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Stability and Effects of Protected Palmitic Acid on In Vitro Rumen Degradability and Fermentation in Lactating Goats

Jenny Nathalia Álvarez-Torres [1], Jacinto Efrén Ramírez-Bribiesca [1,*], Yuridia Bautista-Martínez [2], María Magdalena Crosby-Galván [1], Lorenzo Danilo Granados-Rivera [3], Mónica Ramírez-Mella [4] and Alexis Ruiz-González [5]

1 Programa de Ganadería, Colegio de Postgraduados Campus Montecillo, Texcoco, Texcoco de Mora CP 56230, Mexico; nathalia22.13@gmail.com (J.N.Á.-T.); maria@colpos.mx (M.M.C.-G.)
2 Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas, Km. 5 Carretera a Mante s/n, Ciudad Victoria, Tamaulipas CP 87000, Mexico; ybautista@docentes.uat.edu.mx
3 Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias, Campo Experimental Genera Terán, General Terán, Nuevo León CP 67400, Mexico; dgr_8422@hotmail.com
4 CONAHCyT-Colegio de Postgraduados Campus Campeche, Carretera Haltunchén-Edzná km 17.5, Sihochac, Champotón, Campeche CP 24450, Mexico; monicara@colpos.mx
5 Département des Sols et de Génie Agroalimentaire, Université Laval, Québec City, QC G1V 0A6, Canada; alexis.ruiz-gonzalez.1@ulaval.ca
* Correspondence: efrenrb@colpos.mx; Tel.: +52-59-5952-0200

Abstract: Few studies have evaluated 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) and (b) inclusion of protected PA at three levels: PA3 = 3%, PA6 = 6%, and PA9 = 9% dry matter (DM). The maximum gas production occurred without including protected PA and the low gas production given with the inclusion of protected PA9 ($p \leq 0.05$). Meanwhile, the inclusion with 3 and 6% of protected PA showed a higher level of gas production kinetics than the other treatments ($p \leq 0.05$). DM and organic matter degradation after 72 h of incubation linearly decreased ($p \leq 0.05$) with a high level of protected PA. The level of C16:0 in the protected AP increased with the higher level of supplemented AP ($p \leq 0.05$). The best efficiency in propionic acid, decrease in methane, and increase in biohydrogenation (79%) occurred with the inclusion of protected PA3 ($p \leq 0.05$). In conclusion, supplements with protected PA at 3 and 6% increased PA availability. The results indicate good benefits of protected PA on fermentative variables, and these doses seem ideal for future research in lactating goats.

Keywords: palmitic acid; degradability; rumen fermentation; biohydrogenation; goats

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1. Introduction

Supplementing dairy cattle with various types of fat can increase diet energy density, improve milk fat production, reduce heat stress, enhance reproductive performance, and lower methanogenesis by up to 15% [1]. 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 [2]. In general, supplemental fat contains saturated (SFA) and unsaturated fatty acids (UFA), and one of the most common strategies in livestock units is to supplement more polyunsaturated fatty acids in the diet [3]. The goal is to improve the quality of milk fat and reduce the intake of saturated fatty acids. However, milk fat includes more than 60% saturated fat and has not been associated with cardiovascular diseases in healthy individuals [4].

Diets in lactating cows with more than 2% SFA cause fatty acids (FA) to be inert in the rumen and increase the production [5,6] and yield of fat in milk [7]. Dairy fat is composed of around 70–75% SFA, 20–26% monounsaturated FA, and 4–5% polyunsaturated FA [8,9]. Therefore, there is growing interest in using fat supplements to evaluate the unique effects of individual fatty acids on animal response [10]. At the same time, research on FA supplements in dairy goats is limited [11]. Palmitic acid (PA) supplementation in cows increased milk production and fat without affecting milk protein concentration [7]. Likewise, [12] observed a 5% increase in neutral detergent fiber (NDF) digestibility when a fat supplement enriched with 1.5% PA was administered to lactating Holstein cows.

A meta-analysis [13] 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, favoring bacterial growth, and 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 is transferred to the milk, with a positive correlation (r = 0.65) between palmitic and fat percentage [14]. Goats respond less to diet-induced milk fat content [15], and FA has been shown to increase milk fat secretion in lactating dairy goats with little impact on milk production [16]. Other authors suggest that an unprotected fat-based diet can decrease forage consumption and ruminal fermentation [17]. Protected SFA can prevent energy loss and produce milk [18]. Therefore, in recent years, the livestock industry has developed protected commercial products to correct deficiencies and improve nutrient availability in ruminants [19]. The matrix of the protected product must be resistant to the physical and microbial action of the rumen. Different methodologies have been designed to ensure rumen overflow and increase the nutrient supply to the small intestine [20]. These methods include thermal and chemical treatments and coating with inert fats and polymers [21]. Protection is carried out with microencapsulation techniques that include the formation of microparticles or microcapsules. The materials selected as a coating matrix must have specific properties to protect the active ingredient from rumen degradation. They must be stable at a pH greater than 5.5, soluble in the abomasum (pH 1.5–2), and resistant to microbial attack, and they must have mechanical properties to withstand disintegration [20]. The active ingredient includes an internal coating with an external layer consisting of a delayed-release material, which maintains the integrity of the protective layer in the rumen while it is digested by intestinal enzymes where the active components are released [20,21].

Supplementing palmitic acid without protecting it from the ruminal environment can decrease fiber degradability, affect palatability, and reduce intake [2]. The protected fats marketed in livestock contain, on average, 84% lipids (40% UFA, 9.5% oleic, 1.5% saturated, 44% palmitic, and 5% stearic). UFAs (linoleic acid and linolenic acid) are prevented from ruminal biohydrogenation and protected palmitic acid that bypasses the rumen, improving milk production and milk protein-fat levels [5]. Early lactation cows supplemented with protected palmitic acid have greater energy efficiency due to increased intestinal absorption of long-chain fatty acids. Protected palmitic acid improves energy balance when nutritional demands due to lactation increase [10]. Consequently, few studies have evaluated 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. Particularly, one of the difficulties in protected products is the lack of evaluations within the ruminal environment. There is a need to evaluate protected products; these must contain a high amount of active ingredients, have a high protection capacity, and have limited permanence in the rumen with continuous flow to the small intestine. Some disadvantages of protected products are the low proportion of active material and their limited protection against rumen microorganisms. A bath culture test with goat ruminal fluid 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.

2. Materials and Methods

2.1. 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. This study was carried out in the livestock program and Animal Nutrition Laboratory located at 19°29″ north latitude, 98°53″ west longitude, and an altitude of 2241 m above sea level, with an average annual temperature of 15.2 °C [22].

2.2. 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, and 1.8% mineral premix [23]. The PA used was from the brand WLT® (85%, Winnipeg, MB, Canada), with the particle size ranging from 0.3 to 2.5 mm and protected by butylated hydroxytoluene. Corn grain was replaced by the included PA levels, and the treatments were distributed as follows: control without protected PA (nPA) and treatments with the inclusion of protected PA at three levels: PA3 = 3%, PA6 = 6%, and PA9 = 9% DM.

2.3. Culture Medium, Inoculation, and In Vitro Gas Production and Degradability

The culture medium was prepared as follows: (a) mineral solution I (6.0 g K_2HPO_4 L^{-1} of distilled H_2O), (b) mineral solution II (6.0 g KH_2PO_4, 6.0 g $(NH_4)2SO_4$, 12.0 g NaCl, 2.45 g $MgSO_4$, and 1.6 g $CaClH_2O$ L^{-1} of distilled H_2O), (c) 8% Na_2CO_3 buffer solution, and (d) reduced sulfur solution of cysteine (2.5 g L-cysteine in 15 mL of NaOH (2N) and 2.5 g Na_2S) and 0.1 mL 1% Rezarsurin [24]. Three crossbreed lactating goats (Alpino × Saanen) of 50 kgBW, aged 2 years old, and with a permanent ruminal cannula were used. The animals were fed 2 kg of the base diet (cited above), divided into two feeding times at 6:00 and 15:00 meridian hours. Ruminal fluid (RF) was collected 2 h after feeding and a composite sample was made from all the animals. Then, the RF was filtered through four layers of blanket and placed in a thermos at 39 °C until inoculation. RF was added to the culture medium to a final dilution of 10% [24]. The samples ($0.5^{g^{-1}}$ DM) of the micro-diets were placed in cloth bags measuring 10 cm × 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, 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, R2 = 0.989, subsequently adjusted to mL^{-1} g DM. The bags containing undigested residues at 72 h were removed from the vials, rinsed thoroughly with distilled water, dried at 65 °C by 24 h, and weighed to estimate the DM and nutrient disappearance. The in vitro degradability (IVD) from the batch culture incubation was calculated using 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.

2.4. Laboratory Analysis

All samples were ground and chemical analyses were performed in triplicate, including dry matter (DM), organic matter (OM), fat [25], neutral detergent fiber (NDF), and acid detergent fiber (ADF) [26].

Fatty acid profiles: The samples were analyzed in the long-chain fatty acid profile via the methylation process [27]. Briefly, 0.2 g of sample was weighed and the trans-

esterification process was carried out in 50 mL culture tubes. The gas chromatograph used was an FID Detector and G2613A automatic injector by the HP brand (Hewlett Packard 6890 Herndon, VA, USA), with a 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 via gas chromatograph HP 6890 with an ionization detector [28]. The standard used was FAM6C with 1000 µg mL^{-1} H_2O, with high purity standards.

2.5. Calculations and Statistical Analysis

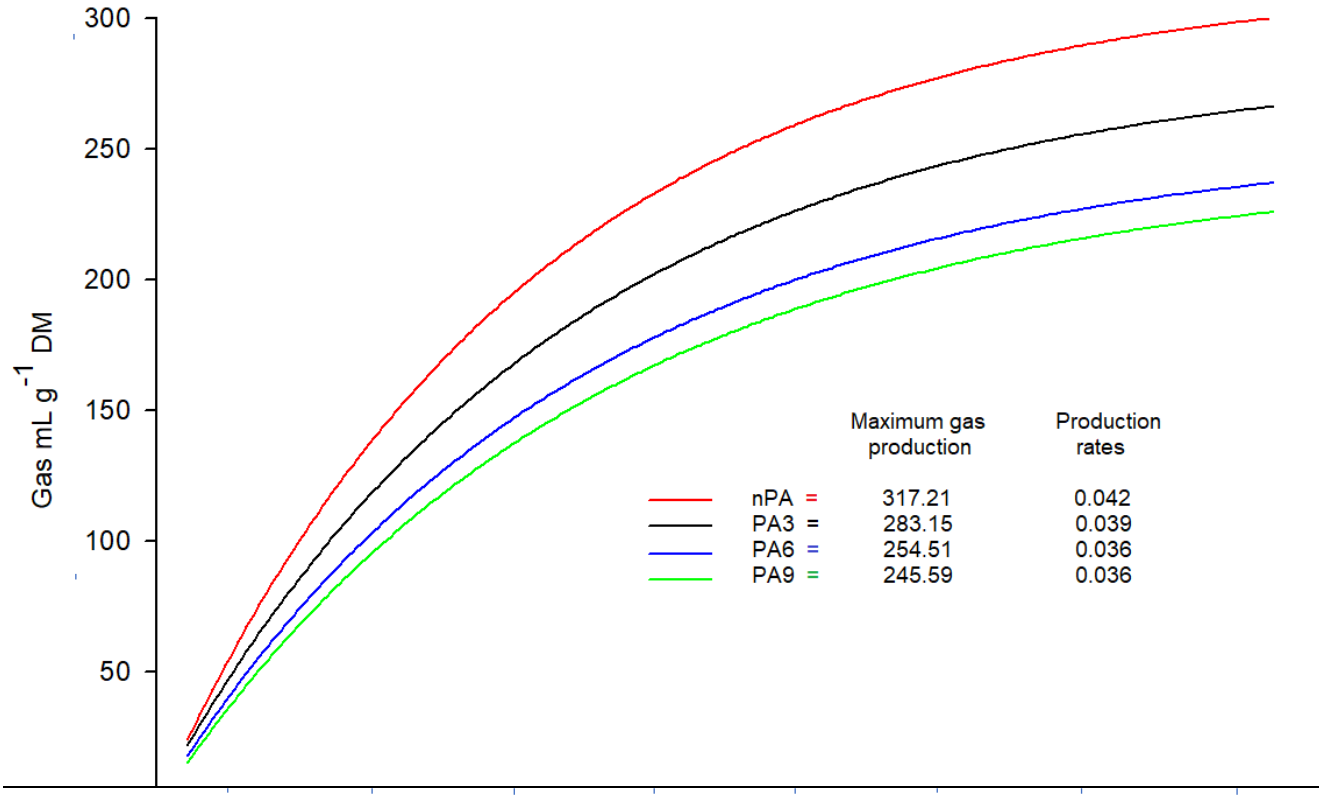
The gas production kinetics (GP) content was calculated using the inverted exponential function [29] using Sigma Plot (10.0): $P = a + b\ (1 - e^{-ct})$, where P is the gas production volume at time t, 'a' and 'b' are mathematical parameters, 'c' is a constant fractional of gas production rates, and a + b is theoretical maximum gas production volume: $\lim_{t \to +\infty} a + b\ (1 - e - ct) = a + b$. Methane production was determined as follows: $CH_4 = 0.45 \times$ (% acetate) $- 0.275 \times$ (% propionate) + $0.40 \times$ (% butyrate) [30]. The biohydrogenation (BH,%) of fatty acids was calculated using the following equation: BH = 100 – 100 [(residual unsaturated FA/total FA in residual digest)/(unsaturated FA feed sample/total FA feed sample)] [31].

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® [32]. The means of the treatments in GP were compared using the Tukey test [33]. The chemical analyses were performed using the GLM procedure, and the values were analyzed using orthogonal polynomials to determine their linear and quadratic effects ($p \leq 0.05$).

3. Results

GP had no differences between treatments at 2 h of incubation (Figure 1). Then, from 4 to 72 h, the three 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 12 h. However, PA6 and PA9 decreased GP from 24 to 72 h by ~13.9% less than PA3. GP indicates a linear effect with a maximum value of nPA = 317.21, with 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 72 h 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 levels (from 35.2 to 43.9). C16:0 presented a linear effect with an increase of ~18.21 units from nPA to PA3, followed by a quadratic effect increasing by ~8.58 units from PA3 to PA6, and finally, ~12.7 units from PA6 to PA9. The C18:0 in the 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%.



Hours/ Treatments	2	4	6	12	24	36	48	72
nPA	20.13 ^{a}	46.83 ^{a}	72.69 ^{a}	124.48 ^{a}	196.06 ^{a}	238.78 ^{a}	273.32 ^{a}	297.71 ^{a}
PA3	19.18 ^{a}	39.96 ^{ab}	61.58 ^{ab}	106.64 ^{ab}	170.42 ^{b}	209.44 ^{b}	239.13 ^{b}	266.45 ^{b}
PA6	15.56 ^{a}	33.45 ^{b}	50.98 ^{bc}	92.48 ^{b}	146.39 ^{c}	187.56 ^{c}	212.45 ^{c}	238.06 ^{c}
PA9	14.78 ^{a}	29.63 ^{b}	48.20 ^{bc}	87.97 ^{b}	141.44 ^{c}	176.14 ^{c}	200.55 ^{c}	224.98 ^{c}
SEM	1.97	1.97	1.98	1.97	1.99	1.97	1.96	1.96
p-Value	0.578	0.019	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

$^{a, b, c}$ Means with another letter within 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, and PA9: inclusion of protected palmitic acid at 9% DM.

Figure 1. Accumulated gas production (mL^{g-1} DM) from in vitro fermentation of diets for lactating goats with three levels of protected palmitic acid.

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 by ~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 by ~0.34 units, and subsequently, there was a decrease (linear effect, $p \leq 0.05$) from PA3 to PA9, decreasing by ~0.83 units. Butyrate and total VFA presented a linear effect ($p \leq 0.05$), decreasing by ~1.3 and ~0.29 units, respectively, from nPA to PA9. BH had a quadratic effect ($p \leq 0.05$) from nPA to PA3, increasing by ~4.5 units, and subsequently, there was a decrease (linear effect, $p \leq 0.05$) from PA3 to PA9, decreasing by ~6.9 units.

Table 1. In vitro degradability of nutritional components of the diet supplemented with protected palmitic acid.

Nutrient Composition	Treatments						
	nPA	PA3	PA6	PA9	SEM	Linear	Quadratic
Initial sample (mg^{-1})							
DM	456.72	456.97	464.35	463.87	0.38		
Nitrogen	8.62	8.43	8.54	8.27	0.01		
OM	384.33	383.17	390.75	390.71	0.32		
NDF	179.13	186.26	203.24	197.14	0.16		
ADF	70.75	78.96	93.10	96.35	0.07		
Fat	9.32	26.60	35.38	48.24	0.02		
C16:0	2.52	18.95	27.33	39.98	0.03		
C18:0	0.21	0.67	0.97	1.35	0.02		
C18:1	2.22	2.92	3.05	3.28	0.02		
C18:2	3.93	3.04	2.65	2.17	0.02		
C18:3	0.22	0.22	0.23	0.27	0.02		
Residual sample in batch culture at 72 h (mg^{-1})							
DM	107.66	134.32	158.65	163.15	3.12	<0.0001	0.002
OM	100.45	124.66	146.28	150.12	2.88	<0.0001	0.002
NDF	68.82	74.64	80.85	77.74	1.90	0.035	0.130
ADF	38.24	45.39	47.90	47.84	1.50	0.018	0.119
C16:0	2.19	20.40	28.98	41.68	0.60	<0.0001	0.024
C18:0	3.02	2.59	3.06	2.81	0.28	0.917	0.800
C18:1	0.67	0.95	1.20	1.33	0.09	0.009	0.498
C18:2	0.40	0.21	0.24	0.18	0.03	0.020	0.184
C18:3	0.03	0.02	0.04	0.02	0.01	0.699	0.451
Degradation in batch culture incubation at 72 h (%)							
DM	76.44	70.61	65.78	64.83	0.68	<0.0001	0.001
OM	73.86	67.47	62.57	61.58	0.74	<0.0001	0.001
NDF	65.64	64.47	65.47	64.64	8.74	0.962	0.986
ADF	37.90	35.21	42.42	43.95	2.12	0.096	0.465
C16:0	12.91	−7.48	−6.02	−4.24	3.39	0.077	0.079
C18:0	−1308.01	−286.17	−215.66	−107.80	38.70	<0.0001	0.001
C18:1	69.93	67.47	60.74	59.59	2.95	0.066	0.854
C18:2	89.81	92.96	90.91	91.85	1.14	0.614	0.540
C18:3	88.70	92.16	87.97	94.37	2.69	0.375	0.637
BH,%	75.09	79.62	74.51	72.65	1.34	0.031	0.042

Linear effect ($p \leq 0.1$), quadratic effect ($p \leq 0.1$), 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, and BH: biohydrogenation.

Table 2. Levels of volatile fatty acids and methane production after 72 h of in vitro degradability.

Items	Treatments						
	nPA	PA3	PA6	PA9	SEM	Linear	Quadratic
VFA (mmol/100 mol)							
Acetate	59.94	61.28	62.82	65.21	0.518	<0.0001	0.33
Propionate	23.99	24.33	23.90	22.64	0.387	0.01	0.05
Butirate	16.07	14.39	13.28	12.13	0.392	<0.0001	0.52
Total VFA	105.01	104.48	104.37	104.14	0.180	0.004	0.46
A:P	2.50	2.52	2.63	2.88	0.061	<0.0001	0.06
CH_4 ($mL^{g^{-1}}$ DM)	26.80	26.64	27.01	27.97	0.278	0.004	0.05

Linear effect ($p \leq 0.05$), quadratic effect ($p \leq 0.05$), 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, Total VFA: total volatile fatty acids, and A:P = acetate: propionate ratio.

4. Discussion

4.1. Gas Production

GP decreased with the inclusion level of protected PA from 4 to 72 h; a similar study with inclusions of 0, 3.5, and 7% of octadecanoic FAs (stearic, oleic, linoleic, and linolenic), also indicates the decrease in GP [34]. This effect was attributed to the decrease in the degradability of the NDF since fat covers the fiber and inhibits the ruminal microbes from carrying out the degradability [35,36]. 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 [37] compared olive, sunflower, and linseed oil at 6% and showed decreased GP. In this case, supplementary oils were associated with decreased methane, and consequently, there was less GP [36]. 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 H_2 ions through the BH process [38]. 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 [35].

4.2. In Vitro Degradability

DM and residual OM increased with the levels of protected PA, contrary to the data of [17], 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 [35]. The energy density of the diet influences the presence of the rumen population [39]; increasing the fat content to cover the animal's energy intake can suppress DM degradability [40].

SFA and UFA supplements do not show differences in DM degradability [41]. However, UFAs are the main inhibitors of the activity of cellulolytic bacteria in the rumen, causing toxic action [42], but it depends on the amount in the diet. A study [43] evaluating a diet high in forage and with oils showed 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 degradability. The attack mechanism of the bacterial population was made up of SAB bacteria (solid-adherent bacteria: firmly adhered to the food particles in the rumen) and LAB bacteria (liquid-associated bacteria: associated with the liquid phase of the rumen); the lipid concentration in the SAB bacteria was twice as high. LAB bacteria [44], in such a way that the amount in the lipid supplement of the diet modifies the microbial population of the rumen, can influence 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 [43]. However, if animals are supplemented with protected fats, there is a lower rumen protozoa content [18], 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 [45]. Another study on goats [36] indicated 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 [46]; 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 [47].

4.3. Saturated and Unsaturated Fatty Acids

A higher rate of residual saturated fat at 72 h indicates that the microorganisms in the rumen provide another significant amount of SFA that can reach the intestine [48]. 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 [11], and with the physiological response being very different when unprotected oils are supplemented, BH is expected, and the amount of SFA increases [43]. Mainly, C18:0 also showed a negative degradability; it is evident that the UFAs were biohydrogenated and the saturation rate increased, although, in this study, a decrease in the residual content of C18:0 was often observed when the inclusion of protected PA increased. Another study indicated that C18:0 decreased as the inclusion of oils in the diet increased [46] since ruminal microorganisms carry out lipolysis and subsequent BH of UFA until reaching the final product, which is the formation of C18:0 [47,48]. BH is generated partially, giving way to intermediate isomers in the rumen and decreasing the use of free hydrogen in the rumen [49,50].

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 [42]. The primary substrates for BH are C18:1 and C18:2, but the BH route in C18:2 requires three steps involving isomerization and, subsequently, hydrogenation. For this reason, the residual content in this study increased with the increase in protected PA, although there was an incomplete BH process of C18:2. In this case, BH decreased with the increase in the level of protected palmitic acid, indicating an adequate protective effect and that the greater amount of BH is due to the fatty acids contained in the ingredients of the diet. The average BH level (75.47%) is considered adequate and coincides with several in vitro studies [35,42].

4.4. 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 [44,51]. In this study, propionate increased with the inclusion of protected PA3, possibly because lipolysis in the rumen releases FA and glycerol from triglycerides. The free glycerol is rapidly fermented by bacteria and protozoa and converted to propionate [42]. The basal diet used in this experiment was isoenergetic and isoproteic, but the replacement of corn with similar proportions of PA increased the energy content. The greater presence of fatty acids contained in the PA provided more free glycerol, 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 [51,52]. Several studies did not report differences in VFA profiles when FA is supplemented [36,50], although other authors also reported an increase in VFA with the use of protected fats [17]. 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 [53].

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 [54,55]. In this study, CH_4 production did not decrease with the protected PA supplement; perhaps the PA was partially used by methanogens, favoring their growth, which was reflected in CH_4 production [56,57]. Consequently, it can be assumed that methanogens can benefit from the protected PA supplement. Methanogenesis occurs due to the reduction of excess H_2 in the rumen. In this case, the PA supplement could interfere with the BH process and perhaps modify the population of protozoans that participate in BH [38], generating greater H_2 in the rumen to be used in the production of CH_4.

5. Conclusions

The inclusion of PA in the in vitro batch culture test showed intermediate levels of gas production kinetics with 3 and 6% inclusion of protected PA. The maximum gas production occurred without the inclusion of protected PA, indicating greater carbon

dioxide and methane formation. Likewise, the low gas production produced in response to the inclusion of protected PA9% was associated with lower degradability of the content and the dietary cell wall. Meanwhile, the best degradation of non-structural carbohydrates and nitrogen sources occurred with the inclusion of protected PA3. On the other hand, the level of C16:0 contained in the protected AP was slightly released and logically increased with the higher level of supplemented AP. The results on the FA profile indicate that the protection efficiency of PA is not efficient; there is an excretion of FA that adheres to the food, affecting the degradability of the cell wall. On the other hand, the best efficiency in propionic acid, decrease in methane, and increase in biohydrogenation (79%) also occurred with the inclusion of protected PA3.

In general, the data show better response with PA3, although, in this study involving goats in vivo, the evaluation of the inclusions of 3 and 6% of protected PA is suggested. The in vitro technique rules out the evaluation of 9% PA levels because the consideration of this amount in lactating goats is unjustified. However, it is known that the limitations of in vitro technique are the lack of control of all environmental and physiological variables in the rumen. The results consider evaluating doses of 3 to 6% of protected AP as ideal for researching lactating goats' metabolic profile, body condition, and the quality of milk fat.

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