total VFA levels (70-150 mM) (McDonald et al., 2010) as a source of carbon skeleton and energy for microbial growth in an aerobic in vitro fermentation ecosystem.

RATION NUTRIENT DIGESTIBILITY

Ration nutrient digestibility (including dry matter digestibility, organic matter digestibility, crude protein digestibility and crude fiber digestibility) of fermented corn cob-based rations and local feed ingredients supplemented with CaS-soybean oil at different levels are presented in Table 4.

DRY MATTER DIGESTIBILITY

The results of the analysis of variance (Table 4) showed that supplementation of various levels of CaS-soybean oil in fermented corn cob-based rations had a very significant effect ($P<0.01$) on the digestibility of all ration nutrient indicators (dry matter digestibility, organic matter digestibility, crude protein digestibility and crude fiber digestibility). Supplementation of different concentrations of CaS-soybean oil (1.5%-4.5%) positively affected the average dry matter digestibility and was higher than the control treatment (without CaS-soybean oil). However, the digestibility of dry matter in all rations supplemented with CaS-soybean oil (R2, R3, R4 and R5) was not significantly ($P>0.05$) different.

ORGANIC MATTER DIGESTIBILITY

The analysis of variance showed that the supplementation

treatment of soybean oil calcium soap had a very significant effect ($P<0.01$) on the digestibility of organic matter of the ration based on fermented corn cob combined with local resource-based feed ingredients. The percentage of organic matter digestibility, shows an increase in organic matter digestibility as the CaS-soybean oil supplementation increases.

The average digestibility value of organic matter obtained in this study followed the trend of the average digestibility value of dry matter. The average digestibility of organic matter of the rations that received CaS-soybean oil supplementation at 1.5%-4.5% was higher than the treatment rations without CaS-Soybean supplementation. However, there was no difference in mean organic matter digestibility between all rations supplemented with CaS-soybean oil. The highest average digestibility of organic matter quantitatively was obtained in treatment R5 and the lowest in treatment R1 (without supplementation of CaS-soybean oil). These results are better than the research by Bain et al. (2018), where the digestibility of organic matter in rations consisting of field grass and concentrates supplemented with 5% calcium soap was 74.31-75.28%.

CRUDE PROTEIN DIGESTIBILITY

The analysis of variance showed that the supplementation treatment of soybean oil calcium soap had a very significant effect ($P<0.01$) on the crude protein digestibility of rations containing fermented corn cob and other local feed ingredients. As with the digestibility of dry matter and organic matter, there appears to be an increase in the digestibility of crude protein as the CaS-Soybean supplementation increases in the rations.

The Duncan Multiple Range Test results showed that the highest average CPD was produced in the ration supplemented with 4.5% CaS-soybean oil. In contrast, the lowest CPD was obtained in the treatment. The more CaS-Soybean oil supplemented positively increases the CPD of research rations based on fermented corn cobs combined with other local feed ingredients. Statistically, the mean CPD values of rations T4 and T5 were not significantly ($P>0.05$) different but higher than those of R2 and R3. The average CPD value of R3 was higher than that of R2 and R1.

CRUDE FIBER DIGESTIBILITY

The analysis of variance showed that the treatment of CaS-soybean oil supplementation at different levels in rations based on fermented corn cobs combined with local feed ingredients had a very significant effect ($P<0.01$) on the average crude fiber digestibility of rations. These results have a similar pattern to that of crude protein digestibility. The results of the DMRT further test showed that the

Table 3: Fermentation characteristics and in vitro digestibility of rations based on agricultural by-product feedstuffs with goat inoculum source.

Fermentation Characteristic	Treatment				
	R1	R2	R3	R4	R5
pH	6.78±0.10	6.85±0.06	6.78±0.10	6.75±0.06	6.80±0.12
NH ₃ -N (mMOL)	11.26 ± 0.10 ^a	13.11±1.41 ^{ab}	14.39±3.48 ^{bc}	15.10±0.78 ^{bc}	16.94±0.49 ^{cd}
Total VFA (mMol)	150.86±5.60 ^a	164.51±10.02 ^b	166.03±10.66 ^b	169.89±4.34 ^b	174.49±8.1 ^b

Note: Different superscripts in the same row indicate significantly different treatment ($P<0.01$). R1 (60% concentrate + 40% forage grass without CaS-soybean oil), R2 (60% concentrate + 40% forage grass containing 1.5% CaS-soybean oil), R3 (60% concentrate + 40% forage grass containing 2.5% CaS-soybean oil), R4 (60% concentrate + 40% forage grass containing 3.5% CaS-soybean oil), R5 (60% concentrate + 40% forage grass containing 4.5% CaS-soybean oil).

Table 4: Nutrient digestibility of corn cob-based rations supplemented with CaS-soybean oil at different levels

Nutrients Digestibility	Treatments				
	R1	R2	R3	R4	R5
DMD	57.72±1.75 ^a	63.32±1.04 ^b	63.85±3.24 ^b	65.31±1.24 ^b	65.76±1.27 ^b
OMD	76.21±2.05 ^a	82.63±1.00 ^b	82.90±3.43 ^b	84.58±1.58 ^b	85.33±1.70 ^b
CPD	52.03±0.80 ^a	54.48±1.37 ^b	64.03±1.81 ^c	71.35±1.58 ^{cd}	77.11±1.17 ^c
CFD	52.16±0.82 ^a	58.13±2.50 ^b	59.97±1.03 ^{bc}	61.08±0.44 ^c	64.98±0.63 ^d

Note: Different superscripts in the same row indicate significantly different treatment ($P<0.01$). R1 (60% concentrate + 40% forage grass without CaS-soybean oil), R2 (60% concentrate + 40% forage grass containing 1.5% CaS-soybean oil), R3 (60% concentrate + 40% forage grass containing 2.5% CaS-soybean oil), R4 (ration (60% concentrate + 40% forage grass containing 3.5% CaS-soybean oil), R5 (60% concentrate + 40% forage grass containing 4.5% CaS-soybean oil). DMD: Dry matter digestibility; OMD: Organic matter digestibility; CPD: Crude protein digestibility; CFD: Crude fiber digestibility

highest average value of CFD digestibility was obtained in the treatment ration supplemented with 4.5% CaS-soybean oil, and the lowest CFD was obtained in ration R1 (without CaS-soybean oil supplementation). The average CFD digestibility of the T4 ratio was higher than that of R1 and R2 but not different from that of the R3 ratio. The positive effect of CaS-soybean oil supplementation was also observed in the average crude fiber digestibility of the rations in this study. The ration based on fermented corn cob without CaS-soybean oil supplementation produced the lowest CFD performance in this study.

DISCUSSION

FERMENTATION CHARACTERISTICS

Fermentation characteristics, including pH level, NH₃-N, and VFA production (total VFA and individual VFA), are essential in creating a fermentation ecosystem for optimal growth and activity of microorganisms in digesting feed nutrients in vitro and in vivo. McDonald et al. (2010) argued that the level of fermentation in the rumen could take place generally if the conditions of the rumen ecosystem have a temperature, pH, N-NH₃-N concentration, and total VFA levels between 38 - 42 °C, 6.0 - 7.0, 6 - 21 mM and, 70 - 150 mM, respectively. The data showed that different ration treatments in the study had relatively similar pH values around 6.75-6.85, an average pH level to support the fermentation process in the rumen. The creation

of neutral pH in vitro incubation in all treatments indicates that CaS-soybean oil supplementation at the level of 1.5% to 4.5% in rations based on fermented corn cobs and other agricultural by-product feedstuffs has a positive impact in creating an ideal incubation ecosystem for growth and in vitro fermentation activity.

Franzolin and Dehority (2010) reported that pH concentration was essential in preserving rumen stability and microbial growth. In addition, pH stability under neutral pH conditions can support the optimization of solid binding of calcium salts-long-chain unsaturated fatty acids so that PUFA compounds are not lysed in the fermentation ecosystem, which can have toxic effects (damage cell integrity) on microorganisms (Maia et al., 2010) during the in vitro incubation process.

Fat inhibition of microbial growth can occur through direct inhibition and or coating activity of unsaturated fatty acids on microbes (Yang et al., 2009). The uninterrupted growth of microorganisms has implications for the good fermentative activity of microorganisms in degrading feed protein, which is useful in supporting microbial protein synthesis (Karsli and Russell, 2002). The positive indication was also shown by the concentration of NH₃-N (13.11 mM-16.94 mM). Even the NH₃-N levels produced in the study increased with the increase of CaS-soybean level (1.5% - 4.5%). The resulting NH₃-N levels were higher than those reported by Bain et al. (2018), which were

The resulting $\text{NH}_3\text{-N}$ concentration reached 16.94 mM, and treatment R5 was an excellent $\text{NH}_3\text{-N}$ concentration (McDonald et al., 2010) to support the growth of microorganisms, which ranges from 85 - 300 mg/l or equivalent to 6-21 mM. The optimal availability of $\text{NH}_3\text{-N}$ in this in vitro process is an essential product as a nitrogen source in microbial body protein synthesis (Bunglavan and Dutta, 2013). The positive impact of using fermented corn cobs combined with agricultural by-products as an energy source feed supplemented with different levels of calcium soap is also demonstrated by the excellent average total VFA production, which ranges from 164.51 mM to 174.49 mM.

The rumen pH level remains neutral, and the ideal $\text{NH}_3\text{-N}$ level, along with the availability of energy from the feed substrate in this study's ration, subsequently stimulates high VFA production by rumen microbial fermentation. The total VFA levels achieved in this study are even higher than those reported by McDonald et al. (2010), which stated that the optimal total VFA level to support anaerobic microbial fermentation in degrading feed ranges from 70-150 mM. VFA products, consisting of acetic, propionic, and butyric acids, are a carbon skeleton source for forming microbial body carbon structures (Bunglavan and Dutta, 2013). Hvelplund (1991) reported that VFA fermentation products provide energy and carbon for the growth and maintenance of the bacterial community.

This phenomenon also indicates that PUFA protection in CaS-soybean oil is adequate in binding PUFA, thus preventing its lysis in the incubation medium. This further implies suppressing the toxic effects of PUFA on microbes (Block et al., 2005) and the anaerobic fermentation ecosystem in vitro. This phenomenon can be understood because the PUFA from vegetable oil released from the calcium mineral bond during the in vitro fermentation process can disrupt cation concentration due to PUFA's ability to bind cations, affecting rumen pH levels (Wina and Susana, 2013). PUFA protection in the form of calcium soap can enhance energy digestibility, maintain normal rumen fermentation, mix easily with other feed ingredients, contribute calcium minerals, and increase PUFA content in the meat and milk of ruminants (Lounglawan et al., 2008). Although calcium soap technology has proven quite effective in binding PUFA, some PUFA compounds inevitably lyse and release in the incubation medium during the in vitro process. This is because the quality and nutritional properties of calcium soap fatty acid products are greatly influenced by the manufacturing method and storage before use (Block et al., 2005).

The lysis of some PUFA from calcium ion bonds contributes to the total VFA production originating from glycerol, derived from PUFA fatty acids degraded by microorganisms (Suharti et al., 2015). Total VFA concentration can be increased by administration of linolenic acid, malic-linolenic acid, and fumaric-linolenic acid (Li et al., 2009). The study results align with Bhatt et al. (2013), which reported that adding 4% rice bran oil in the form of calcium soap in vivo significantly increased total VFA production. Additionally, the contribution of calcium minerals in calcium soap products still lysed during the in vitro incubation process plays a role in the synthesis and stability of microbial cell wall structure and can activate various microbial enzymes such as α -amylase, which is needed by microbes to digest cellulose (Ruckebusch and Thivend, 1980). The increased digestibility of feed fiber fractions subsequently implies an increase in total VFA production.

NUTRIENT DIGESTIBILITY

Nutrient digestibility of the ration describes the utility value of all nutrient components contained in the ration, such as carbohydrates, fats, proteins, and minerals, when the ration is given to livestock. The higher the percentage of dry matter digestibility indicates the greater the amount of nutrient compounds used for various functional benefits of feed nutrition when given to livestock (Suharti et al., 2018). The dry matter digestibility of rations based on fermented corn cobs and other by-products supplemented with calcium soap is higher (63.32% - 65.76%) than rations without calcium soap supplementation. The high dry matter digestibility in the study is likely due to the ideal in vitro fermentation characteristics (pH, $\text{NH}_3\text{-N}$ concentration, and VFA) that support microorganisms (bacteria) in degrading feed nutrient components, especially fibre components.

This condition is closely related to the effectiveness of long-chain unsaturated fatty acid protection in the form of calcium soap products, which do not interfere with the growth and activity of microorganisms in degrading feed fibers. Block et al. (2005) reported that the stable complex of fatty acids and calcium ions that do not decompose in the rumen will prevent unsaturated fatty acids from inhibiting the growth and activity of microbes in degrading feed substrates. The positive indication of the effectiveness of calcium soap products in protecting against the adverse effects of PUFA is also reflected in the study's high organic matter digestibility values, which are more than 80%.

The high organic matter digestibility value aligns with the high dry matter digestibility results. This can be understood because the organic matter components (carbohydrates, proteins, and fats) are the main components of dry matter, so the high digestibility of these organic nutrient

attributes will simultaneously impact the digestibility of dry matter and organic matter. Dewi et al. (2020) reported that organic matter is a component of dry matter, so the increase in organic matter digestibility is in line with the increase in dry matter digestibility.

The positive impact of CaS-soybean oil supplementation on the ideal fermentation characteristics in vitro was also very significant ($P < 0.01$) on the crude protein digestibility of the ration. Crude protein digestibility increased with the level of supplementation. The highest CPD value was obtained with 4.5% CaS-soybean oil supplementation (R5) and the lowest in the ration without CaS-soybean oil (R1). This phenomenon is supported by the high $\text{NH}_3\text{-N}$ level data in the R5 treatment (16.94 mM). This research's significantly ($P < 0.01$) increased crude protein digestibility differs from Behan et al. (2019). However, the high crude protein digestibility with calcium soap supplementation in this study confirms the findings of Schauff and Clark (1992) that there was an increase in CP digestibility with supplementation of the calcium salt of long-chain fatty acid to dairy animals.

The increase in $\text{NH}_3\text{-N}$ concentration with increasing levels of calcium soap depicts the activity and level of feed protein degradation by microbes during in vitro incubation. $\text{NH}_3\text{-N}$ compounds are the end products of feed protein degradation by microorganisms. The high $\text{NH}_3\text{-N}$ concentration also allows for increased microbial protein synthesis in the rumen system, as ammonia is the primary precursor (N source) in the formation of microbial cells (Suharti et al., 2015). Optimal microbial growth can positively affect the availability of protein for various functional uses in the ruminant's body, such as protein turnover originating from dead microorganisms in the digestive tract in the rumen-reticulum. This study's high crude protein digestibility does not align with Behan et al. (2019). However, quantitatively, the crude protein digestibility of rations supplemented with calcium tends to be higher than the control rations in Dorper sheep.

The significant impact of CaS-soybean oil supplementation in rations based on fermented corn cobs combined with agricultural by-products is also observed in crude fiber digestibility. Data shows that crude fiber digestibility increases with the higher levels of CaS-soybean oil in the ration. The crude fiber digestibility of the ration is relatively high at the 4.5% CaS-soybean oil level, followed by the P4 treatment (3.5% CaS-soybean oil). The lowest crude fiber digestibility occurs in rations without CaS-soybean oil supplementation.

The good crude fiber digestibility with the addition of CaS-soybean oil is an implication of the effectiveness of

PUFA protection, ensuring it does not disrupt the in vitro incubation medium ecosystem, which is crucial for supporting the growth and activity of microbes in degrading feed nutrients, especially crude fiber. This is indicated by the very conducive and ideal fermentation attributes (pH, $\text{NH}_3\text{-N}$, and total VFA levels) for continuing the anaerobic fermentation process. This is in line with the use of fatty acid calcium soap, which is to ensure PUFA does not interfere with rumen fermentation (Jenkins and Palmquist, 1984), is not toxic to rumen bacteria, and prevents a decrease in crude fiber digestibility (Palmquist et al., 1986). These positive findings are also confirmed by Behan et al. (2019), who reported that basal diet supplementation plus calcium soap (calcium salts of palm fatty acids) tends to result in higher crude fiber digestibility (54.72%) compared to control rations (without calcium soap), which only showed 43.04%.

The high crude fiber digestibility phenomenon in this study is also an implication of the effective fermentation process of corn cobs (a high-fiber feed ingredient) for microbes to break down the lignin-cellulose structure, making it easier for anaerobic microbes to decompose cellulose and hemicellulose compounds to produce VFA products as an essential energy source for microbial growth. This is shown by the total VFA production in rations supplemented with CaS-soybean oil, which falls within the optimal range of 164.51 mM to 174.49 mM. Fatty acid calcium soap is a chemical protection method for PUFA that withstands rumen digestion and can mitigate the adverse effects of PUFA fatty acids to prevent interference with the rumen fermentation process (Block et al., 2005).

PUFA protection in the form of calcium soap can enhance energy digestibility, maintain normal rumen fermentation, mix easily with other feed ingredients, contribute calcium minerals, and increase PUFA content in the meat and milk of ruminants (Lounglawan et al., 2008). The availability of VFA as a carbon skeleton source for the microbial body and $\text{NH}_3\text{-N}$ as a nitrogen source for microbial protein synthesis (Bunglavan and Dutta, 2013), along with the contribution of Ca minerals from calcium soap products lysed during the incubation process, are essential attributes that support high crude fiber digestibility in this study. The optimal availability of $\text{NH}_3\text{-N}$ in this in vitro process is an important product as a nitrogen source in the microbial protein synthesis process.

CONCLUSION

The results showed that the use of fermented corn cobs in rations and other agricultural by-products supplemented with CaS-soybean oil at different levels significantly affected the variables of fermentation characteristics and ration

nutrient digestibility. Supplementation of CaS-soybean oil at a level range of 1.5%-4.5% of ration dry matter produced optimal fermentation characteristics (pH, NH₃-N and total VFA) to support the growth and activity of microorganisms to digest ration nutrients optimally during in vitro incubation. Supplementation of 4.5% CaS-soybean oil in fermented corn cob-based rations resulted in the best NH₃-N levels and total VFA production, followed by the highest nutrient digestibility (DMD, OMD, CPD and CFD) compared to the other treatment. The study concluded that rations based on fermented corn cobs and other agricultural by-products supplemented with 4.5% CaS-soybean oil produced ideal fermentation characteristics to support optimal growth and activity of microorganisms digesting feed nutrients.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article, either financially or organizationally.

NOVELTY STATEMENT

This recent study presents a novel feed ration for Etawa crossbreed goats based on a combination of fermented corn cob and soybean oil calcium soap. The feed ration has better nutrient quality and digestibility than conventional feed ingredients. The use of corn cob, a widely available and inexpensive agricultural by-product, also signifies the importance of this feed ration in reducing the dependency of goat farming on commercial feed ingredients.

AUTHORS CONTRIBUTION

Conceptualization, A.B., M., N.A.S.; methodology, A.B., M., L.O.M., N.S.A., A.N., W.K.; validation, A.B., M., L.O.N., N.S.A.; statistical analysis, A.B. and L.O.N.; writ-

ing—original draft preparation, A.B., M., L.O.N., N.S.A.; investigation, A.B., M., L.O.M., N.S.A., F.A.A.; preparation of manuscript, A.B., M., L.O.N., N.S.A.; editing and revision, A.B., M., L.O.M., N.S.A., D.Z; supervision, A.B., L.O.N., N.S.A. All authors have read and agreed to the published version of the manuscript.

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