Original Article

Effect of supplementation of oil palm (*Eleis guineensis*) frond as a substitute for concentrate feed on rumen fermentation, carcass characteristics and microbial populations in sheep

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Abstract

Thirty growing cross-bred sheep (20.4 ± 1.9 kg body weight (BW)) were used to determine the effects of dietary supplementation of oil palm (*Eleis guineensis*) frond (OPF) pellets on growth performance, microbial population and carcass characteristics of sheep. Experimental animals were allotted into three treatment groups fed varying levels of OPF pellets and commercial sheep pellets. Treatment diets were control diet (CON group, n=10), 25% OPF pellet in diet (% w/w) (HAF group, n=10) and 50% OPF pellet in diet (OPF group, n=10). After 100 days of feeding, all animals from each group were slaughtered, and carcass and rumen fluid were sampled. Both the HAF and CON groups had much more propionic acid and less acetic acid ($P<0.05$) compared to the OPF group at 8 h of sampling. Both HAF and CON had more marbling compared to OPF ($P<0.05$). The HAF and CON groups had also more bacteria per milliliter (mL) of rumen fluid compared with the OPF group at 0 and 2 h of sampling. Therefore, the supplementation of OPF, which is an easily available oil palm by-product, could be used as a feed ingredient at 25% inclusion level to support sheep farming in tropical countries that lack grazing pasture.

Keywords: carcass, microbe, oil palm frond, rumen, sheep

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Introduction

A major problem in developing ruminant livestock production in some tropical countries like Malaysia is the difficulty in providing feed in sufficient quantity and quality throughout the year, especially during peak cropping periods when most of the land is under cultivation (Ebrahimi et al., 2013). The by-products of oil palm (*Elaeis guineensis* Jacq.), which include oil palm fronds (OPF), palm press fiber, palm kernel cake and palm oil sludge, can be included in animal feeds. Oil palm fronds (OPF), normally available all year round, may be provided as sustainable ruminant feed for the livestock industry in tropical regions (Ebrahimi et al., 2015). As Malaysia has cultivated a large area of approximately 4.48 million hectares of land with oil palm and the total OPF production is approximately 5500 kg/ha/year (Ebrahimi et al., 2015), this by-product can represent an alternative and sustainable source of easily available feed ingredient for ruminants and other herbivores. Its inclusion in animal feeds can also be an effective measure to overcome the lack of grazing pasture for sustaining small ruminant production in oil palm producing countries including Malaysia. Moreover, it has been shown that OPF is a rich source of bioactive compounds, such as tannins and phenolic compounds (Jaffri et al., 2011), of which the effectiveness has been reported to improve rumen metabolism, such as decreased protein degradation in the rumen, increased microbial protein production and protein flow to the duodenum targeting specific groups of rumen microbial populations (Patra and Saxena, 2011). OPF, which contains high level of polyphenols and tannins, can reduce the biohydrogenation in ruminants (Cabiddu et al., 2010). OPF has also been proposed as a potential feed alternative for ruminants with the optimal levels of inclusions in mixed rations (on a DM basis) at 55%, 55% and 50% for cattle, buffalo, sheep/goats, respectively (Dahlan, 2000).

There are studies concerning the effect of OPF on sheep carcass characteristics, as such, carcass weight and dressing percentage were improved with increasing levels of OPF (up to 50%) in the diet on freshly chopped OPF and palm kernel cake-based mixture in sheep (Zahari et al., 2002).

There are a number of studies of the effectiveness of OPF on goat characteristics (Abubakr et al., 2015; Ebrahimi et al., 2015), whereas there is limited information about the effectiveness of OPF on the characteristics of sheep farm animals under tropical condition.

Therefore, by keeping the above facts in view, and by considering the use of possible alternatives to overcome the difficulty in providing feed materials in sufficient quantity and quality, the objective of this study was to evaluate the effects of dietary OPF supplementation on sheep characteristics after 100 days of feeding experiment.

Materials and Methods

Experimental animals and diets: Thirty individually housed growing Barbados Black Belly × Malin cross-bred sheep were allotted randomly into three treatment groups. Animals in the control group (CON group, n=10) were fed a mixture of 100% commercial sheep pellet, those in the HAF group (n=10) were fed commercial sheep pellet with 25% of OPF supplementation (% w/w) and those in the OPF group (n=10) were fed commercial sheep pellet with 50% of OPF supplementation. The ingredients of commercial concentrate were corn (25.44%), soybean meal (19%), palm kernel cake (35.87%), rice bran (11.69%), palm kernel oil (5%), ammonium chloride (1%), vitamins (1%) and minerals (1%). The animals were fed twice daily at 3.5% of body weight on dry matter intake basis. Water was provided *ad libitum*. Ingredients and chemical composition of the experimental diets are shown in Table 1. All sheep were weighed monthly before morning feeding and their care and sacrifice were in accordance with the country standards; the experimental protocol was reviewed and approved by the University Putra Malaysia Animal Care and Use Committee. The overall trial lasted for 14 weeks inclusive of a two-week adjusting period.

Table 1 Ingredients and chemical composition of experimental diets

Ingredients (%)	**Experimental diets**		
	CON	**HAF**	**OPF**
Rice straw	10	7.5	5
Corn grain	65	48.75	32.5
Oil palm frond	0	25	50
Soybean meal	22	16.5	11
Molasses	1.25	0.9375	0.625
Urea	0.75	0.5625	0.375
Salt	0.5	0.375	0.25
Dicalcium phosphate	0.5	0.375	0.25
Chemical composition (g/kg DM)			
Dry matter	928	931.8	942
Crude protein	148.1	116.3	90.2
Crude fiber	99.9	150.4	261.2
Ether extract	59	42.4	21.3
Ash	62.8	73.5	78.7
Gross energy (MJ/kg)	18.15	17.8	17.53

OPF = 50% w/w oil palm frond in diet. HAF = 25% w/w oil palm frond in diet. CON = control.

Slaughtering procedure and sampling: At the end of the 14-week feeding trial, the animals were slaughtered (n=30) at the Meat Science Laboratory, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. Weight and pH of the warm carcass were taken within 45 min post slaughter.

Scoring of subcutaneous fat color, firmness and overall meat color was carried out according to a five-point scale. The carcass was then shrouded and chilled for 24 h at 4^{o}C.

After 24 h, weight and pH of the chilled carcass were obtained. Intact carcass was packed into polyvinyl chloride bags and kept at -20^{o}C until carcass analysis. The dissection techniques used to measure muscle, bone and fat composition of a carcass were as described by Wattanachant (1999). The muscles were individually removed from their attachment and the external fat was then trimmed off. *Psoas major*, *gluteus medius*, *semimembranosus*, *longissimus dorsi* and *triceps brachii* were then weighed and color scored individually. Fats were divided into subcutaneous and intramuscular fats. The channel fat was included in the total fat weight.

Rumen fermentation parameters: Collection of rumen liquor samples was carried out for three animals in each group on the last day of feeding at 0 and 8 h post morning feeding from a nylon string secured from the rumen shortly after evisceration. Two hundred mL of rumen liquor was taken from each animal and strained through 4 layers of surgical gauze to remove feed particles. pH of the rumen fluid was then measured with a Mettler-Toledo pH meter (Mettler-Toledo Ltd., England). Then, the rumen fluid samples were acidified with 25% metaphosphoric acid, centrifuged (10 min, 4^{o}C at 15 000×g) and filtered; the filtrate was used to determine volatile fatty acid (VFA) and ammonia nitrogen (NH_3N). The concentration of NH_3N was determined using the colorimetric method as described by Jafari et al. (2016). The VFA contents of the rumen liquor were determined using gas chromatography (5890 Hewlett-Packard Gas-Liquid Chromatograph Avondale, PA). The centrifuged rumen fluid, 0.5 mL, was added with an equal volume of 20 mM 4-methyl-n-butyric acid (Sigma Chemical Co., St. Louis, Missouri, USA) as the internal standard.

Separation was carried out by a Quadrex 007 Series (Quadrex Corporation, New Haven, CT 06525 USA) bonded phase fused silica capillary column (15m, 0.32mm ID, 0.25 µm film thickness). Identification of the peaks was made by comparison with authentic commercial standards of acetic, propionic, butyric, isobutyric, valeric, isovaleric and 4-methyl-n-valeric acids from Sigma (Sigma Chemical Co., St. Louis, Missouri, USA).

Enumeration of rumen bacteria and protozoa density: Two milliliter of the strained rumen fluid was used for quantification of the microbial population. Bacterial count was carried out using a Petroff-Hauser counting chamber (Leedle et al. 1982). Rumen fluid protozoa count was carried out using a haemacytometer as outlined by Towne et al. (1990) and Hungate (1978).

Statistical analysis: Data from growth performance, fermentation characteristics, carcass traits and microbial populations were analyzed using the one-way ANOVA to investigate effects of the treatment diets. Sampling at different times was analyzed using repeated measures. Significant difference (P<0.05) among means was further tested by Duncan's multiple range test using SPSS for Windows version 16.0 (SPSS Inc. 2007, Chicago, USA).

Results

Effect of treatment diets on growth performance: The body weights of the sheep treated with the three treatment diets at initial point and 14 weeks after feeding are presented in Table 2. The average daily gain (Table 2) and total weight gain (Table 2) were significantly different (P<0.05) between the treatment groups. The mean of total weight gain of the CON group was almost three times that of the OPF group over the 14 weeks of experimental period. The average daily gain was poor for the OPF group, which was at 35 g, while that of the CON group was 113 g. The average terminal body weight was 25.89 ± 3.79 kg for the CON group.

Table 2 Effect of dietary supplementation of oil palm frond on sheep performance after 14 weeks of feeding (Mean±SD)

		Experimental diets			
		CON	**HAF**	**OPF**	***P*-value**
Body weight (Kg)	Initial	16.21 ± 2.97	16.29 ± 2.16	16.07 ± 2.87	0.700
	Week 14th	25.99[a]	22.43 ± 0.88[b]	18.74 ± 2.38[c]	0.032
DMI (g/kg BW $^{0.75}$/day)		75.40 ± 2.18	73.40 ± 2.52	74.30 ± 4.83	0.270
Total gain (kg)*		9.48 ± 3.18[a]	6.48 ± 2.18[b]	2.97 ± 2.18[c]	0.027
ADG (g)*		112.86 ± 3.08[a]	77.24 ± 3.08[b]	35.37 ± 3.08[c]	0.049

DMI, dry matter intake; ADG, average daily gain
*Results related to ADG and total gain are the mean of 14 weeks of feeding.

Effect of treatment diets on carcass traits: The effect of the treatment diets on carcass attributes and also carcass analyses are shown in Tables 3 and 4, respectively. The CON group had significantly higher (P<0.05) warm and chilled carcass weights. The dressing percentage of the OPF group was 32.3%, significantly lower than those of both HAF (40.7%) and CON (43.3%) groups. The carcass pH either warm or chilled was similar (P>0.05) in all groups. Both rib-eye area and back fat thickness differed significantly (P<0.05) among the treatment groups. The back fat thickness of the HAF and CON groups was two times and three times thicker than that of the OPF group, respectively. Subcutaneous fat color and firmness score were not affected (P>0.05) by the treatment diets after 14 weeks of feeding. The overall meat color score and rib-eye area marbling score were significantly different (P<0.05) when the OPF group was compared to both HAF and CON groups. Both HAF and CON groups had similar (P>0.05) meat color and rib-eye area marbling score. The OPF meat was paler and showed less marbling compared to both HAF and CON meats.

The overall meat color scores showed significant differences (P<0.05) between the OPF group and the HAF and CON groups. However, the individual meat color scores, as depicted in Table 4, showed that the meat colors of *longissimus dorsi*, *psoas major*, *gluteus medius*, *semimembranosus* and *triceps brachii* muscles did not differ among the treatment groups. The individual muscle weights, however, differed significantly (P<0.05) due to the treatment effects and were always presented in a decreasing order of CON>HAF>OPF. The OPF animals, being smaller and lighter at slaughter, had a significantly (P<0.05) lower amount of total muscles, total fats, bones and connective tissues compared to the other two groups. Both subcutaneous fats and intramuscular fats were heaviest in the CON group and differed significantly among all treatment groups (P<0.05). In fact, both HAF and CON groups had three times and four times more total fats, respectively, when compared with the OPF group.

Table 3 Effect of dietary supplementation of oil palm frond on sheep carcass attributes after 14 weeks of feeding (Mean±SD)

Attributes	Experimental diets			*P*-value
	CON	HAF	OPF	
Dressing percentage (%)	43.30 ± 2.4[a]	40.70 ± 1.7[b]	32.30 ± 1.2[c]	0.021
Warm carcass weight (kg)	10.60 ± 1.7[a]	9.10 ± 0.6[b]	5.70 ± 0.7[c]	0.032
Chilled carcass weight (kg)	10.20 ± 1.6[a]	8.60 ± 0.6[b]	5.40 ± 0.5[c]	0.035
Warm carcass pH	6.60 ± 0.47	6.79 ± 0.37	6.77 ± 0.45	0.200
Chilled carcass pH	6.43 ± 0.18	6.42 ± 0.34	6.47 ± 0.24	0.430
Rib-eye area (REA) (cm^2)	12.13 ± 1.62[a]	10.11 ± 1.46[b]	7.08 ± 0.76[c]	0.012
Back fat thickness (mm)	3.30 ± 0.9[a]	2.40 ± 0.5[b]	1.00 ± 0.6[c]	0.039
Subcutaneous fat color score[i]	3.00 ± 0.07	3.00 ± 0.05	2.00 ± 0.04	0.340
Subcutaneous fat firmness score[ii]	3.00 ± 0.07	3.00 ± 0.03	3.00 ± 0.05	0.500
Overall meat color score[iii]	4.00 ± 0.1[a]	3.00 ± 0.05[a]	2.00 ± 0.2[b]	0.040
REA marbling score[iv]	3.00 ± 0.1[a]	2.5 ± 0.3[a]	1.00 ± 0.04[b]	0.043

OPF = 50% w/w oil palm frond in diet. HAF = 25% w/w oil palm frond in diet. CON = control.
[a, b, c]Values with different superscript letters within a row differ significantly at P<0.05.
Warm carcass weight and warm carcass pH were measured within one h post-slaughter. Chilled carcass weight and chilled carcass pH were measured at 24 h post slaughter. Rib-eye area (REA) and back fat thickness were measured at locations between 12th-13th ribs.
[i] 1-White → 5-Very yellow
[ii] 1-Firm → 5-Very soft and oily
[iii] 1-Pale → 5-Dark red
[iv] 1-No marbling → 7-Very pronounced marbling

Table 4 Effect of dietary supplementation of oil palm frond on sheep carcass and muscle tissue analyzed after 14 weeks of feeding (Mean±SD)

Parameters	Experimental diets			*P*-value
	CON	HAF	OPF	
Psoas major (g)	82 ± 80[a]	68 ± 60[b]	44 ± 30[c]	0.031
Gluteus medius (g)	181 ± 19[a]	150 ± 90[b]	92 ± 60[c]	0.022
Semimembranosus (g)	1388 ± 21[a]	1264 ± 74[b]	768 ± 57[b]	0.033
Longissimus dorsi (g)	576 ± 66[a]	460 ± 27[b]	250 ± 22[c]	0.036
Triceps brachii (g)	602 ± 21[a]	448 ± 10[b]	440 ± 10[b]	0.020
Subcutaneous fat (g)	600 ± 93[a]	452 ± 47[b]	116 ± 16[c]	0.043
Intermuscular fat (g)	674 ± 87[a]	508 ± 50[b]	144 ± 18[c]	0.012
Total muscles (g)	5398 ± 56[a]	4828 ± 18[b]	2818 ± 19[b]	0.039
Total fats (g)	1420 ± 16[a]	1068 ± 78[b]	352 ± 51[c]	0.034
Total connective tissues (g)	320 ± 36[b]	286 ± 38[b]	401 ± 18[a]	0.0300
Total bone (g)	2952 ± 17[a]	2496 ± 11[b]	2024 ± 10[c]	0.040
Meat to bone ratio (M:B)	1.86 ± 04[b]	1.94 ± 03[a]	1.35 ± 01[b]	0.043
Meat to fat ratio (M:F)	3.77 ± 01[b]	4.91 ± 04[b]	7.55 ± 05[a]	0.039
Meat color score†				
Longissimus dorsi	2 ± 01	2 ± 05	2 ± 01	0.300
Psoas major	2 ± 03	2 ± 03	1 ± 02	0.401
Gluteus medius	2 ± 01	2 ± 01	2 ± 05	0.430
Semimembranosus	2 ± 02	2 ± 01	2 ± 03	0.330
Triceps brachii	2 ± 01	2 ± 04	2 ± 07	0.200

† 1-Pale, 2-Pale red, 3-Pinkish red, 4-Cherry red, 5-Dark red.
[a, b, c]Values with different superscript letters within a row differ at P<0.05.
OPF = 50% w/w oil palm frond in diet. HAF = 25% w/w oil palm frond in diet. CON = control.

However, the total muscle weight was about 1.7-2.0 times greater in the HAF and CON animals than that of the OPF animals. Meat to bone ratio (M:B ratio) and meat to fat ratio (M:F ratio) were not significantly different between the HAF and CON groups. However, these values were significantly (P<0.05) unfavorable for the OPF group. There was a trend indicating that the HAF group had higher M:B and M:F ratios compared to the CON group.

Effect of treatment diets on rumen fermentation parameters: The effects of dietary supplementation of oil palm frond on sheep rumen fermentation characteristics at 0 and 8 h of measurement after 14 weeks of feeding are shown in Table 5. The pH values in all treatment groups were always in a decreasing order (P<0.05) of OPF>HAF>CON at 0 and 8 h of measurement. By 8 hours post feeding, the rumen NH_3N levels in the OPF group were similar to the levels at 0 h post feeding. At this time, the rumen NH_3N level was the highest in the CON animals (P<0.05), followed by the HAF animals. The rumen NH_3N content of the HAF group was almost always midway between those of the OPF and CON groups. The levels of total VFA in both the HAF and CON groups were constantly higher than that of the OPF group at all time points (0 and 8 h) (P<0.05). At 0 h post feeding, the molar proportions of acetic, propionic, butyric acids and other minor VFA were not significantly different (P>0.05) between all treatment groups. The OPF group had much higher level of acetic acid (P<0.05) while both the HAF and CON groups had much more (P<0.05) propionic acid when compared to the OPF group at 8 h of measurement.

Effect of treatment diets on rumen microbial population: The effects of dietary supplementation of oil palm frond on sheep rumen microbial populations at 0 and 8 h of measurement after 14 weeks of feeding are shown in Table 6. The OPF group clearly had the highest number of holotrichs compared to both the HAF and CON groups (P<0.05) at 0 and 8 h of feeding. There was no significant differences (P>0.05) among the treatments in terms of entodiniomorphs at 0 and 8 h of feeding. The OPF group tended to have more total protozoa (P<0.05) compared to both HAF and CON groups at 0 h of feeding. At 0 and 8 h of feeding, the CON animals clearly had the highest number of total bacteria compared to the other animals (P<0.05). The bacteria to protozoa density ratios of all the treatment groups were not significantly different at 8 h of feeding (P>0.05).

Table 5 Effect of dietary supplementation of oil palm frond on sheep carcass and muscle tissue analyzed after 14 weeks of feeding (Mean±SD)

Parameters		Experimental diets			*P*-value
		CON	HAF	OPF	
pH					
	0	6.32 ± 0.21^b	6.54 ± 0.15^{ab}	6.70 ± 0.19^a	0.030
	8	5.79 ± 0.13^c	6.16 ± 0.20^b	6.66 ± 0.09^a	0.040
NH_3N (mM)					
	0	9.21 ± 8.4^a	8.19 ± 7.1^b	17.19 ± 8.2^c	0.021
	8	17.43 ± 13.0^a	13.10 ± 10.0^b	17.0 ± 11.4^c	0.038
Total VFA (mM)					
	0	54.4 ± 2.6^a	51.6 ± 3.7^a	45.1 ± 4.7^b	0.020
	8	6.66 ± 0.13^a	6.16 ± 0.20^a	5.79 ± 0.09^b	0.040
Acetic acid					
	0	0.62 ± 02	0.60 ± 01	0.65 ± 04	0.300
	8	0.59 ± 02^b	0.58 ± 03^b	0.73 ± 03^a	0.040
Propionic acid					
	0	0.27 ± 01	0.28 ± 04	0.21 ± 05	0.403
	8	0.27 ± 04^a	0.27 ± 03^a	0.18 ± 02^b	0.018
Butyric acid					
	0	0.09 ± 02	0.11 ± 01	0.10 ± 02	0.305
	8	0.12 ± 02	0.13 ± 02	0.08 ± 002	0.800
Other VFAs					
	0	0.02 ± 001	0.01 ± 003	0.04 ± 002	0.395
	8	0.02 ± 003	0.02 ± 001	0.01 ± 002	0.100

OPF = 50% w/w oil palm frond in diet. HAF = 25% w/w oil palm frond in diet. CON = control.
[a, b, c]Values with different superscript letters within a row differ significantly at P<0.05.

Discussion

Diets containing a large proportion of concentrate are generally given to ruminants when high performances are expected (Istasse et al., 1986). The average daily gain obtained in this study for the CON group (113 g) was slightly better compared to the 103 g/d reported by Wattanachant (1999) in sheep with similar ages fed 60-80% concentrate and grass *ad libitum* in an intensive production system. Our values were closer to that reported by Basery et al. (1993) at 109 g/d. The average daily gain for the OPF group was found to vary from the sheep reared under an animal-crop integration system at 45 g/d (Wattanachant, 1999). However, contrary to our study, replacing 50% of concentrate in the diet of Kacang cross-bred goats with OPF did not affect growth performance parameters such as average daily gain and final body weight after 100 days of feeding; in fact, in that study, the protein and energy levels for all treatments were adjusted (Ebrahimi et al., 2015). Both dietary protein and energy (AFRC 1993) levels were found to influence growth rates of sheep. Although our diets had similar gross energy content among the treatments, the metabolizable energy was thought to be different as it depends very much on the digestibility of the feed constituent within a dietary mix (McDonald et al., 1991). Clearly, the oil palm frond pellet, with its high fiber content, has less metabolizable energy compared to the highly digestible concentrate (Islam, 1999). It was reported that lambs fed high-energy diets generally had higher live weights, more subcutaneous kidney and pelvic fats, larger rib-eye area and better quality grades compared to those fed low-energy diets (Ebrahimi et al., 2013). Clearly, the growth parameters reflect the effects of the availability of dietary energy and protein of the treatment diets despite their similarity in gross energy content. The availability of energy and protein of diets normally affects both growth performance and carcass characteristics.

Table 6 Effect of dietary supplementation of oil palm frond on sheep rumen microbial populations at 0 and 8 h of measurement after 14 weeks of feeding (mean±SD)

Parameters		Experimental diets			*P*-value
		CON	HAF	OPF	
Bacteria density (×10⁴ mL)					
	0	2.93 ± 0.71[a]	1.30 ± 0.52[b]	0.75 ± 0.21[b]	0.035
	8	1.84 ± 0.39[a]	1.99 ± 0.85[a]	1.13 ± 0.20[b]	0.030
Total protozoa density (×10⁴/mL)					
	0	6.33 ± 4.52[b]	10.67 ± 6.47[b]	19.83 ± 6.24[a]	0.025
	8	9.50 ± 7.89	11.83 ± 5.53	10.83 ± 4.36	0.380
Holotrich density (×10⁴ mL)					
	0	0.50 ± 0.55[c]	3.50 ± 2.26[b]	9.00 ± 2.76[a]	0.022
	8	1.66 ± 0.13[c]	2.50 ± 1.87[b]	4.00 ± 0.89[a]	0.040
Entodiniomorph density (×10⁴ mL)					
	0	5.83 ± 4.31	7.17 ± 4.49	10.83 ± 5.27	0.300
	8	9.50 ±7.89	9.33 ± 4.63	9.83 ± 4.45	0.304
Bacteria to protozoa density ratio					
	0	0.46 ± 52[a]	0.12 ± 04[b]	0.03 ± 05[c]	0.037
	8	0.19 ± 04	0.16 ± 03	0.10 ± 02	0.180

OPF = 50% w/w oil palm frond in diet. HAF = 25% w/w oil palm frond in diet. CON = control.
[a, b, c]Values with different superscript letters within a row differ significantly at P<0.05.

Our results showed that the overall meat color scores were significantly higher (or darker) in the CON and HAF groups compared with that of the OPF group. This is in agreement with the statements by Oliveros et al. (2009) that animals fed concentrate had slightly darker lean color than forage-fed animals, but it was undetectable by consumers. All animals showed low levels of marbling due to their young age. However, both HAF and CON carcasses had considerably better marbling scores in their rib-eye area compared to the OPF carcasses. Consistent with our study, goats fed different levels of concentrate diet had more marbling score than goats without concentrate in their diet (Ryan et al., 2007). There were no significant differences among the treatment groups for the warm and chilled carcass pH. This is contrary to the hypothesis that animals fed commercial pellets intensively tend to have better nutritional status and accumulate more glycogen reserves than those fed primarily forage fiber, and thus have lower ultimate pH compared to carcasses from forage-fed animals (Wiklund et al., 2001). In the current study, the heavier carcass from the CON animals was shown to have a higher amount of total fat compared to those from the OPF animals. It has been stated that decreased inclusion of OPF could increase total fat in the muscle tissues of the animal (Ebrahimi et al., 2015). The overall low amount of fat in the sheep carcass can be explained by the low digestibility of OPF which leads to lower nutrient utilization. Consistent with our results, Yusuf et al. (2014) reported that higher carcass trait such as higher muscle and fat proportion could be explained by more efficient digestion of nutrients while lower visible fat contents could probably be due to the effect of less-digestible products altering the rumen fermentation processes and, hence, helping in maintaining an efficient digestive function.

The CON animals had almost twice rib-eye area than the OPF animals. Similar trends were observed for the back fat thickness as well. However, the carcass fat characteristics (firmness, texture, and color) were not affected by the dietary metabolizable energy, which is again in agreement with the results of Hanekom et al. (2010). The CON and HAF carcasses had significantly better meat to bone ratio compared to the OPF carcass. This ratio is a form of muscling index in live animal or carcass, associated with increased meat production and early maturity in lambs. It could be used as a rough estimate of lean content and, therefore, the higher the better (Gaias, 2012).

High-concentrate diets are known to lower rumen pH, which in turn will inhibit lipolysis and biohydrogenation (De Smet et al., 2000). It was noted that the CON group also had the lowest rumen fluid pH. Taking that the critical pH for effective fiber digestion is between pH 6.0-7.0 (Orskov and Ryle, 1990), it appears that cellulolysis was severely impaired in the rumen of the CON animals. The reduced pH in the CON group was mainly due to fermentation of the sugars and starch supplements within the concentrate feed (Huuskonen et al., 2014). Intermittent feeding results in periods of very low pH after feeding, but separated by periods of higher pH which allows the recovery of cellulolytic microflora provided that adequate dietary fiber is present to maintain rumination and in salivation (Lees, 2016).

This probably resulted in the animals fed HAF diets with equal amounts of concentrate and fiber contents having subtle fluctuations in the rumen fluid pH than the CON animals. This may have helped to preserve the rumen microbial composition which contributed to the mass biohydrogenation of the available dietary unsaturated fatty acids compared to the CON animals. It is sufficient at this stage to know that the rumen pH following the feeding of the treatment diets was in the decreasing order of OPF>HAF>CON at all times (0 and 8 h).

The different concentrations of NH_3N in the treatment groups at different times of measurement were similar to those described by Offer and Percival (1998) for sheep. The concentration of NH_3N in the rumen is known to vary greatly with the diet (Valente et al., 2016). All animals in this trial exceeded the suggested rumen NH_3N levels of at least 50-80 mg nitrogen/L to maximize microbial protein synthesis (Samanta et al., 2003). The levels of available ruminal nitrogen are critical to the protein nutrition and the growth of animals (McDonald et al., 1991). It was expected that the amount of recycled nitrogen would be lower in the CON animals than the OPF animals as the amount of recycled nitrogen could be as much as 70% of the protein intake if the dietary protein intake is low (5% crude protein). When the protein intake is high (20% crude protein), the contribution of recycled nitrogen could be reduced to 11% of the protein intake (Hackmann et al., 2015). This ensures that a higher amount of dietary protein is available for uptake in the hindgut, and therefore the better growth rates among the CON animals. In the HAF and CON animals, the total volatile fatty acids were consistently higher than in the OPF animals.

Consistent with our results, Yi et al. (2015) indicated that replacement of pelletized broccoli by-products with concentrate in the diet at a level of 40% enhanced the molar proportion of acetate and depressed the proportion of propionate in dairy cows. They also concluded that those changes might have a negative effect on energy use because propionate would increase energy use efficiency by decreasing energy losses on inter-species hydrogen transfer and methane gas production. The molar proportion of acetic acid was higher in the OPF animals, while the propionic proportion was higher in the HAF and CON animals. This is in agreement with Ebrahimi et al. (2015) who concluded that a high proportion of acetic acid was a characteristic of a high fiber diet, while high grain diets typically had bigger proportions of propionic acid. It is known that the main VFA constituents and acetic, propionic, butyric acids are utilized with equal energy efficiency in ruminants (Orskov and Ryle, 1990), although their amounts in the rumen are related to the level of feed intakes (Dijkstra et al., 1993).

Previous results in cattle, sheep, and goats showed that, in concentrate feeding, entodiniomorphs were the dominant protozoa family found in the rumen (Ebrahimi et al., 2015) and the results of employing oil palm frond pellets as the sole fiber source in this study confirmed those findings. Hristov et al. (2013) showed that increasing the level of grain inclusion in the diet would result in an increased protozoal count. On the contrary, in the present study, the HAF and CON groups had significantly less protozoa than the OPF group. Holotrich can only survive at pH above 6.0, which corresponds to a diet rich in fiber and high in soluble sugars (Ebrahimi et al., 2015). Thus, the OPF group was observed to have the highest number of holotrichs. The reduced protozoal population in the current study for CON and HAF compared to OPF is consistent with the results of Foiklang et al. (2016). They showed that supplementation of grape pomace powder and mangosteen peel powder could reduce the population of protozoa in steers. They also attributed their results to the presence of bioactive compounds in the mentioned supplements which has been approved in OPF as well (Ebrahimi et al., 2015). It has been shown that protozoa provide hydrogen as a substrate for methane gas production conducted by the methanogens (Foiklang et al., 2016). Therefore, a reduction in protozoa population may lead to a decrease in methanogen population and, subsequently, methane (the second prevalent green-house gas) emission as well, whereas the acetic acid production is concomitantly reduced since it is a product of protozoa metabolism from the fermentation of sugar (McAllister and Newbold, 2008). Conversely, the production of propionic acid is accelerated since the electron transfer reaction has to search for an alternative pathway (an alternative hydrogen sink) (McAllister and Newbold, 2008). The reduced acetic and increased propionic acids observed in CON and HAF compared to OPF could also be due to mentioned factors.

According to the data obtained, it could be concluded that both HAF and CON animals had better performances compared to the OPF animals. This could be due to the higher availability of ruminal nitrogen and higher metabolizable energy content of both dietary mixes. The supplementation of 25% of OPF (HAF) could result in improved rumen fermentation efficiency such as increased propionic acid concentration in sheep. Interestingly, HAF could reduce acetic acid concentration and protozoal population. Therefore, OPF, which is an easily available oil palm by-product, could be used as a feed ingredient at 25% inclusion levels to support sheep farming in tropical countries that lack grazing pasture, with no apparent adverse effects on growth performance if the diets are adjusted on an isocaloric and isonitrogenous basis.

Acknowledgements

The authors would like to thank the Faculty of Veterinary Medicine, Faculty of Agriculture, Universiti Putra Malaysia and Faculty of Agriculture, Tarbiat Modares University.

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บทคัดย่อ

ผลของการเสริมความเข้มข้นของน้ำมันปาล์ม (*Elaeis guineensis*) ทดแทนอาหารข้นต่อการหมักในกระเพาะ คุณภาพซาก และเชื้อจุลินทรีย์ในแกะ

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การศึกษาเพื่อประเมินประสิทธิภาพของใบปาล์มน้ำมันเมื่อใช้เป็นอาหารเสริมเลี้ยงแกะ [oil palm *(Eleis guineensis)* frond (OPF)] โดยประเมินจากการเจริญเติบโตของแกะ ปริมาณประชากรแบคทีเรียในกระเพาะส่วนรูเมน (Rumen) และลักษณะของซากแกะ โดยทำการทดลองในแกะ (น้ำหนัก 20 ± 1.9 กิโลกรัม) จำนวน 30 ตัว แบ่งแกะทดลองออกเป็น 3 กลุ่ม แต่ละกลุ่มได้รับอาหารผสมกับใบปาล์มน้ำมันในปริมาณที่แตกต่างกัน แกะทดลองในกลุ่มแรกได้รับอาหารที่มีส่วนผสมของใบปาล์มน้ำมัน 25 เปอร์เซ็นต์ (% w/w) (กลุ่ม HAF, n=10) แกะทดลองในกลุ่มที่สองได้รับอาหารที่มีส่วนผสมของใบปาล์มน้ำมัน 50 เปอร์เซ็นต์ (% w/w) (กลุ่ม OPF, n=10) และแกะทดลองในกลุ่มที่ 3 (กลุ่มควบคุม) ได้รับอาหารที่ไม่มีใบปาล์มน้ำมัน (กลุ่ม CON, n=10) แกะทดลองได้รับอาหารทดลองเป็นเวลา 100 วัน จากนั้นทำการส่งแกะเข้าสู่โรงเชือดเพื่อเก็บตัวอย่างซากแกะและของเหลวในกระเพาะอาหารส่วนรูเมน การทดลองพบว่า แกะทดลองจากกลุ่ม HAF และ CON มีกรดโพรไพโอนิค (propionic acid) มากกว่าและกรดอะซิติก (acetic acid) น้อยกว่าที่ชั่วโมงที่ 8 ของการเก็บตัวอย่าง ($P<0.05$) เมื่อเปรียบเทียบกับกลุ่ม OPF ในส่วนของการประเมินซากพบว่ากลุ่ม HAF และ CON มีไขมันแทรกภายในกล้ามเนื้อ (Marbling) มากกว่ากลุ่ม OPF ($P<0.05$) นอกจากนี้ พบว่ากลุ่ม HAF และ CON มีปริมาณของแบคทีเรียในของเหลวต่อมิลลิลิตรจากกระเพาะอาหารส่วนรูเมนที่ชั่วโมงที่ 0-2 ของการเก็บตัวอย่างมากกว่ากลุ่ม OPF ใบปาล์มน้ำมันนั้นเป็นวัตถุดิบที่หาได้ง่ายกว่าผลผลิตจากปาล์มน้ำมัน (By-product) ดังนั้น จากผลการทดลอง จึงแนะนำว่าใบปาล์มน้ำมันสามารถใช้เป็นอาหารเสริมในอัตราส่วน 25 เปอร์เซ็นต์เพื่อช่วยเสริมการเจริญเติบโตของแกะในประเทศเขตร้อนชื้นที่ไม่มีทุ่งหญ้าเลี้ยงสัตว์

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