In vitro fermentation with inoculated goat rumen to evaluate the degradation of diets supplemented with three levels of protected palmitic acid

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Abstract

Few studies evaluate the impact of a lipid-rich diet with palmitic acid (PA) in ruminal fermentation. The objective was to evaluate the in vitro bath culture of the protected PA on the ruminal fermentative variables. Four diets were used: a) without protected PA (nPA), b) inclusion of protected PA at three levels: PA3 = 3%, PA6 = 6%, and PA9 = 9% dry matter (DM). The results indicated that protected PA decreased gas production ($p \leq 0.05$). DM and organic matter (OM) degradation after 72h of incubation linearly decreased ($p \leq 0.05$) with a high level of protected PA. Neutral and acid detergent fiber degradation had no significant differences between treatments ($p > 0.05$). PA concentration in ruminal uid had a linear effect with an increase from PA3 to PA9. Propionate had a quadratic effect ($p \leq 0.05$) from nPA to PA3, and total volatile fatty acids had a linear decrease ($p \leq 0.05$) from nPA to PA9. In conclusion, supplements with protected PA at 3 and 6% increased PA availability, and the results indicate good benefits of protected PA on fermentative variables. The doses of 3 and 6% of protected PA are recommended for use and evaluation in lactating goats.

Introduction

Supplements with various types of fat have been supplied to dairy cattle to increase the energy density of the diet, improve milk fat production, reduce heat stress, increase reproductive performance, and reduce methanogenesis by up to 15% (Setiawan et al. 2023). Although many studies indicate that added fat decreases fiber digestion, it mainly depends on the quality and quantity supplied. Supplementary fats in ruminant diets should not be greater than 4% because it reduces intestinal digestibility to 50%, and the other drawback is that lipids cover the forages and prevent the action of the ruminal microbiota from degrading the fiber. In general, supplemental fat contains saturated (SFA) and unsaturated fatty acids (UFA) (Enjalbert et al. 2000), and one of the most common strategies in livestock units is to supplement more polyunsaturated fatty acids in the diet. The purposes are to improve the quality of milk fat and reduce the intake of saturated fatty acids in people (Jenkins and Bridges 2007). However, milk fat is more than 60% saturated fat and has not been associated with cardiovascular diseases in healthy individuals (Chinnadurai et al. 2013).

Diets in lactating cows with more than 2% SFA cause fatty acids (FA) to be inert in the rumen and increase the production (Mosley et al. 2007; Wang et al. 2010) and yield of fat in milk (Lock et al. 2013). Dairy fat is composed of around 70–75% SFA, 20–26% monounsaturated FA, and 4–5% polyunsaturated FA (Jensen 2002; Toyes-Vargas et al. 2013). Therefore, greater interest has been generated in using fat supplements with individual FA to evaluate each FA unique effect on the animal response (De Souza and Lock 2019). At the same time, research on FA supplements in dairy goats is limited (Western et al. 2020). Palmitic acid (PA) supplementation in cows increased milk production and fat without affecting milk protein concentration (Steele 1969; Lock et al. 2013). Likewise, de Souza and Lock (2018) observed a 5% increase in NDF digestibility when a fat supplement enriched with 1.5% PA was administered to lactating Holstein cows.
A meta-analysis by Weld and Armentano (2017) also indicates that feeding a combination of palmitic and stearic increased the digestion of NDF. However, this effect still needs to be understood and requires more research. One possible reason is that PA is incorporated into bacterial membranes, saving ATP and favoring bacterial growth, increasing NDF digestibility. On the other hand, it is believed that there may be a loss of PA in the rumen, and this does not reach the intestine intact and reduces milk fat since around 60% of the total FA absorbed in the small intestine are transferred to the milk, with a positive correlation ($r = 0.65$) between palmitic and fat percentage (Stoop et al. 2008). Goats respond less to diet-induced milk fat content (Chilliard et al. 2014), and FA have been shown to increase milk fat secretion in lactating dairy goats with little impact on milk production (Chilliard et al. 2003). Other authors indicate that fat from the unprotected diet reduces forage consumption and decreases ruminal fermentation (Nam et al. 2014). Protected SFA can prevent energy loss and produce milk (Behan et al. 2019).

Few studies evaluate the impact of a lipid-rich diet with C16:0 on milk production and fat secretion in lactating dairy goats. No studies have evaluated the effects of pure PA supplementation on rumen digestion. Therefore, the objective was to propose an in vitro study where the protected PA is evaluated on the rumen fermentative variables. A bath culture test with goat ruminal uid was used to evaluate three increasing doses of protected PA on the degradability of nutrients in the diet and quantify the products generated from ruminal fermentation.

**Material and Methods**

**Animal ethics standards and localization**

The handling of the animals was carried out in accordance with the Regulations for the use and care of animals intended for research at the Postgraduate College, COBIAN 1009123. The study was carried out in the livestock program and Animal Nutrition Laboratory. This place is located 19° 29" north latitude, 98° 53" west longitude and an altitude of 2241 meters above sea level with the average annual temperature is 15.2°C (Garcia 2004).

**Preparation of treatments**

Four micro-diets were prepared with the following base ingredient: 30% corn grain, 24% corn stover, 12% sorghum grain, 11% alfalfa hay, 12% bran wheat, 8% molasses, 1% soybean meal, 0.2% urea, 1.8% mineral premix (NRC 2007). The PA used was from the brand WLT® (85%, Winnipeg CA) with particle size ranges from 0.3–2.5 mm and protected with Butylated Hydroxytoluene. Corn grain was replaced by the included PA levels and the treatments were distributed as follows: Control without protected PA (nPA), treatments with inclusion of protected PA at three levels: PA3 = 3%, PA6 = 6%, and PA9 = 9% DM.

**Culture medium, inoculation, in vitro gas production and degradability**

The culture medium was prepared as follows: (a) mineral solution I (6.0 g K$_2$HPO$_4$ L$^{-1}$ of distilled H$_2$O); (b) mineral solution II (6.0 g KH$_2$PO$_4$; 6.0 g (NH$_4$)$_2$SO$_4$; 12.0 g NaCl; 2.45 g MgSO$_4$ and 1.6 g CaCl$_2$·2H$_2$O L$^{-1}$)
of distilled H$_2$O); (c) 8% Na$_2$CO$_3$ buffer solution; (d) Reduced sulfur solution of Cysteine (2.5 g L-cysteine in 15 mL of NaOH (2N), 2.5 g Na$_2$S) and 0.1 mL 1% Rezarsurin.

The ruminal fluid was obtained from a cross-breed goats (Alpino x Saanen) with a permanent ruminal cannula, fed with a ration of (2 kg d$^{-1}$), divided into two feeding times at 600 and 3000 postprandial hours. Ruminal fluid was collected in the morning 2h after feeding, filtered through four layers of blanket and placed in a thermos at a temperature of 39°C until inoculation. The ruminal fluid was added to the culture medium to a final dilution of 10% (Menke and Steingass, 1988). The samples (0.5$^9$ d$^{-1}$ DM) of the micro-diets were placed in cloth bags measuring 10 × 8 cm (simulating the ANKOM bags) which were introduced into the 120 mL vials, filled with 90 mL of inoculum and free of O$_2$, and it were covered with a rubber stopper and aluminum ring and incubated in an oven at 39°C. Blank samples only contained the ruminal inoculum.

The incubated vials were punctured with a needle through the rubber stopper and connected to a manometer with a scale of 0–100 PSI, recording the gas pressure from 2 to 72 h of cultivation. The PSI units were transformed into volume under the regression equation y = 7.0245x-1.0849, R$^2$ = 0.989, subsequently adjusted to mL$^{-1}$ g DM (Menke and Steingass, 1988). The bags containing undigested residues at 72h were removed from the vials, rinsed thoroughly with distilled water, dried at 65°C by 24 h, and weighed to estimate the DM and nutrients disappearance. The IVD from the batch culture incubation was calculated by means of the following equation: IVD = 1 – [(R – B) / S] where R is g of nutrients as undigested residue, B is g of nutrients as undigested residue in blank bags, and S is the dry weight of the initial substrate.

**Laboratory analysis**

All samples were ground and chemical analyzes were performed in triplicate, including dry matter (DM), organic matter (OM), fat (AOAC, 2005), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al 1991).

Fatty acid profiles: The samples were analyzed in the long-chain fatty acid profile with methylation process (Palmquist and Jenkins 2003). 0.2g of sample was weighed and the trans-esterification process was carried out in 50 mL culture tubes. The gas chromatograph used was HP brand (Hewlett Packard 6890 USA), FID Detector and G2613A automatic injector, silica capillary column (SPTM-2560, Supelco). The standard used was FAME Mix C4-C24 100 mg (Fatty Acids Methyl Esters).

Volatile fatty acids (VFA) profile: A subsample of 1.5 mL L$^{-1}$ of the remaining liquid fraction was taken and acidified with 4 mL of metaphosphoric acid (0.25; w/v), after removing the bag. VFA levels were analyzed by gas chromatograph HP 6890 with an ionization detector (Erwin et al. 1961). The standard used was FAM6C, 1000µg mL$^{-1}$ H$_2$O, High Purity Standards.

**Calculation and statistical analysis**
The gas production kinetics (GP) content was calculated using the inverted exponential function (Ørskov and McDonald, 1979) using Sigma Plot (10.0): 

\[ P = a + b \left( 1 - e^{-ct} \right) \]

where \( P \) is gas production volume at time \( t \), 'a' and 'b' are mathematical parameters, 'c' is a constant fractional of gas production rates, and \( a + b \) is theoretical maximum gas production volume: 

\[ \lim_{t \to +\infty} a + b \left( 1 - e^{-ct} \right) = a + b. \]

Methane production was determined by:

\[ \text{CH}_4 = 0.45 \times (\% \text{ acetate}) - 0.275 \times (\% \text{ propionate}) + 0.40 \times (\% \text{ butyrate}) \] (Oliveira et al. 2022).

The experiment was repeated three times and each run contained ten replicates per treatment. Cumulative gas production data were analyzed as a completely randomized design using the MIXED model procedure of SAS® (2011). The means of the treatments in GP were compared with the Tukey-test (Steel and Torrie 1992). The chemical analyzes were analyzed with the GLM procedure and the values were analyzed using orthogonal polynomials to determine their linear and quadratic effect (\( p \leq 0.05 \)).

**Results**

GP had no differences between treatments at 2 h of incubation (Figure 1). Then, from 4 to 72 h, the 3 treatments with protected PA decreased GP by \(~ 21.7\% \) (\( p \leq 0.05 \)). The groups supplemented with protected PA had no differences from 2 to 12h. But PA6 and PA9 decreased GP from 24 to 72h by \(~ 13.9\% \) less than PA3. GP indicate a linear effect with the maximum value of \( nPA = 317.21 \) until a minor decrease with \( PA9 = 245.6 \) (\( p \leq 0.05 \)).

Table 1 shows the residual sample in batch culture and the degradability indices at 72h in the different treatments. Residual DM and OM increased linearly (\( p \leq 0.05 \)) around \(~ 25.5 \) and \(~ 22.91 \) units, respectively, from nPA until reaching the maximum in PA6 and subsequently maintained and decreased slightly until PA9 had a quadratic effect (\( p \leq 0.05 \)) of \(~ 4.5 \) and \(~ 3.8 \) units respectively, as protected PA is added to the diet. The average degradability of the DM and MO in the treatments were 69.4 and 66.4, respectively. The residual sample of NDF and ADF presented a linear effect (\( p \leq 0.05 \)) with an increase of \(~ 6.02 \) and \(~ 4.83 \) units, respectively, from nPA to PA9. Only ADF showed a positive linear effect (\( p \leq 0.1 \)) with increasing protected PA level (from 35.2 to 43.9). C16:0 presented a linear effect with an increase of \(~ 18.21 \) units from nPA to PA3, then there was a quadratic effect increasing with \(~ 8.58 \) units from PA3 to PA6 and finally \(~ 12.7 \) units from PA6 to PA9. The C18:0 in residual sample had no significant differences between treatments (\( P >0.05 \)). The negative values of C16:0 and C18:0 in the residual sample indicate an increase in the amount of these FA unlike the initial sample. The degradation of C18:1 decreased (from 69.9 to 59.6\%) linearly (\( p \leq 0.07 \)) with the increase in the level of protected PA. C18:2 and C18:3 did not show significant differences (\( P > 0.05 \)) in the treatments, and additionally, the degradability of both FA was more than 90\%. 
Table 1. Nutritional components from *in vitro* degradability of the diet supplemented with protected palmitic acid

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Treatments</th>
<th>nPA</th>
<th>PA3</th>
<th>PA6</th>
<th>PA9</th>
<th>SEM</th>
<th>Lineal</th>
<th>Quadratic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial sample (mg(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td>456.72</td>
<td>456.97</td>
<td>464.35</td>
<td>463.87</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrogen</td>
<td></td>
<td>8.62</td>
<td>8.43</td>
<td>8.54</td>
<td>8.27</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OM</td>
<td></td>
<td>384.33</td>
<td>383.17</td>
<td>390.75</td>
<td>390.71</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NDF</td>
<td></td>
<td>179.13</td>
<td>186.26</td>
<td>203.24</td>
<td>197.14</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADF</td>
<td></td>
<td>70.75</td>
<td>78.96</td>
<td>93.10</td>
<td>96.35</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td>9.32</td>
<td>26.60</td>
<td>35.38</td>
<td>48.24</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:0</td>
<td></td>
<td>2.52</td>
<td>18.95</td>
<td>27.33</td>
<td>39.98</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:0</td>
<td></td>
<td>0.21</td>
<td>0.67</td>
<td>0.97</td>
<td>1.35</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1</td>
<td></td>
<td>2.22</td>
<td>2.92</td>
<td>3.05</td>
<td>3.28</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:2</td>
<td></td>
<td>3.93</td>
<td>3.04</td>
<td>2.65</td>
<td>2.17</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:3</td>
<td></td>
<td>0.22</td>
<td>0.22</td>
<td>0.23</td>
<td>0.27</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Residual sample in batch culture at 72 h (mg(^{-1}))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td>107.66</td>
<td>134.32</td>
<td>158.65</td>
<td>163.15</td>
<td>3.12</td>
<td>&lt;.0001</td>
<td>0.002</td>
</tr>
<tr>
<td>OM</td>
<td></td>
<td>100.45</td>
<td>124.66</td>
<td>146.28</td>
<td>150.12</td>
<td>2.88</td>
<td>&lt;.0001</td>
<td>0.002</td>
</tr>
<tr>
<td>NDF</td>
<td></td>
<td>68.82</td>
<td>74.64</td>
<td>80.85</td>
<td>77.74</td>
<td>1.90</td>
<td>0.035</td>
<td>0.130</td>
</tr>
<tr>
<td>ADF</td>
<td></td>
<td>38.24</td>
<td>45.39</td>
<td>47.90</td>
<td>47.84</td>
<td>1.50</td>
<td>0.018</td>
<td>0.119</td>
</tr>
<tr>
<td>C16:0</td>
<td></td>
<td>2.19</td>
<td>20.40</td>
<td>28.98</td>
<td>41.68</td>
<td>0.60</td>
<td>&lt;.0001</td>
<td>0.024</td>
</tr>
<tr>
<td>C18:0</td>
<td></td>
<td>3.02</td>
<td>2.59</td>
<td>3.06</td>
<td>2.81</td>
<td>0.28</td>
<td>0.917</td>
<td>0.800</td>
</tr>
<tr>
<td>C18:1</td>
<td></td>
<td>0.67</td>
<td>0.95</td>
<td>1.20</td>
<td>1.33</td>
<td>0.09</td>
<td>0.009</td>
<td>0.498</td>
</tr>
<tr>
<td>C18:2</td>
<td></td>
<td>0.40</td>
<td>0.21</td>
<td>0.24</td>
<td>0.18</td>
<td>0.03</td>
<td>0.020</td>
<td>0.184</td>
</tr>
<tr>
<td>C18:3</td>
<td></td>
<td>0.03</td>
<td>0.02</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
<td>0.699</td>
<td>0.451</td>
</tr>
<tr>
<td><strong>Degradation in batch culture incubation at 72 h (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td></td>
<td>76.44</td>
<td>70.61</td>
<td>65.78</td>
<td>64.83</td>
<td>0.68</td>
<td>&lt;.0001</td>
<td>0.001</td>
</tr>
<tr>
<td>OM</td>
<td></td>
<td>73.86</td>
<td>67.47</td>
<td>62.57</td>
<td>61.58</td>
<td>0.74</td>
<td>&lt;.0001</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 2 shows the effect of the treatments on the production of the main VFA at 72 h of *in vitro* degradability. Acetate and the acetate:propionate ratio showed a linear effect ($p \leq 0.05$) increasing with ~1.76 and ~0.13 units, respectively, from nPA to PA9. Propionate had a quadratic effect ($p \leq 0.05$) from nPA to PA3, increasing ~0.34 units and subsequently there was a decrease (linear effect, $p \leq 0.05$) from PA3 to PA9, decreasing ~0.83 units. Butyrate and total VFA presented a linear effect ($p \leq 0.05$) decreasing ~1.3 and ~0.29 units, respectively, from nPA to PA9.

**Discussion**
Gas production

GP decreased with the inclusion level of protected PA from 4 to 72 h, a similar study (Zhang et al. 2008) with inclusions of 0, 3.5 and 7% of octadecanoic FAs (stearic, oleic, linoleic, and linolenic), also indicates the decrease in GP. This effect is attributed to the decrease in the degradability of the NDF since fat covers the fiber and causes inhibition of the ruminal microbes to carry out the degradability (Buccioni et al. 2012; Nur Atikah et al. 2018). Contrary to the present study, the inclusion of high amounts of protected PA in PA6 and PA9 decreased GP, but NDF was not affected. Another study (Vargas et al., 2020) compared olive, sunflower, and linseed oil at 6% and showed decreased GP. In this case, supplementary oils are associated with decreased methane, and consequently, there is less GP (Nur Atikah et al. 2018). In this study, methane production did not decrease with the increase in protected PA. The lack of effect is because it is a SFA, and it cannot capture H₂ ions through the biohydrogenation process (Sun et al. 2022). The data from this study indicate that diets with SFA such as protected PA do not have total protection; part of the PA is available and used by rumen microorganisms and has an effect of decreasing the GP and consequently decreases the degradability of DM and OM (Buccioni et al. 2012).

In vitro degradability

DM and residual OM increased with the levels of protected PA, contrary to the data of Nam et al. (2014), who supplemented rumen-protected fats (3.62%) in Holstein cows, observing that the degradability of DM in the rumen was not affected in the rumen until 48 h. However, a longer ruminal degradation time causes the protected PA to lose part of its protective integrity, and the rumen microorganisms use this PA for their metabolism (Buccioni et al. 2012). The energy density of the diet influences the presence of the rumen population (Baldwin and Connor 2017); increasing the fat content to cover the animal's energy intake can suppress the DM degradability (Yanza et al. 2021).

SFA and UFA supplements do not show differences in DM degradability (Embaby et al. 2019). However, UFA are the main inhibitors of the activity of cellulolytic bacteria in the rumen, causing toxic action (Martínez et al. 2010). However, it depends on the amount in the diet. A study by Jacob et al. (2012), evaluating a diet high in forage and with oils, observed that diets with sunflower oil and 6% soybean oil decreased the degradability of DM, NDF, and ADF at 48 h, while doses of these oils at 4.5% did not affect the degradabilities. The attack mechanism of the bacterial population is made up of SAB bacteria (firmly adhered to the food particles in the rumen) and LAB bacteria (associated with the liquid phase of the rumen); the lipid concentration in the SAB bacteria is twice as high. LAB bacteria (Bauchart et al. 1990), in such a way that the amount in the lipid supplement of the diet modifies the microbial population of the rumen, and differences are shown in the degradability of DM and OM in the rumen.

In this study, only the degradability of ADF increased as the supplementation with protected PA increased. The reason for not having an NDF effect may be due to the low amount of free and available PA in the rumen. Supplementing oils greater than 6% in the diet in unprotected form reduces the degradability of NDF and ADF (Jacob et al. 2012). However, if animals are supplemented with protected fats, there is a
lower rumen protozoa content (Behan et al. 2019) and the bacterial population has more activity in the degradability of fiber; the protozoa also contribute to their ruminal activity, but by not being able to absorb and transform excess dietary lipids, they can die from lipid toxicity (Czerkawski 1976). Another study in goats (Nur Atikah et al. 2018) indicates that olive, palm, and sunflower oil supplements did not modify the protozoan population. The quality of the fat influences the negative effect on the digestibility of nutrients (Panahiha et al. 2022); medium-chain FA can reduce the population of rumen protozoa and affect the degradability of fiber, while UFA acts negatively on cellulolytic bacteria that degrade fiber (Ibrahim et al. 2021).

**Saturated and unsaturated fatty acids**

A higher rate of residual saturated fat at 72h indicates that the microorganisms in the rumen provide another significant amount of SFA that can reach the intestine (Mizrahi et al. 2021). The data from this study indicate that the purity of the protected PA can be around 85%. Another similar study indicates that the protected PA presented greater intestinal flow (Western et al. 2020), the physiological response being very different when unprotected oils are supplemented, biohydrogenation is expected, and the amount of SFA increases (Jacob et al. 2012). Mainly, C18:0 also showed a negative degradability; it is evident that the UFA were biohydrogenated and the saturation rate increased, although, in this study, there was a tendency to decrease the residual content of C18:0 when the inclusion of protected PA increased. Another study indicates that C18:0 decreased as the inclusion of oils in the diet increased (Jacob et al. 2012) since ruminal microorganisms carry out lipolysis and subsequent biohydrogenation of UFA until reaching the final product, which is the formation of C18:0 (Martínez et al. 2010). Biohydrogenation is generated partially or incompletely, giving way to intermediate isomers in the rumen and decreasing the use of free hydrogen in the rumen (Osorio and Vinazco 2010; Prieto-Manrique et al. 2017).

On the other hand, the residual content of C18:1 c-9 increased with the increase of protected PA, but C18:2 c-6 decreased in this study. The differences can be attributed to the DM content of the forage since it affects the lipolysis process in the rumen, and the composition is also influenced by the presence of glycolipids, phospholipids, and triglycerides (Martínez et al. 2010). The primary substrates for biohydrogenation are C18:1 and C18:2, but the biohydrogenation route in C18:2 requires three steps involving isomerization and, subsequently, hydrogenation (Buccioni et al. 2012; Martínez et al. 2010); For this reason, the residual content in this study increased with the increase in protected PA, although there was an incomplete biohydrogenation process of C18:2.

**Volatile fatty acids and methane**

The levels of VFA can be variable, and it also shows the effectiveness in the fermentation of nutrients. Lipid supplements commonly improve the acetate: propionate ratio (Panahiha et al. 2022; Baeel et al. 2023). In this study, propionate decreased when the inclusion of protected PA was increased, possibly lipolysis in the rumen releases FA and glycerol from triglycerides. The free glycerol is rapidly fermented by
bacteria and protozoa and converted to propionate (Martínez et al. 2010). However, since the diet used in this experiment was isoenergetic and isoproteic, the glycerol content varied by the levels of protected PA, and the differences in energy caused the differences in the amount of VFA. The decrease in butyrate was probably due to the inhibitory action of PA on rumen protozoa since the more significant degradation of carbohydrates generates butyrate as a final product (Abubark et al. 2013; Baee et al. 2023). Several studies do not report differences in VFA profiles when FA are supplemented (Behan et al. 2019; Prieto-Manrique et al. 2017; Nur Atikah et al. 2018), although other authors also report an increase in VFA with the use of protected fats (Nam et al. 2014). The degradability of OM influences the production of total VFA; a lower amount of OM fermented in the rumen causes lower energy production and less VFA production (Clark et al. 1992).

UFA commonly causes a reduction in acetate production, acetate:propionate ratio, and CH$_4$ production, generating an increase in the production of propionate, butyrate, and total VFA (Jacob et al. 2012; Prieto-Manrique et al. 2017; Kholif et al. 2018; Embaby et al. 2019; Arcos et al. 2022). Associated with this study, CH$_4$ production did not decrease with the protected PA supplement; perhaps the PA was partially used by methanogens, favoring their growth, and was reflected in CH$_4$ production (Morgavi et al. 2010). Consequently, it can be assumed that methanogens can benefit from the protected PA supplement. The methanogenesis occurs due to the reduction of excess H$_2$ in the rumen. In this case, the PA supplement could interfere with the biohydrogenation process and perhaps modify the population of protozoans that participate in biohydrogenation (Martínez et al. 2010), generating greater H$_2$ in the rumen to be used in the production of CH$_4$.

**Conclusion**

The inclusion of 9% protected PA in diets for lactating goats slightly decreased gas production and the degradability of DM and OM. However, the digestibility of the cell wall was not affected. Supplements with protected PA at 3 and 6% increased the availability of C16:0 to flow to the intestine and be used as a lipid source in milk. The protected PA offers advantages for its use; however, its protection is not total. In summary, the results indicate good benefits of protected PA on fermentative variables. The doses of 3 and 6% of protected PA must be evaluated with goats in vivo to assess its use on fat quality in milk.

**Declarations**

**Authors contributions** Jenny Álvarez: Conceptualization; Methodology; Data collection; Sample collection; Investigation; Formal analysis; Software. J Efrén Ramírez: Data collection; Visualization; Writing - original draft, final manuscript Writing - review and editing. Yuridia Bautista: Formal analysis; Writing and review. María Crosby: Data curation; Formal analysis. Lorenzo Granados: Resources; Writing and review. Mónica Ramírez: Resources; review and editing Alexis Ruiz: Conceptualization; Funding acquisition; Methodology.
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**Conflicts of interest** The authors declare no conflict of interest.

**Ethics Approval** The first author, Jenny Nathalia Álvarez, is a MSc student of PREGEP-COLPOS.

**Data availability** The datasets generated and analyzed in the current study are available upon reasonable request to the corresponding author for scientific purposes upon written request.

**References**


21. Jacob, A.B., Balakrishnan, V., Kathirvelan, C., 2012. Effect of amount and source of vegetable oils in a high fibrous cattle diet on in vitro rumen fermentation, nutrient degradability and rumen cis-9, trans-


Figures
Figure 1

Accumulated gas production (mL\(^{\text{g}^{-1}}\) DM) from \textit{in vitro} fermentation of diets supplemented with protected palmitic acid.

\[\text{Gas mL g}^{-1} \text{ DM}\]

<table>
<thead>
<tr>
<th>Hours / Treatments</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>nPA</td>
<td>.13(^a)</td>
<td>46.83(^a)</td>
<td>72.69(^a)</td>
<td>124.48(^a)</td>
<td>196.06(^a)</td>
<td>238.78(^a)</td>
<td>273.32(^a)</td>
<td>297.71(^a)</td>
</tr>
<tr>
<td>PA3</td>
<td>19.18(^a)</td>
<td>39.96(^{ab})</td>
<td>61.58(^{ab})</td>
<td>106.64(^{ab})</td>
<td>170.42(^b)</td>
<td>209.44(^b)</td>
<td>239.13(^b)</td>
<td>266.45(^b)</td>
</tr>
<tr>
<td>PA6</td>
<td>15.56(^a)</td>
<td>33.45(^{bc})</td>
<td>50.98(^{bc})</td>
<td>92.48(^b)</td>
<td>146.39(^c)</td>
<td>187.56(^c)</td>
<td>212.45(^c)</td>
<td>238.06(^c)</td>
</tr>
<tr>
<td>PA9</td>
<td>14.78(^a)</td>
<td>29.63(^{bc})</td>
<td>48.20(^{bc})</td>
<td>87.97(^{bc})</td>
<td>141.44(^c)</td>
<td>176.14(^c)</td>
<td>200.55(^c)</td>
<td>224.98(^c)</td>
</tr>
<tr>
<td>SEM</td>
<td>1.97</td>
<td>1.97</td>
<td>1.98</td>
<td>1.97</td>
<td>1.99</td>
<td>1.97</td>
<td>1.96</td>
<td>1.96</td>
</tr>
<tr>
<td>p-Value</td>
<td>0.578</td>
<td>0.019</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

\(^{a,b,c}\) Means with another letter in the column indicate differences. SEM: Standard error of the mean; nPA: Without inclusion of palmitic acid; PA3: Inclusion of protected palmitic acid at 3% DM; PA6: Inclusion of protected palmitic acid at 6% DM; PA9: Inclusion of protected palmitic acid at 9% DM.