Feeding oil palm (Elaeis guineensis, Jacq.) fronds alters rumen protozoal population and ruminal fermentation pattern in goats

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Abstract

Oil palm fronds (OPF), normally available all year round, may provide a sustainable ruminant feed for livestock industry in tropical regions. A feeding trial was conducted to study the effects of feeding OPF on the rumen protozoal population, rumen fermentation and fatty acid profiles of rumen fluid in goats. Twenty-five-month-old Kacang crossbred male goats were individually housed and fed for 100 d with concentrate diets supplemented with oil palm (Elaeis guineensis Jacq.) frond pellets. The treatments were: CON (100% concentrate), MOPF (75% concentrate + 25% OPF, w/w) and HOPF (50% concentrate + 50% OPF, w/w). The diets were adjusted to be isocaloric. The rumen fluid pH is a good indicator of the altered status in the rumen of animals receiving HOPF and MOPF diet. These results were suggestive of a decreased biohydrogenation in the rumen, resulting in higher levels of UFA available for hindgut absorption, and hence their increased incorporation in the plasma and edible tissues of the HOPF animals.

Introduction

Lack of animal feed for ruminant livestock is a serious problem in Malaysia. An effective utilization of oil palm frond (OPF), which normally is abundantly available throughout the whole year, may provide a sustainable ruminant feed for the livestock industry in this tropical region to partly overcome this problem. OPF is rich in secondary metabolites, which might adversely affect the animal performance. OPF contains high amounts of tannins and phenolic compounds (Jaffri et al., 2000). Its inclusion in animal feeds can be an effective measure to overcome the lack of grazing pasture for sustaining small ruminant production in palm oil producing countries including Malaysia.

Oil palm tree by-products could be used to supplement rumen deficient diets, but OPF is rich in secondary metabolites, which might adversely affect the animal performance. The OPF contains high amounts of tannins and phenolic compounds (Jaffri et al., 2011). Tannin is one of the most abundantly available plant secondary metabolites, which has adverse effects on the rumen microbial population, feed digestibility and animal performance (Vasta et al., 2009). High amounts of tannin in the diets usually reduce the digestibility and performance of ruminant animals (Mueller-Harvey, 2006). Rumen microbes can show different reactions to secondary metabolites including tannin (McSweeney et al., 2001).

Microbial biohydrogenation was first recorded by Reiser and Reddy (1956). Many subsequent experiments have shown that the microbial composition and fermentation conditions are important factors regulating the relative rates of lipid lipolysis and hydrogenation in the rumen (Gerson et al., 1983). Ebrahimí et al. (2012) showed that supplementation of concentrate with OPF in the experimental diets had beneficially influenced the fatty acid profiles of the plasma and tissues of the goats. The rumen pH is also affected by the type of feed in the rumen. Diets with high concentrate contents lead to a lower rumen pH of less than 5.62. However, diets with high roughage contents increased the rumen pH to more than 5.90. The rumen fluid pH is a good indicator of the overall rumen functions (Kay, 1983). Unsaturated 18-carbon fatty acids are degraded into saturated 18-carbon fatty acids in the rumen by microbial and protozoal activities (Gurr and Harwood, 1991). The protozoal content is around 1/10 of the rumen volume (Hungate, 1978). Their activities are important for the rumen ecosystem and should be considered in ruminant nutrition studies. Two general groups of protozoa are the Holotrichs and Entodiniomorphs. Holotrichs have complete somatic ciliation whereas Entodiniomorphs have just ciliae in selected regions (Jouany et al., 1988; Dehority, 1998). Holotrichs are known to ingest the long chain fatty acids for direct membrane incorporation (Jenkins, 1993). The Entodiniomorphs are known as digesters or engullers of starch, cellulose and bacteria. The ruminal Entodiniomorphs are included in the
Ophryoscolecidae family, and comprised of six subfamilies (Hungate, 1978; Dehority, 1998). In a ruminant animal, the protozoal population would differ due to the diet, time after feed intake, daily feeding frequency and physico-chemical conditions of the rumen (Jouany et al., 1988). In addition, it has been reported that concentrate feeding would lead to partial defaunation of the rumen protozoal flora, particularly the Holotrichs (Towne et al., 1990).

This investigation was aimed to establish any possible link(s) between the observed rumen conditions (used as an indicator of the status of rumen function) and observed changes in the fatty acids of the rumen fluid. The current objectives were to determine the extent of alterations in the rumen pH, volatile fatty acid concentrations, free fatty acid concentrations, microbial composition and densities due to the dietary modification. It was hypothesized that increasing the level of OPF in the diet would increase the rumen pH, altering the ruminal fermentation patterns and both the composition and size of the rumen microbial populations.

Materials and methods

Animals, experimental design and treatments

In the present study, three dietary treatments were used namely CON (control; 100% concentrate without OPF), MOPF (25% OPF) and HOPF (50% OPF) (Table 1). The pelleted OPF were purchased from a local supplier. Table 1 shows the nutrient and fatty acid composition of the raw OPF and the experimental diets. The diets were adjusted to be isocaloric according to National Research Council (2007) recommendations. The animals were fed, adjusted for bodyweight changes, at 3% bodyweight DM intake, twice daily at 8:00 a.m. and 5:00 p.m. Water was provided ad libitum, and a mineral salt block was always available. They were weighed monthly before the morning feeding to monitor their growth performance.

Chemical analysis

The proximate chemical analysis of the feeds was carried out following standard methods of AOAC (1990). The DM was determined by oven drying in a forced-air oven for 24 h at 105°C. A Kjeltac Auto Analyzer (Tecator, Hoganas, Sweden) was used to determine nitrogen. The amounts of nitrogen then were converted to crude protein (CP = N × 6.25). The ether extract (EE) was determined in petroleum ether (40-60°C) using a 2025 Soxtec Auto Analyzer (Tecator, Hoganas, Sweden). The ash content was determined by ashing the samples in a muffle furnace at 550°C for 4 h. An adiabatic bomb calorimeter (Leco Corp., St. Joseph, MI, USA) was used for the measurement of gross energy (GE). Additionally, samples were analyzed for neutral detergent fibre (NDF), using heat-stable amylase and sodium sulphite, and acid detergent fibre (ADF) according to Van Soest et al. (1991). Values for NDF and ADF are expressed inclusive of residual ash. The animals were slaughtered according

| Ingredients, % | Raw OPF | CON | MOPF | HOPF |
|----------------|--------|-----|------|------|
| OPF            | -      | -   | 25.00| 50.00|
| Corn, grain    | -      | 42.10| 26.15| 9.73 |
| Soybean meal   | -      | 13.50| 17.64| 24.00|
| Palm kernel cake| -  | 25.00| 16.13| 5.28 |
| Rice bran      | -      | 14.00| 9.03 | 3.00 |
| Limestone      | -      | 0.50 | 0.50 | 0.50 |
| Palm oil       | -      | 3.50 | 4.16 | 6.10 |
| Salt           | -      | 0.40 | 0.40 | 0.40 |
| Mineral permix | -      | 0.50 | 0.50 | 0.50 |
| Vitamin permix | -      | 0.50 | 0.50 | 0.50 |

Nutrients

- Crude protein, %: 4.35, 16.50, 17.45, 17.22
- Ether extract, %: 3.14, 5.10, 5.33, 5.67
- Dry matter, %: 94.78, 95.99, 97.25, 97.92
- Neutral detergent fibre, %: 74.06, 28.80, 39.76, 43.80
- Acid detergent fibre, %: 51.72, 14.33, 20.57, 26.11
- Ash, %: 3.20, 7.5, 7.90, 8.10
- Metabolisable energy, MJ/kg 1: 5.65, 10.26, 10.42, 10.26

Fatty acid, g/100 g of total fatty acids

- Myristic (C14:0): 4.97, 5.82, 3.66, 1.46
- Palmitic (C16:0): 37.14, 18.39, 20.98, 21.19
- Stearic (C18:0): 8.44, 2.27, 3.48, 3.77
- Oleic (C18:1 n-9): 7.39, 22.48, 24.43, 30.58
- Linoleic (C18:2 n-6): 16.88, 49.28, 44.71, 38.90
- α-linolenic (C18:3 n-3): 24.97, 1.47, 2.48, 3.77
- Arachidic (C20:0): - 0.29 0.27 0.32
- ∑ saturated: 50.55, 26.77, 28.38, 26.74
- ∑ unsaturated: 49.45, 73.23, 71.62, 73.26
- ∑ monounsaturated: 7.59, 22.48, 24.43, 30.58
- ∑ polyunsaturated n-3: 24.97, 1.47, 2.48, 3.77
- ∑ polyunsaturated n-6: 16.89, 49.28, 44.71, 38.90
- n-6:n-3 ratio: 0.68, 35.41, 18.10, 10.32
- Unsatuated: saturated ratio: 0.98, 2.75, 2.53, 2.75
- Polysaturated: saturated ratio: 0.83, 1.91, 1.67, 1.60

OPF: OPF, CON: 100% concentrate; MOPF: 75% concentrate + 25% OPF; HOPF: 50% concentrate + 50% OPF. ∑ saturated, sum of C14:0 + C15:0 + C16:0 + C18:0 + C20:0; ∑ unsaturated, sum of C18:1 n-9 + C18:2 n-6 + C18:3 n-3; ∑ monounsaturated, C18:1 n-9 only; ∑ polyunsaturated n-6, C18:2n-6 only; ∑ polyunsaturated n-3, C18:3n-3 only; n-6: n-3, C18:2 n-6: C18:3 n-3.

*Calculated value.
to the standard slaughter procedures outlined in the MS 1500:2004 (Department of Standards Malaysia, 2004).

Rumen fermentation parameters

After slaughtering of the animals, their gastrointestinal tracts were quickly excised and approximately 100 mL of rumen fluid (RF) of each animal were collected by sampling the rumen contents from the ventral, caudal and central areas of the rumen, and squeezing through four layers of cheesecloth. The pH of RFs was measured immediately (Mettler-Toledo Ltd., UK). Each frozen RF sample was thawed and centrifuged at 10000 × g at 4°C for 20 min, and the supernatant was collected for volatile fatty acid (VFA) measurement. The VFA contents of the RF were determined using gas chromatography. Briefly, the RF was fixed with 25 % metaphosphoric acid, centrifuged at 3000 rpm and the supernatant collected. The supernatant (0.5 mL) was added with an equal volume of 20 mM methyl n-valeric acid (Sigma Chemical Co., St. Louis, MI, USA). Separation was done on a Quadrex 007 Series (Quadrex Corporation, New Haven, CT, USA) bonded phase fused silica capillary column (15m, 0.32mm ID, 0.25 µm film thickness) in a 5890 gas chromatography. Briefly, the RF was fixed with 25 % metaphosphoric acid, centrifuged at 3000 rpm and the supernatant collected. The supernatant (0.5 mL) was added with an equal volume of 20 mM methyl n-valeric acid (Sigma Chemical Co., St. Louis, MO, USA), to each sample before transmethylation to determine the individual fatty acid concentration within the sample. Transmethylation of the extracted fatty acids to their fatty acid methyl esters (FAMEs) was carried out using KOH in methanol and 14% methanolic boron trifluoride (BF3) according to the methods reported by ADAC (1990). The FAMEs were separated by gas chromatography (Agilent 7890A), using a Supelco SP 2560 capillary column of 100m, 0.25 mm ID and 0.20um film thickness (Supelco, Bellefonte, PA, USA). One microlitre of each sample was injected into the GLC equipped with a split/splitless injector and a FID, using an autosampler (Agilent Auto Analyzer 7683 B series, Agilent Technologies, Santa Clara, CA, USA). The carrier gas was nitrogen at a flow rate of 1.2 ml/min. The split ratio was 1:20 after injection of 1 µL of the FAMEs. The injection temperature was programmed at 220°C, and the detector temperature was 270°C. The column temperature program started to run at 150°C, for 2 min, warmed to 158°C at 2°C/min, held for 28 min, warmed to 230°C at 1°C/min, and then held for 20 min to achieve satisfactory separation. The peaks of samples were identified, and concentrations were calculated based on the retention time and peak areas of known standards. A reference standard (mix C4-C24 methyl esters; Sigma-Aldrich, Inc., St. Louis, Missouri, USA) and CLA standard mixture (O-3507 Sigma-Aldrich, Inc., St. Louis, Missouri, USA) were used to determine recoveries and correction factors for the determination of individual FA composition.

Determination of fatty acid profiles

The total fatty acids were extracted from feeds and RF based on the method of Folch et al. (1957) modified by Rajon et al. (1985) and Ebrahimi et al. (2014) using chloroform/methanol 2:1 (v/v) containing butylated hydroxytoluene to prevent oxidation during sample preparation. One gram of experimental diets or 10 mL of RF were homogenized in chloroform/methanol (2:1, v/v). An internal standard, benecicosaenoic acid (C21:9) (Sigma Chemical, St. Louis, MO, USA), was added to each sample before transmethylation to determine the individual fatty acid concentration within the sample. Transmethylation of the extracted fatty acids to their fatty acid methyl esters (FAMEs) was carried out using KOH in methanol and 14% methanolic boron trifluoride (BF3) according to the methods reported by ADAC (1990). The FAMEs were separated by gas chromatography (Agilent 7890A), using a Supelco SP 2560 capillary column of 100m, 0.25 mm ID and 0.20um film thickness (Supelco, Bellefonte, PA, USA). One microlitre of each sample was injected into the GLC equipped with a split/splitless injector and a FID, using an autosampler (Agilent Auto Analyzer 7683 B series, Agilent Technologies, Santa Clara, CA, USA). The carrier gas was nitrogen at a flow rate of 1.2 ml/min. The split ratio was 1:20 after injection of 1 µL of the FAMEs. The injection temperature was programmed at 220°C, and the detector temperature was 270°C. The column temperature program started to run at 150°C, for 2 min, warmed to 158°C at 2°C/min, held for 28 min, warmed to 230°C at 1°C/min, and then held for 20 min to achieve satisfactory separation. The peaks of samples were identified, and concentrations were calculated based on the retention time and peak areas of known standards. A reference standard (mix C4-C24 methyl esters; Sigma-Aldrich, Inc., St. Louis, Missouri, USA) and CLA standard mixture (O-3507 Sigma-Aldrich, Inc., St. Louis, Missouri, USA) were used to determine recoveries and correction factors for the determination of individual FA composition.

Statistical analysis

Statistical analysis of experimental data was performed by the least-squares means method using GLM procedure of SAS (Statistical Analysis System, SAS Institute Inc., Cary, NC, USA) version 9.2. The following model was used: Y ij = μ + Di + Ei j, where Y ij represents an observation; μ is the overall mean; D i is the effect of the diet (i = CON, MOPF or HOPF); and E i j is the residual error. Significantly different means were then further differentiated using the least significant difference comparison procedures. All statistical tests were conducted at 95% confidence level.

Results and discussion

Growth performance

The growth performance of the goats fed experimental diets is presented in Table 2. There was no significant difference between the goats fed the three dietary treatments in terms of their initial or final weight, average daily weight gain or total feed intake.

Rumen fluid pH

The pH values of the RF in all treatment groups were always in the order of HOPF>MOPF> CON (Figure 1). Hence, the pH tended to increase by increasing the levels of oil palm fronds in the diet. However, all groups had pH values below 6.0. There was a significant difference (P<0.05) between HOPF and CON groups in terms of ruminal fluid pH.

The rumen pH can range from approximately 8 to less than 5, with the lower values occurring principally in animals fed high concentrate diets (Styter et al., 1966; Cerrato-Sanchez et al., 2008). At more acidic pH, VFA production is often reduced and acetic acid accumulation is increased (Dunlop and Hammond, 1965; Calsamiglia et al., 2008). However, pH varia-

Table 2. Growth performance of goats fed the experimental diets.

| Experimental diets | CON | MOPF | HOPF |
|-------------------|-----|------|------|
| Initial weight, kg | 22.50±0.97 | 22.57±0.76 | 21.88±1.09 |
| Final weight, kg | 30.42±1.23 | 30.14±1.31 | 27.53±1.22 |
| Average daily gain, g/day | 79.17±8.98 | 75.71±7.27 | 56.50±4.43 |
| Total feed intake, kg | 74.63±6.32 | 74.32±7.21 | 70.73±8.54 |

CON, 100% concentrate; MOPF, 75% concentrate+25% OPF, w/w; HOPF, 50% concentrate+50% OPF, w/w.
tions have generally been attributed to different dietary regimes and different substrates in the feed. This was clearly confirmed by observations in the present study that animals fed with 100% concentrates (CON group) had a consistently lower pH than those fed only just 50% concentrates (HOPF group). Changes in rumen pH could have a significant impact on the rumen microbial composition and digestive functions (Calsamiglia et al., 2008) since the rumen microflora and the environment are very sensitive to wide changes of the rumen pH (Istasse et al., 1986). Reduction of the rumen pH could also alter the rumen bacterial population and composition significantly (Van Soest, 1994). In the present study, the lowered pH of the rumen fluid observed in the CON animals would lead to partial destruction and washing out of mainly rumen bacteria, and inhibition of cellulolysis (Mould and Orskov, 2004).

Volatile fatty acids

The molar proportions of the VFs found in the RF of the experimental animals are shown in Table 3. The molar proportions of acetic acid were significantly different between all treatment groups (P<0.05) and was in the order of HOPF>MOPF>CON. The MOPF and CON groups had similar amounts of propionate but the HOPF group had higher (P<0.05) amounts. Propionate tended to increase from the HOPF group to the CON group as the concentrate in the diet increased. The molar proportions of butyric acid and other minor VFs remained similar between all the treatment groups. The acetate: propionate ratio in the HOPF group was significantly higher (P<0.05) than the MOPF and CON group (Table 3). There was no significant difference in the acetate: propionate ratio between the MOPF and CON groups.

The results of the present study showed that the rumen fluid of the HOPF animals contained more acetic acid compared to the other groups, whilst propionate was higher in the MOPF and CON group. These results were expected since higher amounts of fibre in the HOPF diet would result in the production of higher amounts of acetate (Beever and Mould, 2000).

Fatty acid profile of rumen fluid

Fatty acid profiles of RF for different treatment groups are presented in the Table 4. The concentrations of different fatty acids varied among different treatment groups. The HOPF, MOPF and CON groups contained 10.03, 20.62 and 30.46 mg/100 mL myristic acid, respective-

| Table 3. Molar proportions of volatile fatty acids in the rumen fluid of goats (mol/100 mL; mean±SE; n=8). |
|----------------|----------------|----------------|
| VFA            | Dietary treatment | P      |
| Acetate        | CON             | MOPF | HOPF |
| 53.88±0.77     | 56.36±0.94      | 61.54±0.54 | 0.001 |
| Propionate     | 30.38±0.89      | 29.93±0.75 | 25.07±0.66 | 0.048 |
| Butyrate       | 11.13±0.74      | 10.07±0.24 | 9.86±0.25  | 0.063 |
| Others         | 4.63±0.18       | 3.64±0.28  | 3.53±0.28  | 0.077 |
| Acetate:propionate | 1.79±0.07    | 1.89±0.08  | 2.49±0.08  | 0.039 |

| Table 4. Fatty acid concentrations (mg/100 mL) of the rumen fluid in goats (mean±SE; n=8). |
|----------------|----------------|----------------|
| FA             | Dietary treatments | P      |
| Myristic (C14:0) | 10.03±0.96      | 20.62±1.89 | 30.46±5.60 | 0.031 |
| Pentadecanoic (C15:0) | 17.13±3.23     | 27.79±3.90 | 36.26±6.41 | 0.042 |
| Palmitic (C16:0) | 18.81±1.76      | 27.79±1.03 | 24.66±8.33 | 0.025 |
| Stearic (C18:0) | 22.61±1.61      | 27.31±2.60 | 25.52±3.20 | 0.259 |
| Vaccenic (C18:1-t-11) | 10.28±0.56     | 12.28±1.21 | 12.87±1.32 | 0.006 |
| Oleic (C18:1-n-9) | 21.59±1.59      | 37.37±3.54 | 35.60±4.65 | 0.045 |
| Linoleic (C18:2-n-6) | 20.57±2.48     | 28.65±2.98 | 35.05±4.28 | 0.025 |
| -linolenic (C18:3-n-3) | 10.34±0.97     | 14.5±1.21  | 14.76±1.43 | 0.013 |
| CLA c-9 t-11     | 5.75±0.48       | 6.50±0.79  | 7.05±0.64  | 0.504 |
| CLA t-10 c-12    | 2.31±0.19       | 3.12±0.24  | 2.76±0.32  | 0.650 |
| Arachidonic (C20:4-n-6) | 10.31±0.61    | 17.24±1.46 | 19.60±3.25 | 0.041 |
| Saturated FA     | 68.58±4.57      | 104.27±5.51 | 116.90±6.64 | 0.012 |
| Unsaturated FA   | 73.09±3.59      | 107.99±7.30 | 128.69±11.66 | 0.001 |
| Monounsaturated FA | 21.59±1.59   | 37.37±3.54 | 35.60±4.65 | 0.017 |
| Polyunsaturated n-6 FA | 30.87±2.38    | 45.89±3.21 | 54.65±7.41 | 0.036 |
| Polyunsaturated n-3 FA | 10.34±0.97   | 12.45±1.21 | 14.76±1.43 | 0.020 |
| Unsaturated FA saturated FA | 1.07±0.04   | 1.04±0.04 | 1.10±0.07  | 0.212 |
| Polyunsaturated FA saturated FA | 0.60±0.02   | 0.56±0.02 | 0.59±0.04  | 0.770 |

FA, fatty acids; CON, 100% concentrate; MOPF, 75% concentrate+25% OPE, w/w; HOPF, 50% concentrate+50% OPE, w/w.

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acid (UFA) biohydrogenation in the rumen results in stearic acid production (Keele et al., 1989). The extent of biohydrogenation depends on the availability of UFAs in the rumen (Jenkins and Bridges, 2007). The present observations showed that there were significantly more UFAs (meaning less biohydrogenation of UFAs) in the RF of the HOPF and MOPF animals than the CON group. These UFAs would then be available for absorption in the gut. This would eventually lead to a higher content of UFAs in the goat’s plasma and muscles as reported earlier by Ebrahimi et al. (2002). The results of this study confirmed an earlier report by Goh (2002) who also found that by increasing the levels of OPF in the diet of sheep, the rumen ultimate pH and the rumen protozoa increased, and the rumen fatty acid profile changed. In the present study, changes in fatty acid profiles of RF of the animals that received OPF were strongly related to the altered microbial population of their rumen. The changes in the microbial composition of RF could be due to the final fermenting pH in the rumen which possibly increased the predation of biohydrogenation-capable bacteria by the large numbers of *Entodiniomorphs* in the HOPF animals. The OPF also contains polyphenolic compounds which might act as inhibitors of some microbes that participate in the biohydrogenation process (Vasta et al., 2009). In addition, the high concentration of MUFA, mainly oleic acid (C18:1n-9), in the rumen of both MOPF and HOPF animals which were significantly higher (P<0.05) than the CON animals also provided indirect evidence of a lowered biohydrogenation process. These observations agree with the theory of reduction in the biohydrogenation process by increasing UFA in the diet (Gunstone, 1996; Jenkins and Thies, 1997).

**Protozoal population**

Table 5 shows the population of protozoa in the rumen of the three treatment groups. Both HOPF and MOPF groups had a significantly (P<0.05) higher population of *Entodinium* sp. than the CON group. The HOPF and MOPF animals had similar numbers of *Entodinium* sp. (17.75 and 17.13 x10⁴/mL, respectively). Although the populations of *Holotrichs* and total protozoa between the three treatment groups did not show any significant difference (P>0.05), the populations were in the numerical order of HOPF > MOPF > CON. The changes in the total protozoal count were heavily influenced by the number of *Entodiniomorphs* in all the treatment groups. The HOPF goats numerically had the highest number (8.13x10⁴/mL) of *Holotrichs* compared to both MOPF (7.06x10⁴/mL) and CON (5.94x10⁴/mL) goats although the differences were not significant (P>0.05).

The presence of a high population of protozoa may contribute to stabilizing ruminal pH and slow ruminal fermentation of carbohydrates (Hungate, 1966). Although the populations of *Holotrichs* and total protozoa in HOPF group were only numerically higher than that in the CON animals, population of *Entodinium* sp. in the HOPF animals was significantly higher than that in the CON group. This led to a higher rumen pH in the HOPF group compared to the CON group, which agreed with the results of Rajon et al. (2001).

The current results also showed that the RF of the HOPF group was clearly dominated by the *Entodiniomorph* population. On the contrary, the population of *Holotrichs* was not significantly different among the three treatment groups. These findings were similar to those of Jouany and Ushida (1990) who reported that when the rumen pH was below 6.0, small numbers of *Entodiniomorphs* started to dominate and at pH below 5.5, very few species of them were able to survive (Eadie and Mann, 1970). The lower population of *Holotrichs* compared to *Entodiniomorphs* observed in this study could be explained by the low tolerance of the former to acidic pH (Dehority, 2005). The *Holotrichs* can only survive in a pH above 6.0, which is obtained by diets containing high levels of fibre and soluble sugars (Jouany and Ushida, 1990). Hence, the HOPF diet, which caused the highest pH value of the RF, showed the highest number of *Holotrichs*. The high numbers of *Holotrichs* and in turn total protozoa count in the HOPF group immediately after feeding was expected as Abe et al. (1981) have shown that a pre-feeding stimulus will encourage the migration of protozoa from the reticulo-rumen to the rumen itself.

Results of this study clearly showed that with increasing levels of concentrate feeding (from 50% w/w to 100% w/w), the protozoal numbers were clearly affected and the composition shifted towards an *Entodiniomorph*-dominant flora. Finally, the present observations confirmed that protozoal numbers in the rumen fluid of goats immediately after slaughtering (mean±SE; n=8).

![Figure 1. Rumen fluid pH in goats immediately after slaughtering (mean±SE; n=8). Error bar=±1 SE. Bars with different letters differ significantly at P<0.05. CON, 100% concentrate; MOPF, 75% concentrate+25% OPF, w/w; HOPF, 50% concentrate+50% OPF, w/w.](image)

### Table 5. Protozoal population in the rumen fluid of goats (mean±SE; n=8).

| Dietary treatments | CON | MOPF | HOPF |
|--------------------|-----|------|------|
| Total protozoa, x10⁴/mL | 20.82±2.59 | 24.19±4.48 | 25.88±2.52 |
| Entodinium sp., x10⁴/mL | 14.88±1.71 | 17.13±3.70 | 17.75±2.70 |
| Holotrichs, x10⁴/mL | 5.94±0.95 | 7.06±1.10 | 8.13±1.12 |

CON, 100% concentrate; MOPF, 75% concentrate+25% OPF, w/w; HOPF, 50% concentrate+50% OPF, w/w. *Values with different superscripts within a row differ significantly at P<0.05.*
individual animals differed greatly. Diets containing 50% concentrate (like HOPF group in the present study) would support maximal protozoal numbers with a diverse fauna containing species in most of the genera (Towne et al., 1998; Rajion et al., 2001; Dehority, 1998, 2005). When diets with high levels of concentrate or diets completely composed of concentrate are fed and ruminal pH decreases below 6.0, the numbers of protozoa would decrease and primarily the Entodinium morphs would dominate.

Conclusions

In conclusion, the inclusion of OPP in the diet of goats resulted in changes in the rumen environment toward reduction of the UFA biohydrogenation process. The above-mentioned evidence suggests that supplements of OPP in the diets of goats increased significantly the concentration of Entodinium morphs in the rumen microbial population. However, production-type experiments are needed to confirm the possibility of such benefits from the OPP supplements and to determine the effect of the OPP on other microbes such as bacteria and fungi.

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