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In Vitro Goat Fermentation of PUFA-Diet Supplemented with Yeast and *Curcuma xanthorrhiza* Roxb

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ABSTRACT

This *in vitro* experiment was conducted to evaluate the ruminal performances of polyunsaturated fatty acid (PUFA)-diet (containing PUFA with 80% concentrate and 20% King grass) supplemented with yeast and *C. xanthorrhiza* Roxb. Experimental design was completely randomized block design of 4 x 4 with ruminal liquor derived from 4 slaughtered goats and 4 treatments (PD0-no supplement, PDY- 0.5% yeast, PDC-2% curcuma, and PDM- 0.5% yeast + 2% curcuma). Variables measured were pH, N-NH_3, total and partial VFA (volatile fatty acid), protozoa population, and CH_4 (methane). Results showed that the lowest ($P<0.05$) organic (59.63%) and dry matter (58.00%) digestibilities were found in PDM. In *in vitro*, this diet was also showing quantitatively low in N-NH_3 (8.73 mM) and protozoa population (7.90±4.09 10^3 cfu/mL). On the other hand, it showed numerically high in VFA production (45.27 mM) and pH (6.74), yet low in CH_4 (13.43% v/v). Based on these data, PDM was considered the most potential diet to improve nutrient metabolism in rumen of goat, *in vitro*.

Key words: PUFA- diet, yeast, curcuma, in vitro fermentation

ABSTRAK

Penelitian *in vitro* ini dilakukan untuk mengevaluasi performans pakan mengandung *polyunsaturated fatty acid* (PUFA)- (terdiri atas 80% PUFA-konsentrat dan 20% rumput raja) yang disuplementasi ragi dan *C. xanthorrhiza* Roxb. Desain eksperimen yang digunakan adalah rancangan acak kelompok 4 x 4, yang terdiri atas 4 macam cairan rumen yang berasal dari 4 ekor kambing yang dipotong dan 4 perlakuan (PD0-tanpa suplemen, PDY- 0,5% ragi, PDC-2% curcuma, dan PDM- 0,5% ragi + 2% curcuma). Variabel yang dianalisis adalah pH, N-NH_3, VFA (volatile fatty acid) total dan parsial, populasi protozoa, dan CH_4 (metan). Kandungan PUFA pada PDM tampak tinggi (17,59%) diantara diet lainnya. Hasil penelitian menunjukkan bahwa PDM signifikan ($P<0,05$) menurunkan kecernaan bahan organik (59,63%) dan bahan kering (58,00%). Hasil *in vitro* menunjukkan bahwa ransum ini secara kuantitatif rendah N-NH_3 (8,73 mM) dan populasi protozoa (7,90±4,09 10^3 cfu/ml). Namun demikian, PDM secara kuantitatif menghasilkan VFA yang tinggi (45,27 mM) dan rendah CH_4 (13,43% v/v). Berdasarkan data ini, ransum PDM dapat dipertimbangkan sebagai pakan yang potensial untuk memperbaiki metabolisme nutrien dalam rumen kambing secara *in vitro*.

Kata kunci: PUFA- diet, ragi, curcuma, fermentasi in vitro

INTRODUCTION

Ruminal fermentation performance is affected mostly by diet being ingested. Diet containing high concentrate with PUFA may increase production, improve milk fat and milk fatty acid in ruminants through microbial hydrogenation of PUFA in the rumen. However, to make such improvements, it needs any bioadditives, such as yeast and curcuma to be supplemented into diet that would determine the kinetics of rumen fermentation.

Yeast has been known as a rumen enhancer that improves digestion and nutrient metabolism. *In vitro* fermentation of diet added with Diamond- V XP yeast (XPY) showed higher dry matter digestibility (DMD),

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organic matter digestibility (OMD), and total VFA than those of diet with A-Max yeast with the same dose (57 g/d) (Miller *et al.,* 2002). Feeding XPY ruminally in 56 g/d (to the concentration of 6 10^{11} cfu/steer/d) also tended to increase OMD and acid detergent fiber (ADF) (Lehloenya *et al*., 2008). Supplementation of *Saccharomyces cereviseae* culture (1.16 10^4 cfu/g) of 0.35g/L produced higher concentration of acetate (C_2) than that of higher dose of 0.73g/L (Lynch & Martin, 2002). Results of meta-analysis on the influence of *S. cerevisiae* supplementation were variable; however, in average, it increased rumen pH and VFA but did not affect acetate/propionate ratio (Desnoyers *et al.,* 2009). In transition dairy cows, feeding 50 g/d of *S. erevisiae* fermentation product (Diamond V Original XP) can improve feed intake and milk production than that of no supplement as reported by Zaworski *et al*. (2014). Besides yeast as a supplement, some herbals containing bio actives such as tannin and curcumin could affect ruminal dynamics.

Tannin derived from *Peltiphyllum peltatum* was also utilized to manipulate rumen by decreasing protein degradability in the rumen and make it available in small intestine (Jayanegara *et al*., 2010). Tannin extract could decrease protozoal population, methane (CH_4) gas production, NH_3, and VFA; on the other hand, it increased bacterial population in the rumen of dairy cow (Khiaosa- ard *et al.,* 2009). Plants containing both hydrolyzable and condensed tannins were reported to be more effective in suppressing methanogenesis than those containing either one (Bhatta *et al*., 2009). Tannin (13.84%) extracted from Pakar leaves (*Ficus infectoria*) was added into diet fed for goat and revealed some higher levels of N-NH_3, acetate, and total fungi with lower levels of total VFA and propionate (Singh *et al*., 2011).

Curcumin found in *C. xanthorrhiza* Roxb was reported to have a potential as an antibacterial for certain strain (Wiryawan *et al*., 2005). It was also reported that in early identification, curcumin and xanthorrhizol showed very significant activities as antibacterial and effectively slowed down the growth of *Staphylococcus aureus*, *Salmonella paratyphi*, *Trichophyton gypseum*, and *Mycobacterium tuberculosis* (Benson, 2012). *Curcuma xanthorrhiza* Roxb supplemented for 1.5% in PUFA-concentrate was apparently interacted with the sources of the PUFA (corn oil or roasted ground corn) in decreasing dry matter and organic matter digestions as well as total digestible nutrient (TDN) in dairy cow (Sulistyowati *et al.,* 2010). Therefore, tannin, curcumin, and yeast that were supplemented into PUFA-diet were expected to work synergistically in the rumen system.

Based on these data, a study on PUFA-diet supplemented with yeast and curcuma was conducted to observe *in vitro* rumen fermentation performance of goats.

MATERIALS AND METHODS

Preparation of Yeast, Curcuma Powder, and PUFA-Diet

Yeast, curcuma powder, and PUFA-diet were prepared according to protocol reported in the previous result (Sulistyowati *et al.,* 2013). The PUFA-diet was reformulated as it was in the PUFA-concentrate and then was combined with soybean by-product from local tofu industry. Ground corn was half roasted; while soy bean meal was completely roasted in 80 °C for about 20 min. These ingredients together with corn oil were designated as PUFA sources in the diets.

Basic PUFA-diet (PD) composed of rice bran (18.42%), ground corn (15.79%), soybean meal (7.89%), cassava meal (7.89%), soybean byproduct (27.49%), corn oil (2.11%), minerals (0.53%), and King grass (19.88%). Yeast and or *Curcuma* powder were then added to the diet as a treatment. The treatments were:

PD0 : PUFA-diet without supplementation
PDY : PUFA-diet with 0.5% yeast supplementation
PDC : PUFA-diet with 2% curcuma powder supplementation
PDM : PUFA-diet with 0.5% yeast and 2% curcuma powder supplementations

Chemical Analyses

Nutrient contents were assessed based on proximate analysis (AOAC, 1990); ADF (acid detergent fiber) was analyzed according to Van Soest (1990). Curcumin was analyzed by using HPLC separation performed on a C_{18} column by using three solvents i.e., methanol, 2% AcOH, and acetonitrile, with detection at 425 nm (Jayaprakasha *et al*., 2002). Tannin was analyzed by modification of Folin-Ciocalteu method (Harborne, 1987). Fatty acid content was detected as fatty acid methyl esters (FAME) by using gas chromatography (GC) (Shimadzu 2010 series), after extraction of diet fat samples according to the method conducted by Palmquist & Jenkins (2003). Individual fatty acid was quantified by using specification of column (SP™ -2560, 100 m x 0.25 mm ID, 0.2 μm film), gas carrier (helium, 20 cm/s), oven temperature (140 °C in 5 min to 240 °C at 4 °C/min), detector (FID, 260°C), and inject (1 μL, 2600C, split 100:1) and each was identified according to a mixed FAME standard (Supelco 37 component, Supelco Inc).

In vitro procedure was based on Tilley & Terry (1963). Fermentor tubes were loaded with 0.5 g of samples and added with 40 mL of Mc. Dougall solution. Tubes were put into shaker bath in 39°C and added with 10 mL rumen liquor of slaughtered goats. Tubes were soaked and flowed with CO_2 for 30 s and the pH was checked (6.5-6.9) by using pH meter. The tubes were then probed with ventilated rubber caps and fermented for 48 h. When fermentation was stopped at 4 h, the caps were taken off from the tubes, and 2-3 drops of $HgCl_2$ was added to stop microbial growth (at this point, analyses were conducted for protozoal population and CH_4 production). Fermenter tubes were then centrifuged at 4.000 rpm for 10 min. Substrates were separated as solid in the bottom and supernatant on the top. The supernatant was used for VFA, and N-NH_3 analyses, while the solid was then added with 50 mL pepsin-HCl 0.2%, centrifuged in 4.000 rpm for 15 min. This substrate was then reincubated for 48 h without probes; and then strained through Whatman paper no 41 (the weight was recorded) by using vacuum pump. The solid left in the paper was transferred into baker glass, put in oven in 105 ^oC for 24 h. The glass, paper, and residue were taken out from the oven, and put in exicator before weighing to get dry matter content. Afterward, these samples were fired in electrical oven for 6 h in 450-600 ^oC. Then the samples were weighed to get their dry and organic matters to calculate the *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD). Blanco was prepared from the residue of the fermentation without sample. The digestibility was calculated as if there was a hydrolytic post ruminal fermentation after two timed 24 h (Tilley & Terry, 1963).

Analysis of N-NH_3 was conducted by using micro diffusion Conway. Analysis of VFA in partial containing of acetate (C_2), propionate (C_3), isopropionate, butyrate (C_4), isobutyrate, valerate, and isovalerate were detected by using gas chromatography (GC) (Chromopack 9002) with centrifuge (IEC micromac RF type 3593), column capillary WCOT fused silica ID coating FFAP-CB. Methane (CH_4) was measured by using GC Shimadzu 8-APT detector TCD and C- R6A Chromatopack.

Counting of protozoal population was done by adding 0.5 mL methyl formalin saline (MFS) into the reaction tubes then mixing it with rumen liquor and homogenized. Sample was dropped onto hematocytometer and covered it with glass securely. Protozoa population was evaluated under microscope by using 40x objective lense with 10x ocular lense.

Experimental Design and Data Analysis

Experimental design being applied was completely randomized block design with 4 ruminal liquors derived from four slaughtered goats and 4 treatments (PD0, PDY, PDC, PDM), in 3 replications. Data were tabulated and analysis of variance (Anova) was conducted, any significant differences were then tested by using Duncan Multiple Range Test (DMRT) according to Lentner & Bishop (1986).

RESULTS AND DISCUSSION

Chemical Composition of Diets

Average content of protein in these diets was 12.96±0.23% (Table 1). This level was fulfilled the protein requirement of 12% for 30 kg dairy goat with 1 kg milk production (NRC, 1981). Other result showed that protein content in diet with extruded soybean in dairy goat was much higher i.e., 18.5±0.38% or 42.75% higher (Schmidely *et al*., 2005). In quantity, PDM, diet with a mixture of yeast and curcuma, contained higher organic matter, lower ADF, and higher fat than other diets. This result strongly approved that these two additives could improve nutrient content.

Diet with high concentrate in this experiment (80%) could decrease ether extract of the diet that eventually would decrease milk fat. However, since this research used fat sources (roasted ground corn, roasted soy bean meal, and corn oil), the ether extract of these diets were relatively high (4.0%-4.43%), except in control diet (3.75%). This result was in accordance with others reports that diet without extruded soybean contained very low fat (1.38%) while the one with that ingredient showed a high fat content (5.19%) (Schmidely *et al*., 2005). Diet with corn oil was reported to have higher fat i.e., 5.62% (Bouattour *et al*., 2008). It seemed that bioactives, curcumin and tannin did not affect the fat content of the diets; however, these supplements did affect fatty acid content in dairy goat milk (Sulistyowati *et al*., 2013).

Fatty Acid Content in PUFA-Diets

As can be accessed in Sulistyowati *et al.* (2013), diets in all treatments were not significantly different. However, diet with a mixed additives (PDM) quantitatively had high PUFA (17.59%), high long chain fatty acid (LCFA) (40.7%), low unsaturated fatty acid (UFA) (81.18%), and low UFA/SFA ratio (4.31). However, curcuma supplementation in diet (PDC) numerically decreased monounsaturated fatty acid (MUFA) (11.88%) and low atherogenicity (0.87).

It has been known that curcumin plays a role in disturbing lipid peroxidation, when the process of demethylation finished, consequently its function as antioxidant was over as well. It means that, curcumin will be functioning depends on the availability of the substrate that is going to be catabolized. It was reported that curcuminoid as much as 0.0133 mg/mL could slow down peroxidation of linoleic acid (Tonnesen & Karlsen, 1988). The implication of the result in this research was that curcumin in *C. xanthorrhiza* Roxb functioned much stronger in fatty acid reduction when it was singly. In contrast, its effect would be weaker when it was combined with yeast. This result might be due to the level of curcumin that was not high enough to catabolize the increasing substrate resulted from yeast fermentation in the diet, when both supplements were combined.

Short chain fatty acids (C10 and C11) were not detected in control, yeast, and curcuma diets; while very little level was shown in the mixed PUFA-diet. Long chain fatty acids (C18:0 to C18:3n3) were higher in both yeast (PDY) and yeast + curcuma diet (PDM). On the other hand, these fatty acids were reported high in diet with supplementation of forage tannin, tannin extract,

Table 1. Nutrient contents of PUFA-diet supplemented with yeast and *C. xanthorrhiza* Roxb fermented *in vitro* in goat rumen liquor

Nutrient	PD0	PDY	PDC	PDM
Dry matter (%)	93.14	93.07	94.42	94.46
Organic matter (%)	84.54	85.97	86.81	88.27
Protein (%)	12.86	13.11	13.18	12.67
Ether extract (%)	3.75	4.20	4.43	4.00
Crude fiber (%)	27.32	27.69	26.93	27.74
ADF (%)	54.68	50.77	52.78	43.20
Gross energy (Mcal/kg)	3.84	3.86	3.83	3.90
Tannin (%)	0.487	0.492	0.491	0.491
Curcumin (%)	-	-	0.13	0.11

Note: PD0: PUFA-diet with no additives; PDY: PUFA-diet with 0.5% yeast; PDC: PUFA- diet with 2% curcuma; PDM: PUFA-diet with a mixture of 0.5% yeast and 2% curcuma.

or saponin extract i.e., about 83.72% (Khiaosa-Ard *et al*., 2009). Ruminant diet with high linoleic acid will be transformed into rumenic acid (cis-9 trans-11 18:2) through ruminal biohydrogenation (Jenkins *et al*., 2008) that along the process will produce abundant amount of intermediates, such as vaccenic and stearic acids (Palmquist *et al*., 2005). Biohydrogenation involving rumen microbes depends upon the type of diet. Lipid supplementation stimulated bacteria from the family of Lachnospiraceae (Belenguer *et al*., 2010).

Fermentation Characteristics

In vitro fermentation characteristics (Table 2) of diet containing – PUFA concentrate, yeast, curcuma, and a mixture of these additives in goat rumen liquor were mostly not different significantly, except in IVDM and IVOM. Both digestibilities of diet without additives (PD0) were significantly higher than those of PDM; while the yeast (PDY) or curcuma (PDC) diet was remained the same. Other results showed that IVDMD (53.8%-56.9%) and total VFA (43.8- 59.5 mM) with PMX70SBK live cells yeast decreased with the increasing yeast level from 0.37 to 0.73g/l in rumen liquor of young male cattle fed alfalfa hay (Lynch & Martin, 2002). Supplementation of *S. cereviseae*[1026] significantly decreased N-NH_3, yet increased protozoa population (Al-Ibrahim *et al*., 2010). Lower ruminal N-NH_3 with yeast supplementation were also found in other reports (Marden *et al*., 2008; Moallem *et al*., 2009).

The A/P ratios in these diets (3.54-3.88) were higher than that of with the same high concentrates (2.08-2.31) in Rusitec fermentors as reported by Martinez *et al*. (2010). It was known that the type of diet will determine the production of propionate and acetate. Carbohydrate produces higher propionate, while fiber will produce higher acetate. The A/P ratio increased with the longer fermentation, suggesting that the more structural carbohydrate was degraded, therefore, the higher acetate and lower propionate production.

Tannin contents in all PUFA-diets were relatively the same (0.487%-0.491%). Tannin and curcumin added in PDM yielded the highest total VFA (45.27 mM) and the lowest isobutyrate (0.66%). A different result showed that tannin (13.84%) from Pakar leaves (*Ficus infectoria*) fed to goats showed some lower variables, such as in total VFA (4mM) and propionate (11.86%), but higher in N-NH3 (5.29 mM), acetate (83.56%), and total fungi (28.16%) than those found in feed lower in tannin (8.6%) (Singh *et al*., 2011). Increasing doses of essential oil (0 to 5000 mg/L) such as limonene were reported to decrease total VFA, acetate and A/P in in vitro system (Castillejos *et al*., 2006).

Concentration of N-NH_3 did not significantly decrease from 11.26 mM in PD0 to 8.73 mM in PDM. This level of NH_3 was much higher than that of NH_3 (8.5-9.6 mM) with pH of around 6.7, in goat fed with extruded soybean with or without $NaHCO_3$ (Schmidely *et al*., 2005). The N-NH_3 concentration in dairy cow fed diet containing tannin extract was detected to reach the lowest level (5.1 mM) as compared to that of control diet (17.2 mM) with pH around 7.05-7.09 (Khiaosa- Ard *et al*., 2009). Decreased N-NH_3 was also detected in *in vitro* system with essential oils (limonene, guaiacol, thymol, and eugenol), suggesting that deamination of amino acid was disrupted (Castillejos *et al*., 2006).

Protozoa Population

Protozoa population (Table 3) was not significantly low in PDM (7.90 10^3 cfu/mL), compared to control diet (PD0), that was (32. 10^3 cfu/mL). These results suggested that yeast or curcuma or their combination worked well in protecting the PUFA-diet by suppressing the growth of protozoa that eventually, otherwise will increase biohydrogenation of PUFA sources. The PUFA protection of feed source means to elevate ruminal bypass of α-linolenic acid without being modified by microbial biohydrogenation (Khiaosa-Ard *et al*., 2009). *In vitro* bacteria and protozoa counts were significantly higher in goats than that of in sheep. However, fungi population was higher in sheep and fungi existence in rumen system was crucial in digesting dry matter as described by Li & Hou (2007). Supplementation

Table 2. *In vitro* dry matter and organic matter digestibility (IVDMD and IVOMD) and fermentation characteristics of PUFA-diet supplemented with yeast and *C. xanthorrhiza* Roxb in goat rumen liquor

Variables	PD0	PDY	PDC	PDM
pH	6.71±0.02	6.73± 0.07	6.73± 0.05	6.74± 0.04
IVDMD (%)	62.72±1.66^{a}	60.26± 1.90^{ab}	60.77± 1.63^{a}	58.00± 1.05^{b}
IVOMD (%)	64.85±2.22^{a}	61.74± 2.07^{bc}	62.82± 1.36^{ab}	59.63± 0.59^{c}
NH3 (mM)	11.26±3.82	10.28± 3.82	9.31± 3.75	8.73± 3.16
VFA total (mM)	42.13±3.45	43.95±11.09	44.82±12.30	45.27±12.92
Acetate (A), %VFA	67.13±6.39	67.68± 4.43	68.82± 4.98	68.46± 5.73
Propionate (P), %VFA	19.78±4.24	19.35± 2.60	18.25± 3.20	18.64± 3.10
Butyrate (B), %VFA	11.33±2.90	11.21± 3.23	11.13± 2.85	11.05± 3.86
Iso Butyrate (B),%VFA	1.27±0.17	1.11± 0.24	1.08± 0.18	0.66± 0.58
Valerate (V), %VFA	1.76±0.66	1.76± 0.81	1.80± 0.79	1.86± 0.85
IsoValerate , %VFA	2.66±0.30	2.48± 0.58	2.46± 0.48	2.08± 0.87
A:P	3.54±0.97	3.55± 0.55	3.88± 0.82	3.76± 0.77

Note: Means in the same row with different superscripts differ significantly ($P<0.05$). PD0: PUFA-diet with no additives; PDY: PUFA-diet with 0.5% yeast; PDC: PUFA- diet with 2% curcuma; PDM: PUFA-diet with a mixture of 0.5% yeast and 2% curcuma.

Table 3. Protozoa and methane gas production of PUFA-diet supplemented with yeast and *C. xanthorrhiza* Roxb in goat rumen liquor fermentation *in vitro*

Variables	PD0	PDY	PDC	PDM
Protozoa (10^3 cfu/mL)	32.00 ±24.70	28.50 ±25.31	25.50 ±24.95	7.90 ±4.09
CH_4 (% v/v)	12.90 ± 1.25	12.49 ± 1.33	14.20 ± 2.79	13.43 ±2.26
H_2	0.04 ± 0.02	0.06 ± 0.04	0.14 ± 0.17	0.04 ±0.02
O_2	0.006± 0.00	0.006± 0.00	0.006± 0.00	0.006±0.00
CO_2	87.07 ± 1.24	87.45 ± 1.30	85.67 ± 2.82	86.51 ±2.22

Note: PD0: PUFA-diet with no additives; PDY: PUFA-diet with 0.5% yeast; PDC: PUFA- diet with 2% curcuma; PDM: PUFA-diet with a mixture of 0.5% yeast and 2% curcuma.

of *Hibiscus tiliaceus* leaves (saponin source) for 5% significantly decreased rumen protozoa population (4.50 10^3 cfu/mL), compared to control (16.25 10^3 cfu/mL) as reported by Istiqomah *et al*. (2011).

Protozoa population in this research was affected by tannin. Addition of tannin extracted from *Acacia mearnsii* was effectively decreased protozoa population to a very low level (1.91 10^3 cfu/mL) and the protozoa population could be higher when the additive was not in the form of extract (Khiaosa-Ard *et al*., 2009). Meanwhile, protozoa population in this research was lower than that found in diet added with saponin generated from *Yucca schidigera* (6.17 10^5 cfu/mL) and *Quillaja saponaria* (6.17 10^5 cfu/mL) in rumen feremementation of dairy cow (Holtshausen *et al*., 2009). Total protozoa (*Entodinium* spp, *Isotrica* spp., *Dasytrica* spp., *Epidinium* spp., *Ophryoscolex* spp., and *Diplodinium* spp.) detected in rumen fermentation of dairy cow supplemented with yeast XP 56g/d was much higher (8.03 10^5 cfu/mL) (Hristov *et al.,* 2010). This result indicated that the type of plants or additives, the method of administration, and the right dose will determine the effectiveness of the bioactives during the rumen fermentation.

Gas production (CH_4) in a mix diet (PDM) and curcuma diet (PDC) was higher (around 13%-14%, respectively) as compared to PDY and PD0 diets (about 12%). This implied that supplementation of tannin and curcumin was not effective in reducing methane (CH_4) production in this study. On the other hand, Khiaosa-Ard *et al.* (2009) reported that tannin extract was able to decrease gas production (CH_4, CO_2, and H_2) to the lowest level (0.454 L/d). Decreased gas production was also found in diet containing silage and concentrate added with *Peltiphyllum peltatum* rich in phenol and tannin (Jayanegara *et al*., 2010). The use of rhubarb (milled rhizomes of *Rheum* spp.) in the diets of ruminants was also reported effectively to improve ruminal fermentation by reducing methane emission that eventually potentially benefits animal production and environment (Gonzales *et al*., 2010).

Production of methane gas was reported higher (10.3 g/d) with XP *yeast* supplementation diet than that in control (9.7 g/d) in dairy cow (Hristov *et al*., 2010). Methane productions with supplementations (curcuma and mix) were not significantly lower than that in control (12.90% v/v). This result was contradicted to the results that CH_4 emission (g/d) of lactating dairy cows fed dietary supplements (fat, fatty acid, and Ca) decreased 10%. However, it remained unchanged when it was correlated to milk production (Zijderveld *et al*., 2011). Emissions of daily CH_4 was also affected by the level of crude fiber in the diet; the increasing corn silage (0% to 27%), the higher the production of CH_4 (487 to 540 g/d) in lactating dairy cow (Benchaar *et al*., 2014). Methane gas was produced through a mechanism starting from anaerobic degradation; triglyceride hydrolysis, saturation of unsaturated fatty acid (C18:2→C18:1→C18:0→C16:0→C14:0), and successive β-oxidation of saturated fatty acid (C10-C18). All these reactions produced acetate that eventually reacted with H_2 to produce CH_4 (Sage *et al.,* 2008).

CONCLUSION

In spite of having the lowest organic and dry matter digestibilities as well as $N\text{-}NH_3$ production, as was supported by low protozoa population and high VFA production in the goat rumen fluid, the PUFA-diet with a mixture of yeast (0.5%) and curcuma (2%) additives was considered to be the most potential diet.

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