ANIMAL SCIENCE

Effects of dietary palm oil supplementation on ruminal degradation and apparent digestibility of nutrients in sheep

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Abstract: The aim of this study was to evaluate the effects of the inclusion of palm oil on the ruminal environment and nutrient digestibility of sheep diets. Twenty rumen-cannulated sheep were kept in individual stalls equipped with feeding and drinking troughs The animals were fed five diets based on Elephant grass (*Pennisetum purpureum* Schum. cv. Roxo) silage and supplemented with 0, 25, 50, 75, or 100 g kg^{-1} of palm oil (based on total DM). The Elephant grass was harvested at 90 days of regrowth and the concentrate was based on ground corn grain, soybean meal and mineral mix (20 g kg^{-1} DM), offered to the sheep at a ratio of 1.5 g kg^{-1}d^{-1} of body weight (restricted intake) to maintain a forage-to-concentrate ratio of 1:1, based on DM. There were no differences (P = 0.324) in ruminal disappearance and degradability parameters with up to 75 g of oil per kg of DM. Organic matter showed a linear reduction in apparent digestibility, while ether extract increased linearly. Palm oil affected the digestibility and nutritional parameters in ruminant diets.

Key words: degradability, lipid, ruminal fermentation, ruminants.

INTRODUCTION

The use of lipids in ruminant feed provides important benefits, such as increased dietary energy densities, lower caloric increments, better carcasses in the finishing phase, increased reproductive indices, reduced enteric methane production, and changes in the fatty acid profile of the carcass, among other benefits. However, its use at high levels can cause disorders in the development of ruminal microorganisms, affecting fiber degradation and consequently reducing dry matter intake (Manso et al. 2006).

However, there are limiting factors in the use of vegetable oils in ruminant diets. The oil content should not exceed 70 g kg^{-1} of dietary dry matter (NRC 2007). Higher values affect the degradation of fiber, with a consequent reduction in voluntary intake and a decrease in nutrient digestibility (Sullivan et al. 2004). Larger inclusion levels are usually achieved when lipids are offered with some form of protection, such as whole grains or protected fats (Palmquist & Jenkins 1980). The magnitude of the limit of inclusion of free lipids in the ruminant diet depends on the fatty acid profile and the fat source. In addition, short-chain fatty acids cause greater negative effects than long-chain fatty acids, and unsaturated fatty acids cause greater deleterious effects than saturated fatty acids (Fiorentini et al. 2015).

Palm oil (*Elaeis guineensis*, Jacq) is the most widely produced and consumed vegetable oil in the world. It is used for diverse purposes, ranging from use in foods including margarine, ice cream, industrial frying oil and confectioneries to cosmetics and cleaning products. It also serves as a lubricant and, more recently, has been used in the production of biofuel (Benami et al. 2018).

Palm oil is characterized by a high concentration of saturated fatty acids, which distinguishes it from other vegetable oils. It contains approximately 90% triglycerides, with more than 440 g kg^{-1} of saturated fat (Raiol et al. 2012); palmitic (16:0) and oleic (18:1) fatty acids are most abundant, with concentrations of around 400 and 420 g kg^{-1}, respectively (Grimaldi et al. 2005), with a profile similar to that of bovine tallow. Such characteristics may make it less harmful to the ruminal environment, which would allow for a greater proportion of inclusion compared to other vegetable oils, thus allowing an increase in the energy density of the diet without the commonly observed disorders.

We, therefore, tested the hypothesis that, due to the high concentration of saturated fatty acids in palm oil, it is possible to include higher than recommended levels of lipids in the diets of ruminants. The overall aim of this work was to evaluate the parameters of *in situ* degradability and apparent digestibility of nutrients in sheep fed diets with different levels of palm oil.

MATERIALS AND METHODS
Animal care
Data were obtained from a study conducted in Castanhal, Pará, Brazil (1°17'49"S/47°55'19"W; 41 m above sea level). The study was approved by the Ethics Committee in Animal Use of the Veterinary Department of the Federal University of Pará (process number 120/2013).

Animals, experimental design, and diets
Twenty rumen-cannulated cross-breed sheep, weighing 35.8 ± 9.46 kg, were kept in individual stalls (0.8 m x 1.5 m) with cement floors, covered by a bed of sawdust and equipped with feeding and drinking troughs. The experiment consisted of five treatments, with four animals in each treatment. The animals were fed five isoproteic and isofiber diets based on Elephant grass (*Pennisetum purpureum* Schum. cv. Roxo) silage and supplemented with 0, 25, 50, 75, or 100 g kg^{-1} of palm oil (based on total dry matter, DM), noted as Palm0, Palm25, Palm50, Palm75, and Palm100, respectively (Table I).

Grass was cut after 90 days of regrowth. The concentrate, containing ground corn grain, soybean meal and mineral mix (20 g kg^{-1} DM), was offered to the sheep mixed with the forrage at a ratio of 1.5 g kg^{-1} body weight (restricted intake) per day (NRC 2007), maintaining a forage-to-concentrate ratio of 1:1 based on DM. Palm oil was mixed with the concentrates to facilitate distribution and intake. Feed was supplied in two equal meals, at 07:00 h and 18:00 h. Prior to data collection, the animals were given a period of 21 days to adapt to the diets and the experimental conditions. The fatty acid profile of the palm oil is outlined in Table II.

Degradability
The silage was dried in a forced ventilation oven at 55°C for 72 h and ground with a Willey mill equipped with 5-mm mesh sieves. Duplicate samples (4 g DM) were weighed, placed in 12 x 5 cm nylon bags with a porosity of 50 μm (Nocek 1988) and introduced through in the rumen. After different incubation times (6, 12, 24, 48, 72, and 96 h), the nylon bags were removed from the rumen, immediately immersed in cold water, washed in running water until colorless, and transferred to a forced ventilation oven at 55°C, where they remained until a constant weight

Table I. Ingredients and chemical compositions (g kg^{-1}of DM) of the experimental diets.

Ingredients		Palm oil levels (g kg^{-1} of DM)				
		Palm0	Palm25	Palm50	Palm75	Palm100
Elephant grass silage		500.0	500.0	500.0	500.0	500.0
Palm oil		0.0	25.0	50.0	75.0	100.0
Corn		355.0	320.0	290.0	260.0	225.0
Soybean meal		125.0	135.0	140.0	145.0	155.0
Mineral and vitamin suplement[1]		20.0	20.0	20.0	20.0	20.0
Chemical composition	**Silage**					
Dry matter	211.4	542.9	543.7	549.2	549.2	551.0
Organic matter	907.8	924.0	918.4	916.0	919.6	921.7
Crude protein	46.5	108.4	116.9	111.7	118.8	119.5
Ether extract	26.1	38.5	71.2	90.5	113.5	125.2
Ash	92.2	76.0	81.6	84.0	80.4	78.3
NDFap[2]	757.2	468.4	467.2	465.9	457.8	464.1
ADFap[3]	425.1	228.9	227.5	228.6	227.8	230.4
Lignin[4]	74.0	39.0	38.9	38.7	38.6	39.6

[1]Contained per kg: 140 g of Ca, 65 g of P, 10 g of Mg, 760 mg of F, 12 g of S, 1,000 mg of Fe, 3,000 mg of Mn, 60 mg of I, 80 mg of Co and 130 g of Na; Palm0, Palm25, Palm50, Palm75 and Palm100 correspond respectively to the inclusion of 0.0, 25.0, 50.0 75.0 and 100.0 g of palm oil per kg of DM; [2]Neutral detergent fibre corrected for ash and protein; [3]Acid detergent fibre corrected for ash and protein; [4]Lignin in sulfuric acid.

Table II. Fatty acid profile of palm oil (g kg^{-1}).

Lauric acid (C12:0)	4.0
Myristic acid (C14:0)	10.0
Palmitic acid (C16:0)	410.0
Palmitoleic acid (C16:1)	5.0
Stearic acid (C18:0)	50.0
Oleic acid (C18:1)	415.0
Linoleic acid (C18:2)	110.0
Linolenic acid (C18:3)	< 10.0
Arachidonic acid (C20:0)	< 5.0
Total fatty acids	>900.0
Saturated fatty acids	416.0
Unsaturated fatty acids	584.0

was reached. Disappearance at time zero were determined by washing un-incubated bags in the same manner as described for the incubated bags.

DM, OM, CP, NDF an ADF degradability parameters were fitted according to the following model (Ørskov & McDonald 1979):

$$P = a + b(1 - e^{-ct})$$

where: P = rumen disappearance of DM (%) at time t (h); a = rapidly soluble fraction (%); b = insoluble, but potentially degradable fraction (%); c = fractional degradation of fraction b at constant rate (h^{-1}); and t = incubation time (h).

To estimate effective degradability (ED), we used the following formula (Ørskov & McDonald 1979):

$$ED = a + \left[\frac{bc}{c + k} \right]$$

where k is the flow rate of particles out of the rumen and ED is calculated using an outflow rate of 0.02 h^{-1} (AFRC 1993).

To estimate lag time, the following equation was used (McDonald 1981):

$$TC = -\left(\frac{1}{c}\right) \times \ln\left[\frac{a+b-s}{b}\right]$$

where a, b, and c are the same parameters as in the previous equation and S is the fraction soluble at time zero. In the neutral detergent fiber (NDF) and acid detergent fiber (ADF), the soluble fraction S was considered zero.

Apparent digestibility

Indigestible neutral detergent fiber (iNDF) was used as an internal marker for fecal production to estimate the apparent digestibility coefficients of nutrients. The NDF was collected using non-woven-textile (NWT, 100 g m^{-2}) after 240 h (Casali et al. 2008) of *in situ* ruminal incubation with the supplied feed, residual feed, and feces. The samples were incubated in triplicate (20 mg DM cm^{-2}) in the rumen of a cannulated buffalo kept under grazing conditions and supplemented with palm cake at 0.5 g kg^{-1} of body weight. After removal from the rumen, the remaining incubated material was extracted with neutral detergent (Van Soest et al. 1991) to measure the indigestible NDF content. Fecal excretion of dry matter was calculated using the relationship between intake and the fecal concentration of indigestible NDF (Sampaio et al. 2011).

Ruminal fluid sampling and fermentation pattern

Ruminal content was sampled using rumen cannula at 0, 1, 3, 6, 9, and 11 h after feeding. The sample pH was measured immediately using a portable pH meter (Model pH 221, Lutron Electronic Enterprise Co., Taipei, Taiwan). Aliquots of 40 mL of ruminal fluid were added to 1,00 mL of sulfuric acid (1:1) and frozen (-20°C) until

further determination of NH$_3$-N concentrations by distillation in magnesium oxide and calcium chloride using Kjeldahl distillation; this was performed using 40 g kg^{-1} of boric acid stock solution and 0.05 N hydrochloric acid (Fenner 1965).

Methylene blue reduction time (MBR) was assessed by adding one part of 0.003 g kg^{-1} to 20 parts of strained ruminal fluid in a glass blood collection tube and determining the time (in min) necessary to return to normal staining compared to the control sample, without the addition of methylene blue (Radostits et al. 2002).

Analyses

Chemical analyses of incubation residues, feed and feces samples were pre-dried in a forced air ventilation oven (55°C for 72–96 h) and milled with a knife mill equipped with 1.00 mm mesh sieves. Samples were analyzed for DM content (method 930.15) (AOAC 2005), ash (method 924.05) (AOAC 2005), crude protein (CP) (method 984.13) (AOAC 2005), ether extract (EE) (method 920.39) (AOAC 2005), neytral detergent fiber (NDF), and acid detergent fiber (ADF) (Van Soest et al. 1991) contents, with heat-stable amylase and without sodium sulfite. Neutral detergent insoluble nitrogen (NIDIN) and acid detergent insoluble nitrogen (ADIN) were determined with results free of ash and residual nitrogen (Licitra et al. 1996).

Statistical analysis

For *in situ* degradability, a completely randomized design was adopted, using a using a split-plot design which the plots were the treatments and the subplots were different incubation times (Zanton & Heinrichs 2009). The data were analyzed by nonlinear mixed effects model through PROC NLMIXED in SAS® (Statistical Analysis System, version 9.2.), fitting

the model of Ørskov & McDonald (1979), with the *Gauss-Hermite* quadrate integration method and a *Gauss-Newton* optimization technique. As the interactive process of the maximum likelihood method is complex and dependent on good initial estimates, they were obtained through PROC NLIN in SAS® (Statistical Analysis System, version 9.2.), using the Gauss-Newton method. The predicted equations were compared by the identity test (Regazzi & Silva, 2004). Equations were considered identical to those presented by equation between all the parameters of the equations by the hypotheses predicted in the methodology described by the authors cited above and parallelism (Freese 1970). the equations considered parallel when the mean differences between the equations at all incubation times are equal.

The apparent digestibility assay was developed in a completely randomized design. Data were analyzed with the general procedure of linear models, in a completely randomized experimental design, according to the following equation:

$$Y_{ij} = \mu + T_i + \varepsilon_{ij}$$

where μ is the average mean, T_i is the effect of the experimental treatment, and ε_{ij} is the random error. Apparent digestibility values were analyzed with an analysis of variance and regression using a F-test where the linear, quadratic effects were evaluated at a probability level of α = 0.05.

RESULTS
Ruminal disappearance of nutrients
There was no interaction between levels of oil supplementation and incubation times. Dry matter, OM, NDF, and ADF ruminal disappearance were not affected by the inclusion of 75 g of oil per kg of DM; however,

values reduced when 100 g of oil were included in the diet. Nutrient disappearance as a function of incubation time progressively increased up to 72 h, at which point stabilization occurred, with similar results at 96 h (Table III).

Degradability parameters
The parallelism test indicated that there was no influence of palm oil on the pattern of degradation curves for DM nor for OM. To determine whether the result is globally affected, despite similar patterns, an identity test was applied, which measures the distance between the curves. The identity test showed that the inclusion of palm oil reduced DM degradation in the Palm25, Palm50 and Palm75 treatments, which were similar to each other, but differed from the Palm100 treatment, which had a reduced potential degradation. For OM, the identity test indicated that there was a reduction in the potential degradation at each level of palm oil supplementation (Table IV).

Lag times for DM did not change significantly with oil inclusion, but for OM, colonization times gradually increased with increasing increments of palm oil supplementation. Effective degradability (ED) represents real degradability with respect to the passage rate, which, for this study was considered to be 0.02 h⁻¹. The mean ED value for animals in maintenance (AFRC 1993) showed no variation betwen the treatments. However, for OM, there was a gradual reduction in ED with the inclusion of lipids. The ED of crude protein showed no effect on supplementation levels as well as DM (Table IV).

For the potential degradation of NDF, the parallelism test indicated that there was no change in the patterns of the degradation curves (P = 0.324). However, the identity test indicated that there was a reduction in the potential degradation of NDF in the Palm50 and Palm75 treatments (*P* = 0.023), which did not differ

Table III. Disappearance in the rumen of dry matter (DM), organic matter (OM), crude protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF), as a function of palm oil inclusions and incubation times.

	Disappearance (g kg^{-1} DM)				
Treatment	**DM**	**OM**	**CP**	**NDF**	**ADF**
Palm0	312.0[a]	276.4[a]	431.5[a]	244.8[a]	212.5[a]
Palm25	311.4[a]	273.7[a]	480.8[a]	241.6[a]	203.9[a]
Palm50	299.1[a]	263.6[a]	442.7[a]	228.0[a]	203.5[a]
Palm75	294.4[a]	256.0[a]	481.1[a]	226.1[a]	194.9[a]
Palm100	267.4[b]	225.5[b]	455.4[a]	191.4[b]	160.2[b]
Time (hours)	**DM**	**OM**	**CP**	**NDF**	**ADF**
06	118.5[e]	67.7[e]	267.8[e]	31.3[e]	4.8[e]
12	162.6[d]	115.9[d]	368.4[d]	76.7[d]	47.3[d]
24	249.1[c]	208.4[c]	433.6[c]	158.9[c]	127.7[c]
48	360.0[b]	326.5[b]	496.4[b]	305.1[b]	276.0[b]
72	440.3[a]	412.6[a]	590.6[a]	385.3[a]	355.5[a]
96	450.6[a]	423.1[a]	594.8[a]	398.3[a]	364.5[a]
P* - Palm oil supplementation (POS)	0.023	0.034	0.278	0.012	0.038
P* - Incubation time (IT)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
P* - POS x IT	0.534	0.340	0.234	0.521	0.125
SEM	1.23	1.32	1.23	1.39	1.37

Palm0, Palm25, Palm50, Palm75 and Palm100 correspond respectively to the inclusion of 0.0, 25.0, 50.0 75.0 and 100.0 g of palm oil per kg of DM; Values with different letters in the columns differ from each other (p <0.05) by the Tukey test. *P-value error I.

between themselves. Therefore, for the Palm100 treatment, we observed greater effects of the inclusion of palm oil, showing lower potential degradability. For the ADF, there was no change in the degradation pattern for the Palm0, Palm25, Palm50 and Palm75 diets; however, the parallelism test indicated that there was an alteration in the standard of the degradation curve for the highest level of palm oil (Palm100), with a reduction in potential degradability. The identity test also indicated that the inclusion of oil did not significantly affect degradation at the first inclusion level (Palm25), but that degradation levels were reduced in the Palm50 and Palm75 diets; this differed from the Palm100 diet, which was most strongly affected by the presence of oil in the diet (Table V).

Ruminal fermentation parameters

The parameters of ruminal fermentation, ammoniacal nitrogen (NH_3-N), pH, and methylene blue reduction time (MBR), showed no differences ($P = 0.493$) in the regression analysis for oil supplementation levels. However, NH_3-N presented a fourth-order effect as a function of collection time, differing from pH and MBR, for which a cubic effect was observed for collection time (Table VI). The pH varied ($P = 0.004$) between the sampling times (Table VI).

Apparent digestibility

The apparent digestibilities of DM, CP, NDF, and ADF were not affected ($P = 0.376$) by the inclusion of palm oil in the diet. However, the apparent digestibility of EE showed a linear increase of

Table IV. Regression equations of ruminal degradation parameters of dry matter (DM), organic matter (OM) and crude protein (CP), of Elephant grass silage (*Pennisetum purpureum*) in diets with palm oil inclusion.

DM				
	Equations	**R²**	**LT**	**ED**
Palm0	$PD = 9.45 + 52.47 (1 − e^{0.018t})$ aA	0.9723	1.39 [a]	34.75 [a]
Palm25	$PD = 8.87 + 50.07 (1 − e^{0.016t})$ aB	0.9798	2.42 [a]	31.30 [a]
Palm50	$PD = 9.07 + 48.05 (1 − e^{0.016t})$ aB	0.9698	2.23 [a]	30.71 [a]
Palm75	$PD = 9.28 + 48.19 (1 − e^{0.015t})$ aB	0.9874	2.19 [a]	29.22 [a]
Palm100	$PD = 9.19 + 45.84 (1 − e^{0.017t})$ aC	0.9760	2.00 [a]	30.80 [a]
P* - Palm oil supplementation (POS)			0.542	0.117
OM				
Palm0	$PD = 9.00 + 55.22 (1 − e^{0.017t})$ aA	0.9693	3.34 [d]	34.51 [a]
Palm25	$PD = 8.11 + 54.27 (1 − e^{0.016t})$ aB	0.9712	4.69 [c]	32.40 [b]
Palm50	$PD = 8.11 + 45.54 (1 − e^{0.017t})$ aC	0.9708	5.22 [b]	29.34 [c]
Palm75	$PD = 8.78 + 42.15 (1 − e^{0.014t})$ aD	0.9769	5.60 [b]	26.56 [d]
Palm100	$PD = 7.74 + 40.73 (1 − e^{0.014 \times t})$ aE	0.9434	7.73 [a]	24.92 [d]
P* - Palm oil supplementation (POS)			0.017	0.023
CP				
Palm0	$PD = 41.06 + 26.03 (1 − e^{0.049t})$ aB	0.8456	0.002 [a]	59.55 [a]
Palm25	$PD = 41.06 + 26.09 (1 − e^{0.053t})$ aB	0.8832	0.002 [a]	60.02 [a]
Palm50	$PD = 41.07 + 25.37 (1 − e^{0.060t})$ aB	0.8743	0.006 [a]	60.13 [a]
Palm75	$PD = 41.09 + 26.70 (1 − e^{0.074t})$ bA	0.8360	0.013 [a]	62.08 [a]
Palm100	$PD = 41.09 + 26.17 (1 − e^{0.081t})$ bA	0.8942	0.014 [a]	62.12 [a]
P* - Palm oil supplementation (POS)			0.482	0.234

Palm0, Palm25, Palm50, Palm75 and Palm100 correspond respectively to the inclusion of 0.0, 25.0, 50.0 75.0 and 100.0 g of palm oil per kg of DM; PD = a + b x (1 - e-ct). PD, potential degradability at time t (hours) of rumen incubation; a, rapidly soluble fraction; b, potentially degradable fraction; c, rate of degradation of fraction b; t, incubation time (hours). Equations following by equal lowercase letters in the column are parallel by the curve parallelism test; Equations following by equal capital letters in the column are identical by the curve identity test 5% error probability. ED, effective degradability; LT, lag time. *P-value error I.

0.96% for each 1.00% inclusion of oil in the diet. OM, on the other hand, had a negative linear effect, with a reduction of 0.44 units for each percentage unit of oil supplementation (Table VII).

DISCUSSION

The inclusion of up to 75 g kg^{-1} DM of palm oil to the diet of sheep, which resulted in a total EE of 111.35 g kg^{-1} DM (Table III), did not affect ruminal disappearance of the evaluated nutrients; average values were 304.3 and 235 g kg^{-1} for DM and NDF, respectively. At this level of supplementation, EE was practically twice the recommended amount for ruminant diets, which is around 60 to 70 g kg^{-1} of DM (Mir et al. 2001, Sullivan et al. 2004). The low observed effect of palm oil on nutrient disappearance may be related to the high saturated fatty acid profile of palm oil. Palm oil predominantly consists of palmitic acid (C16:0, 410 g kg^{-1}) and oleic acid (C18:1, 415g kg^{-1}) (Table II), which together consist of more than 80% of the fatty

Table V. Regression equations of ruminal degradation parameters of neutral detergent fiber (NDF) and acid detergent fiber (ADF) of Elephant grass silage (*Pennisetum purpureum*) in diets with palm oil inclusion.

	Equations	R^2	LT	ED
NDF				
Palm0	$PD = 11.07 + 57.15\ (1 - e^{0.017t})$ aB	0.9476	10.27	37.53
Palm25	$PD = 10.57 + 60.72\ (1 - e^{0.017t})$ aA	0.9443	9.49	38.40
Palm50	$PD = 10.33 + 62.11\ (1 - e^{0.013t})$ aC	0.9243	11.70	34.97
Palm75	$PD = 11.07 + 58.10\ (1 - e^{0.015t})$ aC	0.9543	11.38	36.28
Palm100	$PD = 10.65 + 43.06\ (1 - e^{0.020t})$ aD	0.9142	10.83	32.41
ADF				
Palm0	$PD = 14.16 + 53.65\ (1 - e^{0.018t})$ aA	0.8943	12.98	39.60
Palm25	$PD = 13.75 + 52.75\ (1 - e^{0.018\ t})$ aA	0.9143	12.57	39.05
Palm50	$PD = 13.97 + 51.60\ (1 - e^{0.016t})$ aB	0.9134	14.54	37.29
Palm75	$PD = 15.08 + 50.97\ (1 - e^{0.015\ t})$ aB	0.9465	16.98	37.15
Palm100	$PD = 13.08 + 40.08\ (1 - e^{0.022t})$ bC	0.9732	12.96	35.16

Palm0, Palm25, Palm50, Palm75 and Palm100 correspond respectively to the inclusion of 0.0, 25.0, 50.0 75.0 and 100.0 g of palm oil per kg of DM; $PD = a + b\ x\ (1 - e^{-ct})$. PD, potential degradability at time t (hours) of rumen incubation; a, rapidly soluble fraction; b, potentially degradable fraction; c, rate of degradation of fraction b; t, incubation time (hours). Equations following by equal lowercase letters in the column are parallel by the curve parallelism test; Equations following by equal capital letters in the column are identical by the curve identity. ED, effective degradability; LT, lag time.

Table VI. Ruminal parameters of sheep fed diets with palm oil inclusion.

Parameters	Sampling time in hours						Mean	RE	P-value	SEM
	0	**1**	**3**	**6**	**9**	**11**				
[1]NH_3-N (mg dL^{-1})	14.49[c]	20.95[a]	17.63[b]	10.88[d]	9.83[d]	9.26[d]	13.84	Q	0.001	0.468
[2]pH	7.08[a]	6.68[c]	6.53[d]	6.67[cd]	6.86[b]	7.03[a]	6.81	C	0.004	0.028
[3]MBR (min)	4.31[ab]	3.27[c]	3.55[bc]	4.34[a]	4.54[a]	4.95[a]	4.16	C	0.003	0.102

NH_3-N - ammonia nitrogen; MBR, methylene blue reduction time; RE, regression equation; Q, fourth-order effect; C, cubic effect; SEM, standard error of the mean. [1]$\hat{Y} = -0.017x^4 + 0.4288x^3 - 3.4396x^2 + 8.1169x + 14.916\ (R^2 = 0.9800)$. [2]$\hat{Y} = -0.0027x^3 + 0.0588x^2 - 0.3246x + 7.0325\ (R^2 = 0.9300)$. [3]$\hat{Y} = -0.0066x^3 + 0.1261x^2 - 0.5121x + 4.0622\ (R^2 = 0.8100)$.

Table VII. Apparent digestibility coefficients in sheep fed diets with palm oil inclusion.

Variable	Apparent nutrient digestibility (g kg^{-1} DM)					RE	P-value	SEM
	Palm0	**Palm25**	**Palm50**	**Palm75**	**Palm100**			
DM	627.8	618.3	611.8	606.5	595.2	ns	P>0.05	1.42
EE[1]	778.1	795.7	805.3	827.1	882.4	*	P=0.02	3.29
OM[2]	658.7	647.8	630.0	625.4	614.8	*	P=0.03	1.35
CP	576.1	616.2	619.0	630.7	615.3	ns	P>0.05	2.75
NDFap	583.0	574.6	580.5	573.4	530.1	ns	P>0.05	1.71
ADFap	555.4	545.6	538.7	543.6	519.3	ns	P>0.05	1.49

Palm0, Palm25, Palm50, Palm75 and Palm100 correspond respectively to the inclusion of 0.0, 25.0, 50.0 75.0 and 100.0 g of palm oil per kg of DM; * significant at 5% probability; P = p value; RE, regression equation; R^2, ns, not significant; SEM, standard error of the mean; DM, dry matter; EE, ether extract; OM, organic matter; CP, crude protein; NDFap, Neutral detergent fibre corrected for ash and protein; ADFap, Acid detergent fibre corrected for ash and protein. [1]$Y = 76.974 + 0.96X\ (R^2 = 0.8892)$. [2]$Y = 65.736 - 0.44X\ (R^2 = 0.9713)$.

acids present in palm oil. Oleic acid only has one double bond, and is rapidly converted to stearic acid by mixed ruminal microorganisms (Jenkins & Bridges 2007); stearic acid has a lower suppressive effect on ruminal microorganisms. This could explain the low observed effect on ruminal disappearance, mainly of the fibrous fraction, with results similar to those observed for Elephant grass silage cut at 100 days of regrowth (Santos et al. 2012).

The absence of an effect of palm oil on CP disappearance may be related to the lower sensitivity of the proteolytic microorganisms to the presence of lipids (Palmquist et al. 1993) or to an increase in microbial synthesis efficiency (Messana et al. 2013). The latter is often caused by the provision of vegetable oils in the ruminal environment. Vegetable oil supplements can affect rumen microbial protein synthesis by preventing the supply of fermentable energy for rumen microbes or through a defaunation effect on the rumen protozoa; however, this can increase microbial production efficiency in the rumen, since it reduces the predation of bacteria by protozoa (Dewhurst et al. 2000, Fiorentini et al. 2015).

Ruminal disappearance of the nutrients, as a function of incubation time, was reduced in the fractional rate of degradation; this is a naturally observed effect due to the reduction of the faster degradable fractions. Values did not differ with the oil supplementation levels, corroborating the results of other studies with Elephant grass silage cut between 70 and 100 days of regrowth, in diets without lipid inclusion (Rêgo et al. 2010, Santos et al. 2012).

Dry matter is the fraction of food that is composed of all other fractions, and the fiber fraction represents most of the DM content in forage. Thus, it was expected that palm oil inclusion would have an effect on the diet due to a reduction in the degradation of

these fractions, which are most affected by the presence of fat in the diet. However, no influence of oil supplementation was observed on the degradation pattern, which was similar between the diets. On the other hand, potential degradability was affected by the inclusion of palm oil; this was reduced in the Palm25 diet, which did not differ from the Palm50 and Palm75 diets, but differed from the Palm100 diet. This diet had the lowest results for potential degradability, indicating that the presence of oil at this inclusion level is capable of appreciably affecting the ruminal environment.

The Palm75 diet did not differ from the Palm25 and Palm50 diets, and at this level of inclusion, the EE was 111.35 g/kg DM, which is much higher than the recommendation for the inclusion of free fat in the diets of ruminants (Mir et al. 2001). This demonstrates that palm oil is less aggressive in the ruminal environment probably due to the high concentration of saturated fatty acids. The high rate of efficiency of biohydrogenation to stearic acid (C18:0) in the rumen reduces the duodenal flow of polyunsaturated fatty acids and increases saturated fatty acids, thus allowing for higher inclusion levels than are normally recommended because the diet becomes richer in saturated fatty acids, which cause fewer deleterious effects on fiber digestibility when compared with unsaturated fatty acids (Raiol et al. 2012).

The ED results are due to degradation rates and influenced by variations in ruminal fermentation; when ruminal fermentation is appropriate, it favors microbial proliferation, and consequently, higher degradation of the food. In the present study, the observed mean ED was 305.0 g kg^{-1} DM h^{-1} for the Palm25, Palm50, Palm75 and Palm100 diets, which is lower than that of the control diet (347.5 g kg^{-1} DM h^{-1}). The observed reduction was expected since the ED is obtained from ruminal degradation parameters (a, b and

degradation rate, parameter c), thus, when these parameters were affected by the inclusion of palm oil, the ED was directly affected.

In the present study, the low quality of the forage used may have also contributed to the observed results. Upon evaluation of the degradation parameters of the fibrous fractions, we observed that there was influence of the presence of oil on the degradation patterns. The degradation curves were similar for both NDF and ADF in the Palm0, Palm25, Palm50 and Palm75 treatments, differing only at the maximum inclusion level (Palm100) for ADF, which presented a change in the degradation curve.

Results indicate that the degradation values of the fibrous fractions were influenced by the presence of oil in the diet (Table V). There was no difference in the degradation between the Palm50 and Palm75 diets, when compared using the identity test. This effect was described by Palmquist (1996) and by Mertens (1992), who demonstrated that the effect of lipids on fiber depends not only on the type of fatty acid incorporated into the diet, but also on the quality of the fibrous fraction, with lower effects when lower quality fibrous fractions are used. Moreover, the effects of oil inclusion on fiber are more intense in diets with low fiber (Palmquist & Conrad 1980), since the degradability of the fiber and its constituents is strongly related to the adhesion of bacteria to food particles. Another possible explanation is the formation of insoluble salts (saponification) occurring at pH > 6 (Chalupa et al. 1984), which would reduce the effects of lipids on microorganisms by forming micelles in aqueous media (Chalupa et al. 1984). In the present study, there was no fiber limitation in the diets, and the pH was always higher than 6 in all treatments (Table VI), thus permitting an adequate environment for microbial development.

In relation to the effective degradability of the fibrous fractions, there was a similar decrease for the fibrous fractions as the oil increased in the diets (Table V). The ED is the mathematical result of the interaction of the potentially degradable fraction and the rate of degradation as a function of a certain passage rate. Thus, ED represents the effect of the factors that are involved in the degradation process. In this case, the observed effects of the inclusion of palm oil are related to physical effects, such as particle envelopment, which may have resulted in an increase in colonization time; this is reflected in the reduction of the ED with increasing levels of palm oil in the diet (Doreau & Ferlay 1994).

The concentration of NH_3-N, an indicator of proteolytic activity in the rumen, had an overall mean of 13.8 mg dL^{-1}, 20.9 mg dL^{-1} 1 h after feeding, and a minimum of 9 mg dL^{-1} 11 h after feeding. The minimum concentration of NH_3-N should be 5 mg per 100 mL of ruminal fluid to avoid the limitation of microbial fermentation (Satter & Roffler 1975). Even the lowest value, found at 11 hours of feeding time, was not below the amount that limits microbial growth. The study represents an extreme condition with a longer than traditional feeding time, confirming that the concentrations can be maintained above the general average needed for full microbial growth.

The observed fourth order effect only reflects the oscillation in the degradation of the protein fractions of the food and consonance with the microbial death over time between the feeding intervals. The results observed in this study are higher than those described for cattle fed with different types of oil (Fiorentini et al. 2015).

In the present work, the mean value of the pH between sampling times was 7.03, (Table VI) which is considered adequate for optimal cellulolytic activity (Church 1998). The observed

results corroborate with those of a previous study (Fiorentini et al. 2015).

The determination of methylene blue reduction time is a simple test, usually used to evaluate real-time microbial activity (Soriano et al. 2000). Considering the influence of the lipids on the ruminal environment, the MBR could indicate whether a greater effect of the lipids is occurring in the ruminal environment; for example, it could indicate the physical involvement of the food particles, microbial suppression, or both. Because MBR was not affected by sampling time and did not differ between degradation parameters, the reduction in ruminal degradation of the nutrients was mainly due to the physical involvement of the particles; this led to an increase in colonization times, rather than a direct effect of palm oil on ruminal microorganisms.

The observed cubic effect for collection times portrays the natural oscillation of microbial activity over time, which is influenced by the availability of nutrients from food and by microbial death.

Palm oil supplementation increased dietary EE to levels higher than those recommended in the literature for ruminant diets; this occurred as early as the first inclusion, 71.2 g kg^{-1} DM, reaching 125.2 g kg^{-1} for the Palm100 diet. However, this did not reduce DM and apparent CP digestibility.

However, the OM digestibility presented a linear reduction of 0.44% for each gram of oil inclusion, while the digestibility of EE was linearly increased by 0.96% for each 1% inclusion; the apparent digestibility of EE for the Palm100 diet was 882.4 g kg^{-1}, close to the maximum, 900 g kg^{-1} (Palmquist et al. 1986).

Although it showed no reduction due to palm oil supplementation, DM digestibility was relatively low, probably due to the low quality of the silage used (Elephant grass silage cut at 90 days of regrowth); this represented half of the DM of the diet. The reduction of OM digestibility may have occurred as a function of the reductions in the digestibility of fibrous fractions. In addition, this may be related to the fatty acid profile of palm oil, which has a low effect on ruminal degradation, but high effects on digestion and absorption of EE, culminating in its high apparent digestibility; this may have favored new fermentation in the large intestine (Murphy et al. 1987, Jenkins 1993) compensating for the observed reduction in ruminal degradation parameters for the Palm100 diet. However, results from this study corroborate with those obtained by Raiol et al. (2012), who found no difference in the apparent digestibility of lambs fed with the inclusion of 50 g of palm oil per kg of DM.

Considering that, in many cases, the forage available for animal feed is often of reasonable or even low quality, the inclusion of palm oil would greatly contribute to an increase in the energy density of the diet without depressing the degradation of the diet's fibrous fractions. Another benefit of palm oil supplementation is the fact that fatty acids do not undergo ruminal fermentation, which decreases the production of heat, in addition to being incorporated directly into adipose tissue and/or milk, dispensing the metabolic pathways for the production of fatty acids from acetate; this decreases the caloric increment and the associated energy loss and metabolic pathway (Baldwin et al. 1980).

CONCLUSIONS

Palm oil affected the digestibility and nutritional parameters in ruminant diets.

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