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Original Article

Kinetic and *in vitro* ruminal fermentation characteristics of copra meal diets with different fat levels

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ABSTRACT

This study aimed at evaluating the chemical composition and *in vitro* ruminal characteristics of diets using copra meal with different fat contents. Three levels of copra meal inclusion (10, 20 and 30%) and two levels of fat (high-fat copra meal – HFCM and low-fat copra meal – LFCM) were used in replacement of soybean meal. The addition of copra meal increased EE and reduced CP and NFC of diets, especially when HFCM was included. Treatments with the inclusion of 30% copra meal had a higher colonization time (L), independently of fat content. The maximum gas production was reduced from 59.06 to 39.21 mL/g DM with the addition of HFCM, but was not affected when diets contained LFCM. Digestibility was also reduced with the addition of copra meal, the highest reduction being with the addition of 30% copra meal. Copra meal inclusion has reduced the ammonia concentration from 29.75 mg/100 mL (control diet) to 17.05 mg/100 mL (30% copra meal) but did not affect significantly methane production. Copra meal impacts the chemical composition and ruminal fermentation characteristics of diets, especially when containing high oil content.

INTRODUCTION

Copra meal is a byproduct of the coconut industry that can be used as a cheap source of nutrients, compared to those normally used, to supply part of the requirements of animal diets.

Meals obtained after oil extraction can have a varied nutritional composition based on the industrial process utilized for extraction. If the industrial oil extraction process is not efficient, high quantities of coconut oil can stay in copra meal and influence the ruminal fermentation of this ingredient. A reduction in methane production can be obtained when refined coconut oil is used in diets (JORDAN et al., 2006; JORDAN et al., 2007; MACHMÜLLER; KREUZER, 1999).

The final ruminal fermentation products are influenced by diets offered to ruminants because each microorganism has its specificity in digesting nutrients (CHURCH, 1988). Fermentation parameters can be modified with the addition of different fat sources, though the intervention depends on the source and the addition percentage (HOMEM JÚNIOR et al., 2010; O'BRIEN et al., 2014).

Thus, the objective of this study was to evaluate the chemical composition and estimate the kinetic and *in vitro* ruminal fermentation characteristics of diets where soybean meal was replaced by copra meals containing different levels of fat.

MATERIALS AND METHODS

Copra meal was obtained from two coconut-processing plants and was included in diets to replace soybean meal. The copra meals contained different fat levels and were classified according to the level of fat into: high-fat

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copra meal (HFCM) and low-fat copra meal (LFCM) (Table 1).

Table 1 – Nutritional composition of high-fat and low-fat copra meal.

	MM	DM	CP	EE	NDF	ADF
HFCM	6.33	92.57	12.97	49.88	38.33	21.15
LFCM	12.42	92.48	26.32	10.5	62.57	31.04

HFCM – high-fat copra meal; LFCM – low-fat copra meal; MM – mineral matter; DM – dry matter; EE – ether extract; CP -– crude protein; NDF – neutral detergent fiber; ADF – acid detergent fiber.

Diets were formulated with 60% forage and 40% concentrate. The forage portion was composed of Tifton hay and the concentrate portion was composed of corn, soybean meal and copra meal (CM) in the proportions shown in Table 2.

Table 2 – Centesimal composition (g/kg) of experimental diets.

Diet	Tifton hay	Corn + MMix	Soybean meal	Copra meal
Control	600	100	300	0
10% CM	600	100	200	100
20% CM	600	100	100	200
30% CM	600	100	0	300

* MMix – mineral mix; CM – copra meal.

The classification of the experimental treatments is shown in Table 3.

Table 3 – Classification of experimental treatments.

Processing plant	% Copra meal	Classification
Control	0	Control
1	10	HFCM 10%
1	20	HFCM 20%
1	30	HFCM 30%
2	10	LFCM 10%
2	20	LFCM 20%
2	30	LFCM 30%

High-fat copra meal (HFCM) and low-fat copra meal (LFCM).

Percentages of dry matter (DM), mineral matter (MM) and ether extract (EE) were obtained according to the methodology described by Silva; Queiroz (2002). Crude protein (CP) was determined by Dumas combustion method in a nitrogen analyzer Leco 528LC (Leco Corp., St. Joseph, MI, USA). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to the methodology described by Van Soest et al. (1991).

For the calculation of total carbohydrates (TC), the equation TC = 100 - (CP% + EE% + MM%), described by Sniffen et al. (1992), was applied. Non-fiber carbohydrates (NFC) were estimated by the equation NFC = 100 - (CP% + NDF% + EE% + MM%) (SNIFFEN et al., 1992).

In vitro gas production was carried out using the methodology described by Theodorou et al. (1994) and modified by Mauricio et al. (1999), using a pressure gauge. A total of 0.2 g of each experimental diet was added to 100 mL glass flasks containing 24 mL of buffer solution (MCDOUGALL, 1948) and 6 mL of rumen inoculum. The flasks were kept hermetically sealed in a water bath at 39 ± 1°C under stirring. Seven replicates were prepared for each diet. Additionally, seven flasks containing only the inoculum and the buffer solution were incubated to function as a "control", thus quantifying the production of gases originating from the inoculum fermentation alone. The inoculum was collected from a male castrated bovine, with a fistulated rumen, and subjected to a Tifton hay-based diet (approved by Ethics, Bioethics and Animal Welfare Committee – CEBEA - FCAV–UNESP–Jaboticabal – Protocol number 021119/11). The inoculum was obtained by the filtration, in two layers of a diaper-type fabric, of the ruminal liquid collected in the ventral sac of the animal's rumen in a fasted state. The inoculum was kept at a temperature of 39 °C and a CO_2 saturation throughout the process. Pressure readings, in millivolts, were made at 1, 2, 3, 4, 5, 6, 9, 12, 18, 24, 30, 36, 48, 60, 72, 84, 96, 120 and 144 hours after the start of incubation.

The levels of CP, MM and EE and gas production data during the 24 hours of incubation were used to predict the energy value according to the equations recommended by Menke; Steingass (1988): DIVMO = 14.88 + ((0.889*gas24) + (0.045*CP17) + (0.065*MM)); ME (MJ/KgDM) = 2.20 + (0.136*gas24) + (0.0057*CP) + (0.00029*EE) Where: ME is metabolizable energy; gas24 is *in vitro* gas production in 24 hours in mL/0.2 gDM; DE (Mcal/KgDM) = ME/0.82; TDN=DE/4.409*100. For the conversion of ME from MJ to Mcal, the following factor was used: ME (Mcal/KgMS) = ME (MJ/KgDM)/4.184.

The fermentation characteristics, namely pH, NH_3 and CH_4, were analyzed at 24 h incubation. At the appointed time, gas samples were taken for analysis of CH_4, and samples of 3 and 2 mL of fluid were collected for analysis of NH_3. Analyses of CH_4 was made using gas chromatography, using methane as standard.

A factorial arrangement 3x2+1 (three copra meal proportions, 2 levels of fat and 1 control diet) was used with seven replications. To estimate the patterns of microbial fermentation, the model by France et al. (1993) was adopted based on the average of accumulated gas production of each sample. It was given by:

$$A = Af \times \left\{1 - e^{[-b\times(t-t_0)-c\times(\sqrt{t}-\sqrt{t_0})]}\right\}$$

Where: A is the accumulated volume of gases produced until the time t; Af is asymptotic volume of produced

gases; b and c are model parameters; and t_o is the discrete lag time.

The fractional gas production rate was calculated according to the equation:

$$\mu = \frac{b + c}{2 \times \sqrt{t}}$$

All statistical analyses were performed using statistical analysis software R development Core Team (2013) (Informer Technologies, Inc.). The Tukey test and F-Snedecor were used to compare means of gas production parameters and mean comparisons of production of pH, NH_3, CO_2 and CH_4.

RESULTS AND DISCUSSION

The nutritional composition of the experimental diets is shown in Table 4. The increase in the level of copra meal resulted in an increase in the percentage of ether extract and a reduction of crude protein concentration, especially when high-fat copra meal was used.

Ether extract values exceeding 6–7% in the diet can inhibit ruminal fermentation (JENKINS, 1993) due to the formation of a physical barrier on the fiber preventing access of bacteria to the subtracts and by changes in rumen microbial flora caused by the toxic effect of lipids. When low-fat copra meal was used to compose the diet, ether extract values did not exceed 5%, even with a 30% inclusion. On the other hand, when high-fat copra meal was used, the inclusion of 10% made ether extract values reach the limit (6.81% EE).

Table 4 – Nutritional composition of diets containing different proportions of copra meal

DIETS	MM	DM	EE	CP	NDF	TC	NFC
Control	12.88	86.52	2.01	22.69	44.53	62.42	17.89
HFCM 10%	12.23	87.03	6.81	19.25	46.44	61.71	15.27
HFCM 20%	11.57	87.53	11.61	15.81	48.36	61.01	12.65
HFCM 30%	10.92	88.04	16.41	12.36	50.27	60.31	10.04
LFCM 10%	12.83	87.02	2.87	20.58	48.87	63.72	14.85
LFCM 20%	12.79	87.52	3.73	18.48	53.21	65.00	11.79
LFCM 30%	12.74	88.02	4.59	16.37	57.54	66.29	8.755

HFCM – high-fat copra meal; LFCM – low-fat copra meal; MM – mineral matter; DM – dry matter; EE – ether extract; CP – crude protein; TC – total carbohydrates; NDF – neutral detergent fiber; NFC – non-fiber carbohydrates.

The percentages of protein in diets were reduced by the inclusion of copra meal but ranged from 12.36 to 20.58% of CP, theoretically ensuring microbial growth and reaching, in some cases, values close to those obtained for the control diet (22.69% CP/DM).

The highest value observed for total carbohydrates (TC) was with the inclusion of 30% LFCM in the diet and the lowest with 30% inclusion of HFCM. The lowest TC values were obtained with HFCM diets, due to the high ether extract content in meals. On the other hand, the highest TC values obtained with LFCM diets were related to high NDF values in copra meal with low fat levels, which reduced the NFC in the meal. NDF data ranged from 44.53 to 57.54% DM. The inclusion of copra meal in the diet increased NDF values, especially when LFCM was used. Braga et al. (2009) observed the opposite effect, a reduction in NDF levels with the inclusion of 6, 12 and 18% of copra meal in Tifton hay diets.

Differences in nutritional values in diets including copra meal are related to variations in the composition of the meals. This variation might be due to the type of oil extraction, coconut tree species and industry standards, demonstrating thus the importance of a previous analysis of this byproduct.

The fermentation characteristics of the experimental diets are shown in Table 5. The maximum potential of gas production (A) was influenced by the type of copra meal. Diets with high-fat copra meal differed statistically to the control treatment. These treatments had higher percentages of EE and lower percentages of NDF and TC when compared to low-fat treatments. The high percentages of EE may have prevented the degradation of the fiber due to the physical barrier that surrounds it, as well as changes in pH and ruminal microflora due to the EE toxic effect (BYERS; SCHELLING, 1988). Low percentages of TC may have provided little energy to rumen microorganisms, reducing the maximum point of gas production of these treatments. Treatments with low-fat copra meal did not show any significant difference.

It was observed that the addition of 30% of copra meal, independent of the fat content, had a longer lag time compared to the control, since these treatments had more EE than other diets with different fat contents. Therefore, high levels of fat should be avoided in the ruminant diet, because they prevent the adherence of microorganisms to food particles (VAN SOEST, 1994). The lag time is favored by the presence of readily fermentable dietary ingredients and by physical and

chemical characteristics of cell walls, which can facilitate microbial colonization (MAGALHÃES et al., 2006).

Table 5 – France's model parameters for the fermentation characteristics of the experimental diets.

Parameters	Control	HFCM 10%	HFCM 20%	HFCM 30%	LFCM 10%	LFCM 20%	LFCM 30%
A (mL/gDM)	59.06[a]	55.40[b]	48.19[b]	39.21[b]	59.03[a]	59.83[a]	55.17[a]
L(h)	0.8015[a]	0.9278[a]	1.0930[a]	1.1744[b]	1.0449[a]	0.5674[a]	1.0455[b]
B	0.0165	0.0119	0.0112	0.0106	0.0033	0.0179	0.0020
C	0.1216	0.1419	0.1698	0.2233	0.1623	0.0732	0.1186
R^2	0.9889	0.9919	0.9893	0.9740	0.9852	0.9806	0.9800
Error	0.3400	0.3572	0.4598	0.3711	0.2326	0.6534	0.2400
DIVMO	61.18	44.38	41.96	39.33	42.77	43.92	35.91

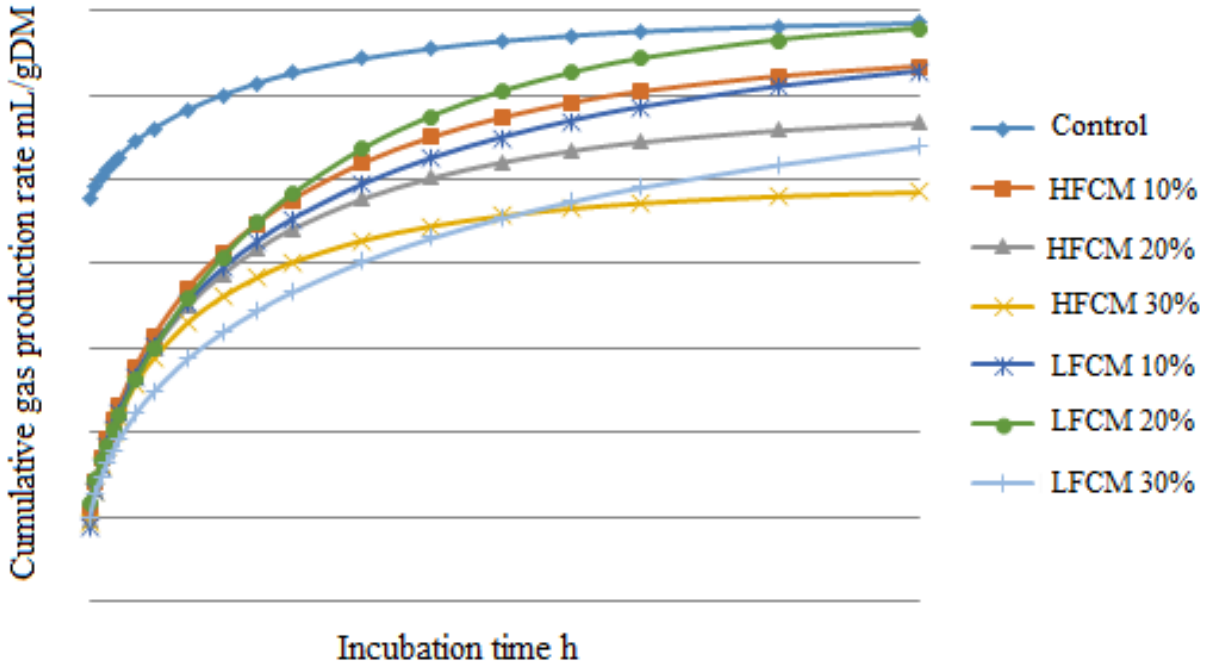
HFCM – high-fat copra meal; LFCM – low-fat copra meal.
A = maximum potential of gas production (mL/gDM); L = lag time, B and C – parameters of DIVMO model = *in vitro* digestibility of organic matter; averages followed by different superscript letters in lines indicate differences of treatments compared to the control group, F-Snedecor test ($P < 0.05$).

In vitro digestibility of organic matter ranged from 35.91 to 61.18%. The control had the highest value.

However, the effect of lipids on rumen digestibility depends on the fat composition and the way it is released in the rumen (DOHME et al., 2001). High-fat-content diets can reduce methanogenesis by inhibiting protozoa and Archaea, and by the biohydrogenation of unsaturated fatty acids, which act as receptors of hydrogen (JOHNSON; JOHNSON, 1995). Soliva et al. (2011) showed that garlic oil is efficient in mitigating methane without noticeably harming microbial nutrient fermentation. O'Brien et al. (2014) have shown that the effectiveness of the fatty acids in mitigating methane production or inhibiting *in vitro* rumen fermentation is both diet- and dose-dependent.

Figure 1 shows the cumulative gas production (mL/gDM). The HFCM 30% treatment had the lowest cumulative gas production – 38.46 mL/gDM – while the control had the highest – 58.53 mL/gDM. There was a decrease in gas production when the concentration of high-fat copra meal was increased in the diet. Regardless of fat type, the treatments with 30% of copra meal had the lowest gas production.

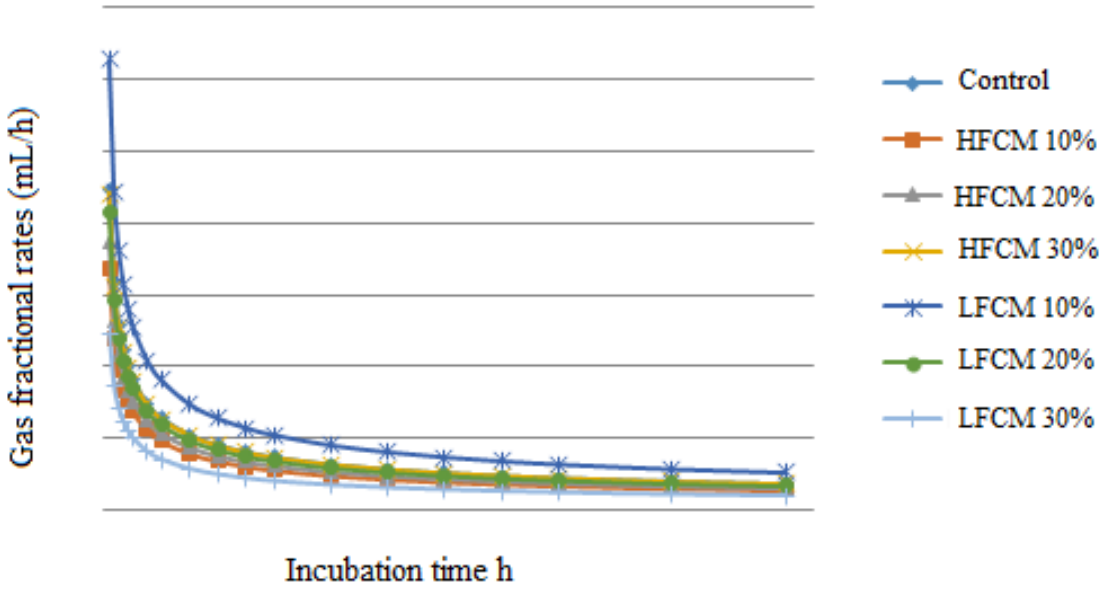
Figure 1. Cumulative gas production (mL/gDM) of copra meal experimental diets with high-fat copra meal (HFCM) and low-fat copra meal (LFCM).



The lowest cumulative gas production of the diets with a high percentage of copra meal confirmed the inhibition potential of coconut oil on ruminal fermentation. Hollmann; Beede (2014) concluded that diets with 2.5% coconut oil decreased lactational performance of Holstein cows by depressing DMI and NDF digestibility.

Figure 2 displays the gas fractional rate of the experimental diets. The highest rates, regardless of diet, were obtained in the first hours. At the beginning of the fermentation there were more substrates, which resulted in higher fractional rates, then with the time lapse the substrates became scarce reducing the fractional rates.

Figure 2. Gas fractional rates of copra meal experimental diets with high-fat copra meal (HFCM) and low-fat copra meal (LFCM).



The fermentation characteristics, namely, NH_3, pH and CH_4, were analyzed at 24 h incubation and the results are show in Table 6. Ammonia concentrations of HFCM 30%, LFCM 10, 20 and 30% treatments were lower and significantly different to the control treatment.

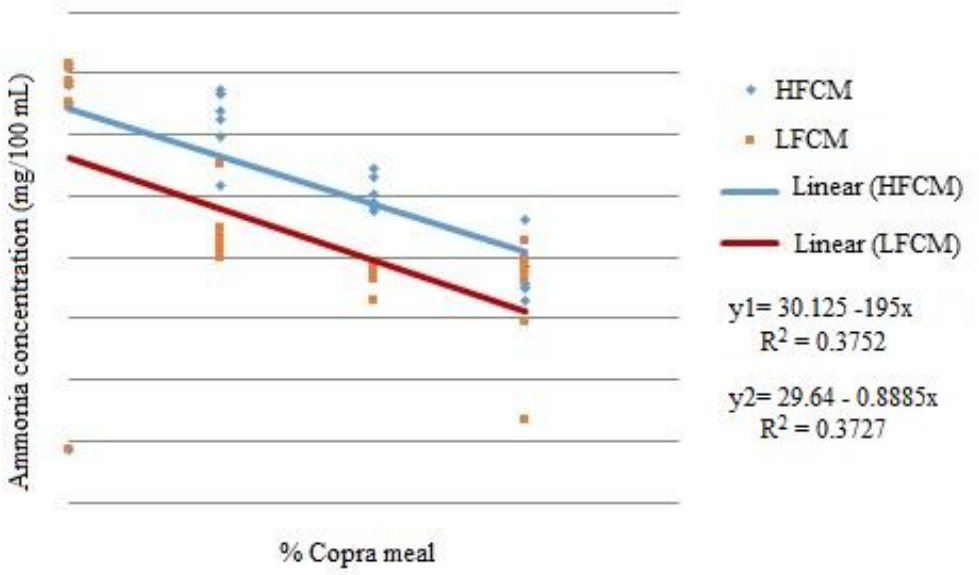
Table 6 – Values of the final fermentation products of the copra meal experimental diets

Treatments	Ammonia (mg/100mL)	pH	Methane (mmol/mol)
Control	29.75 ^{ab}	7.12 ^{ab}	17.85
HFCM 10%	31.25 ^{a}	7.19 ^{a}	13.64
HFCM 20%	25.10 ^{abc}	7.18 ^{a}	17.90
HFCM 30%	18.80 ^{c}	7.09 ^{b}	07.79
LFCM 10%	22.00 ^{bc}	7.06 ^{bc}	13.14
LFCM 20%	18.50 ^{c}	7.05 ^{bc}	10.18
LFCM 30%	17.05 ^{c}	6.98 ^{c}	12.30

HFCM – high-fat copra meal; LFCM – low-fat copra meal. Means followed by different letters in columns differ by Tukey test ($P<0.05$).

Figure 3 shows a decreasing linear regression between the quantity of copra meal in the diet and ammonia concentration. The addition of 1% of high-fat copra meal led to 0.195 mg/100mL reduction in ammonia concentration. The addition of low-fat copra meal led to a higher reduction, 0.8885 mg/100mL for every 1% of inclusion.

Figure 3. Linear regression of ammonia concentration in copra meal experimental diets with high-fat copra meal (HFCM) and low-fat copra meal (LFCM).



The reduction in ammonia concentration might be due to the reduction of the CP and the increase in EE as a consequence of the inclusion of copra meal in the diets.

Other authors (PIMENTEL et al., 2012; SILVA et al., 2007) have reported reductions in ammonia concentrations when using fat in the diet.

A reduction in ammonia concentration in the rumen could be interesting when associated to a good nitrogen utilization. Nevertheless, Faciola; Broderick (2013; 2014) have shown that lauric acid and coconut oil, with lauric acid being responsible for about 50% of the fatty acid composition, reduced ruminal ammonia by reducing ruminal protozoa. However, it did not improve nitrogen utilization since the digestibility of DM, OM, NDF, and CP, as well as milk production and milk components in dairy cows was also reduced.

The pH average obtained in our study varied from 6.98 to 7.19. There were significant differences among treatments, but there was no interaction among the type of meal and concentration. The pH variation shows that the inclusion of copra meal may change the rumen pH, but the values obtained are in an adequate range for microbial activity in the rumen, therefore the inclusion of up to 30% of copra meal did not seem to affect negatively the *in vitro* ruminal pH.

There was no significant difference in the analysis of variance for methane production. However, the high coefficient of variation (30.03 to 65.38) may have influenced the results. Many studies show the reduction of methane when using fat in the diet (CASTAGNINO et al., 2014; JOHNSON; JOHNSON, 1995; JORDAN et al., 2006; JORDAN et al., 2007; MACHMÜLLER; KREUZER, 1999; O'BRIEN et al., 2014; SOLIVA et al., 2011).

CONCLUSION

Copra meal impacts the chemical composition and ruminal fermentation characteristics of diets, especially when it has a high fat content.

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