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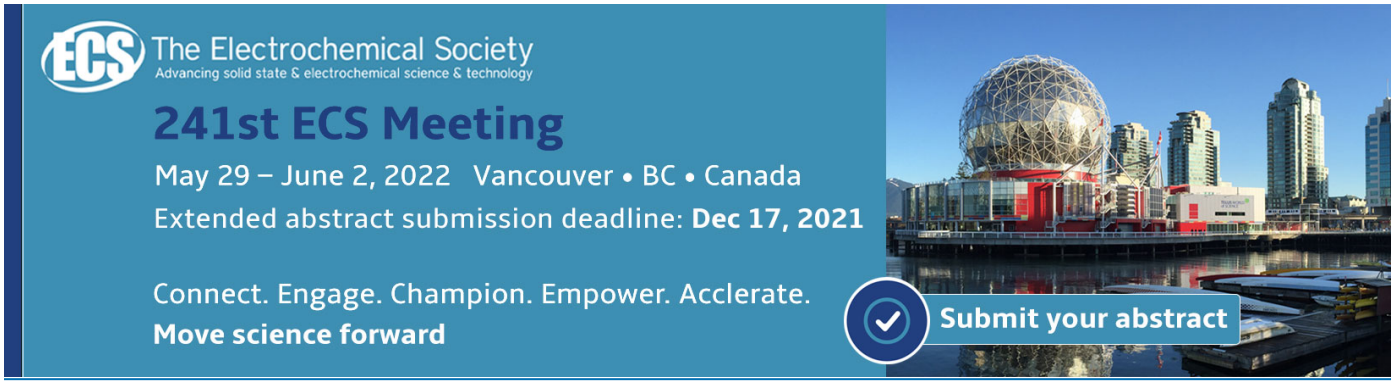

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Fatty acid profile of meat goats fed pre-treated oil palm frond

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Abstract. The main factor affecting the fatty acid (FA) profile of ruminant-derived products is diet composition. In order to determine the effect of pre-treated oil palm frond (OPF) on FA profiles of muscle tissues and subcutaneous fat, twenty crossbred male Boer goats were used for the animal feed trial. Five treatment diets were dietary control consisted of Napier grass (CON); Napier grass with non-treated OPF (NON); Napier grass and physically pre-treated OPF (PHY); Napier grass and biologically pre-treated OPF (BIO) and Napier grass and combined pre-treated OPF (COM). All groups were supplemented with 30% commercial goat pellet. The animals were slaughtered for sampling meat tissues after 130 days of feeding. The saturated fatty acid (SFA) (% of the total in FA) the *longissimus dorsi* muscle and subcutaneous fat of the CON group were significantly ($p<0.05$) higher than the pre-treated OPF groups, but no significant difference in *biceps femoris* muscle. In *biceps femoris*, stearic acid (C18:0) was higher in CON and NON groups than PHY, BIO and COM ($p<0.001$). In *longissimus dorsi*, C18:0 was found higher in NON ($p<0.05$), while in subcutaneous fat, C16:0 was found higher in CON and NON groups. For UFA, *biceps femoris*, *longissimus dorsi* and subcutaneous fat had higher UFA in BIO and COM groups ($p<0.05$). In addition, biological and combination pretreatments of OPF increased the PUFA: SFA ratio significantly compared with CON, NON and PHY groups ($p<0.05$). This finding suggested that biological pretreatment with enzyme extract from *Ganoderma lucidum* effectively enhances the nutritional value of OPF promoting the UFA in meat.

1. Introduction

A strategy emerging at the intersection between food science and animal science is the use of nutritional approaches for improving the quality of livestock feed. Nutritional approaches such as dietary intervention to change the composition of fatty acid in ruminant-derived meat products tend to be of concern due to the correlation of saturated fatty acids (SFA) with atherosclerosis-related diseases as the leading cause of mortality across the globe [1]. In ruminants, dietary polyunsaturated fatty acids (PUFA) are extensively biohydrogenated, resulting in increased SFA levels in the tissue and blood

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circulation. Consequently, beef and dairy products are high in SFA, which may adversely affect human health [2]. Based on the previous study, oil palm frond (OPF) increased the total n-3 PUFA in sheep plasma, which may improve human health [3]. However, another study reported the contradictory finding of the inclusion of OPF diet on fatty acid metabolism in which the OPF feeding did not improve the fatty acid composition of ruminants [4; 5].

The oil palm by-product, which is OPF, is available throughout the year and sustainable for ruminant feed in the tropical countries [6]. However, OPF contains poor nutritive values with high lignin content (205 g/kg DM) which can impair its digestibility and intake of the animal. Positive effects of OPF supplementation on certain characteristics (performance, digestion and microbial populations) in goats and sheep have been documented in previous studies [5; 7]. However, there is still little evidence to date on the effects of pre-treated OPF supplementation on fatty acid composition and meat quality of goats. Therefore, the aim of this experiment was to investigate the efficacy of dietary manipulation using pre-treated OPF on the fatty acid profile of goat muscle tissues.

2. Materials and Methods

2.1 Animal management

Twenty 5-month old male, cross-bred Boer goats were individually housed and allotted randomly into five treatment groups after a two-week acclimatisation period; Group 1 (CON) was a standard diet-based control group (70% Napier grass and 30% goat pellet) and four treatment groups with different OPF pretreatment; Group 2 (NON) consisted of 20% non-treated OPF, 50% Napier grass and 30% goat pellet; Group 3 (PHY) consisted of 20% physical pre-treated OPF following the pressing technique using sugarcane machine, 50% Napier grass, and 30% goat pellet; Group 4 (BIO) consisted of 20% biological pre-treated OPF with enzyme extract from *Ganoderma lucidum*, 50% Napier grass, and 30% goat pellet; Group 5 (COM) consisted of 20% of combined pre-treated OPF (10% physical OPF + 10% biological OPF), 50% Napier grass and 30% goat pellet. The animal feed trial was carried out at the Agrotechno Park, Universiti Malaysia Kelantan (UMK), Jeli Campus as per the guidelines and approval on animal ethics (UPM/IACUC/AUP-R039/2016) and UMK. The nutritional values of the experimental diet as shown in Table 1.

Table 1. Chemical composition of experimental diets in all groups.

Experimental groups	CON	NON	PHY	BIO	COM
Dry matter (%)	26.41±0.71	24.27±0.69	33.03±0.43	38.16±0.19	39.20±1.31
Ether extract (%)	2.01±0.10	1.62±0.03	1.00±0.02	1.80±0.02	1.72±0.08
Crude fibre (%)	33.26±0.09	22.60±1.00	22.71±0.57	20.12±0.21	20.25±1.10
Crude protein (%)	12.31±0.52	9.23±0.19	6.14±0.02	12.87±0.80	13.16±0.76
Ash (%)	5.16±0.01	2.36±0.06	2.10±0.02	4.00±0.01	4.21±0.06
Metabolisable energy (MJ/kg DM)	11.31±0.15	11.47±0.11	11.52±0.07	11.32±0.10	11.58±0.21
Hemicellulose (%)	24.36±0.10	22.10±0.30	18.19±0.21	16.07±0.54	15.08±1.20
Cellulose (%)	34.61±0.32	30.10±0.86	30.08±0.41	35.78±0.22	31.23±0.75
Lignin (%)	6.9±0.05	10.89±0.45	10.90±0.04	8.9±0.04	9.12±0.02

Notes: CON: control diet or basal diet (70% Napier grass and 30% goat pellet); NON: 20% Non-treated OPF (FOPF), 50% Napier grass and 30% goat pellet; PHY: 20% Physical pre-treated OPF (POPF), 50% Napier grass and 30% goat

pellet; BIO: 20% Biological pre-treated OPF (BGL), 50% Napier grass and 30% goat pellet; COM: 20% combination pre-treated OPF, 50% Napier grass and 30% goat pellet.

2.2 Slaughter procedure and sampling

The animals were humanely slaughtered at the end of the feeding trial (day 130) in Agrotechno Park, Jeli Campus, UMK, according to the HALAL procedure as outlined in Malaysian Standard MS1500:2009 (Department of Standards Malaysia, 2009). Following overnight cooling at 4°C, the muscles of *longissimus dorsi* and *biceps femoris* were collected from the left side of the carcass. The subcutaneous fat was stripped from the surface of the meat, wrapped in aluminum foil, covered in plastic bags of polyvinyl chloride (PVC) and preserved at -20°C before analysing the fatty acids.

2.3 Chemical analysis of feed

The feeds were analysed for dry matter (DM), ash, crude fibre (CF) and crude protein (CP) [8] contents. Ether extract (EE) was determined by Foss Extraction system (Foss, Gerhardt, Germany) by extraction with petroleum ether. Acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL) were determined as described by Van Soest et al. [9] and expressed inclusive of residual ash. Hemicellulose was calculated as NDF – ADF and cellulose as ADF – ADL. The metabolisable energy (ME) values of the feeds were calculated based on the following equation: ME (MJ/kg DM) = 0.016 × digestible organic matter.

2.4 Fatty acid composition

Chloroform-methanol (2:1 v/v) was used to extract fatty acid from the muscles of *longissimus dorsi* and *biceps femoris* as well as subcutaneous fat following the procedure of Folch et al.[10], modified by Rajion et al.[11]. In brief, the meat sample was placed in a 50 ml extraction tube and homogenized with the Ultra-Turtax T5 FU homogeniser in 40 ml chloroform-methanol 2:1 (v/v). To determine the individual concentration of fatty acids within the sample, an internal standard, heneicosanoic acid, C21:0 was applied to the samples prior to transmethylation. Potassium hydroxide in methanol and 14% boron trifluoride in methanol were used for transmethylation of derived fatty acids to their fatty acid methyl esters (FAME). The FAME was separated by gas chromatography (Agilent 7890A) with a film thickness of 0.2 μm using a 30 cm 0.25 mm ID capillary column (Supelco, Bellefonte, PA, USA).

2.5 Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science 20.0 (SPSS, USA). All parameters were statistically tested using a one-way ANOVA at a significance level of 5%. Tukey test was performed to explicate significant differences.

3. Results and Discussion

3.1 Fatty acid profile of goat muscles

The FA profiles for different goat muscles; *longissimus dorsi* and *biceps femoris* are shown in Tables 2 and 3, respectively. The highest FA in the goat muscles (Tables 2 and 3) were palmitic acid (C16:0) (24-50%), stearic acid (C18:0) (18-25%) and oleic acid (C18:1 n-9) (8-44%). No significant differences were detected in the proportions of C16:0, C18:1 n-9 and arachidonic acid (C20:4 n-6) in *biceps femoris* muscle ($p<0.05$). However, stearic and linoleic acids in *biceps femoris* muscle showed a lower level of stearic and linoleic acids in BIO and COM than CON and NON ($p<0.05$) groups, meanwhile no significant difference of stearic acid was observed between PHY, BIO and COM groups. In *longissimus dorsi* muscle, palmitic, linoleic and arachidonic acids were not affected by the pretreatments of OPF. Still, the proportions of stearic and oleic acids were significantly greater in the BIO and COM groups, as compared to the control and other treatment groups (i.e. NON and PHY).

The main FAs in goat tissues were oleic, stearic and palmitic acids, as reported recently [12; 13]. The main factors that affect fatty acid composition of the ruminant tissue are breed, diet, sex and

environment. Previously, it was reported that as the inclusion levels of OPF increased, the total SFA in the muscles of Katjang crossbred male goats decreased [14], consistent with the findings in the muscles of sheep fed OPF diet [15]. Such finding could be explained by the increase of fibre feeding from OPF diet and the presence of secondary metabolites in OPF such as tannins and phenolic compounds which could interfere the biohydrogenation of PUFA, resulting in lower production of SFA [16]. The findings observed in the current study mirror those of the previous studies aforementioned [14]. However, no scientific study reported the effect of the biological pretreatment of OPF on the fatty acid composition of goat tissues.

Based on Table 2, there are no significant differences of total SFA (% of total FA) in *biceps femoris* muscle, but the UFA (% of total FA) was highest in COM ($p<0.05$) group. Nevertheless, Table 3 shows that BIO and COM groups in *longissimus dorsi* muscle had low amount of SFA (% of total FA) and high amount of UFA (% of total FA) as compared to CON, NON and PHY groups ($p<0.05$). Thus, the UFA: SFA ratio was always significantly high in all meat tissues of animals fed biological pretreated OPF. However, CON, NON and PHY groups were consistently high in SFA. This finding indicated that the increase in SFA may be attributed to palmitic acid growth. The higher dietary fibre intake for animals may be a potential reason for these findings, as the fibre content of these diets was higher than that of the OPF group's biological pretreatment. The findings were supported by Rusli et al., [17] who reported that non-treated OPF and physical pretreatment of OPF contained higher CF than other pre-treated OPF. This finding may be explained by the fact that high fibre diet increases rumen activity, resulting in the extent of biohydrogenation of PUFA gradually increasing by the rumen microbes [18]. The result was also consistent with the previous *in vitro* work which stated that OPF stimulates biohydrogenation of the rumen [5].

Biological pretreatment of OPF either alone or combined with physical pretreatment decreased the proportions of the SFA in muscles and subcutaneous fat. This was balanced out by increasing total UFA, thus all tissues had a significantly better fatty acid unsaturation index among all treatment groups. In the current study, the *biceps femoris* and *longissimus dorsi* muscles presented greater total UFA (% of total FA) which consist of oleic, linoleic and arachidonic acids in goats fed biological pretreatment OPF ($p<0.05$) when compared to control, non-treated OPF and physical pretreatment OPF groups. The UFA: SFA ratio was always significantly higher in all the muscles and subcutaneous fat of animals fed biological pre-treated OPF compared to control, non-treated OPF and physical pre-treated OPF. These results match those observed in our previous study that biological pretreatment with enzyme extract of *G. lucidum* improved the *in vitro* rumen degradability and increased total gas production, ARDC and VFA [17].

3.2 Fatty acid profile of subcutaneous fat

The FA profiles for subcutaneous fat is shown in Tables 4. The main FAs in the subcutaneous fat, similar to muscles, were oleic, palmitic, and stearic acids. In the subcutaneous fat, the oleic acid ranged between 17 and 29 g/100g. The higher proportion was shown by COM compared to control and other treatment diets ($p<0.05$). In addition, the level of palmitic acid ranged between 9.8 and 21.5 g/100g, with no significant differences across treatments except BIO who was significantly lower than all groups. The stearic acid in the subcutaneous fat ranged between 6.5 and 18.9 g/100g with the control group and PHY scored the highest proportion with a significant difference compared to the other groups.

Nevertheless, as the OPF has low-fat content in the current study, there was a low concentration of PUFA in goat tissues. The PUFA could be increased when an external PUFA source is supplemented with the diet. The PUFA: SFA ratio for a healthy diet in meat was around 0.4 g/100 g and the minimum recommended value is 0.45 for human consumption [19]. However, it is difficult to increase PUFA: SFA ratio unless to include oil supplements in the diet, such as whole linseed or linseed oil. The mean ratio of PUFA: SFA in the current study was from 0.25 to 0.34 in *biceps femoris* muscle and

0.30 to 0.44 in *longissimus dorsi* muscle. However, the biological and combination pretreatments of OPF increased the PUFA: SUFA ratio significantly compared with control, non-treated OPF and physical pretreatment of OPF groups ($p<0.05$). This finding suggests that biological pretreatment of OPF improved the quality of goat diet, hence improved the fatty acid composition of meat.

Table 2. Fatty acid composition of the *biceps femoris* muscle of goats fed diets containing pre-treated oil palm frond.

Fatty Acids	Control	NON	PHY	BIO	COM	p-value
	g/100 g of total fatty acids (Mean ± SE)					
Palmitic, C16:0	0.56±0.04	0.56±0.45	0.51±0.03	0.43±0.03	0.5±0.02	0.177
Stearic,C18:0	0.42±0.01^{b}	0.42±0.01^{b}	0.40±0.00^{a}	0.32±0.01^{a}	0.34±0.02^{a}	<0.001
Oleic, C18:1 n-9 cis	0.82±0.03	0.79±0.01	0.86±0.04	0.80±0	0.81±0	0.368
Linoleic, C18:2 n-6	0.18±0.01^{b}	0.20±0.01^{b}	0.18±0.01^{b}	0.11±0^{a}	0.12±0.01^{a}	0.002
Arachidonic, C20:4 n-6	0.06±0.03	0.10±0	0.11±0.00	0.14±0.03	0.12±0.01	0.259
SFA (% of total FA)	48.07±1.58	47.31±1.73	44.02±0.83	41.53±1.47	44.27±1.53	0.057
UFA (% of total FA)	47.88±1.83^{a}	52.69±1.73^{b}	55.97±0.80^{b}	55.72±1.53^{b}	63.05±1.34^{c}	0.000
PUFA n-6	0.25±0.05	0.306±0.01	0.29±0.02	0.25±0.02	0.24±0.02	0.484
UFA:SFA	1.0±0.07^{a}	1.12±0.07^{b}	1.27±0.04^{c}	1.52±0.09^{ab}	1.26±0.07^{c}	0.005
PUFA:SFA	0.25±0.06	0.31±0.02	0.32±0.03	0.34±0.05	0.29±0.03	0.690
TOTAL FA	2.05±0.04^{b}	2.08±0.04^{b}	2.07±0.64^{b}	1.81±0.03^{a}	1.90±0.05^{a}	0.008

Notes: CON: control diet or basal diet (70% Napier grass and 30% goat pellet); NON: 20% Non-treated OPF, 50% Napier grass and 30% goat pellet; PHY: 20% Physical pre-treated OPF, 50% Napier grass and 30% goat pellet; BIO: 20% Biological pre-treated OPF, 50% Napier grass and 30% goat pellet; COM: 20% combination pre-treated OPF, 50% Napier grass and 30% goat pellet

^{abc} values with dissimilar superscripts within a row show significant difference at $P<0.05$

Saturated fatty acids (SFA) = Palmitic (C16:0) + Stearic (C18:0)

Unsaturated fatty acids (UFA) = Oleic (C18:1n-9 cis)

PUFA n-6 = Arachidonic (C20:4 n-6) + Linoleic (C18:2 n-6)

Table 3. Fatty acid composition of the *longissimus dorsi* muscle of goats fed diets containing pre-treated oil palm frond.

Fatty Acids	Control	NON	PHY	BIO	COM	p-value
	g/100 g of total fatty acids (Mean ± SE)					
Palmitic C16:0	0.56±0.04	0.56±0.45	0.51±0.03	0.43±0.03	0.5±0.02	0.177
Stearic, C18:0	0.23±0.04^{b}	0.3±0.01^{ab}	0.11±0.01^{a}	0.23±0.02^{b}	0.20±0.03^{b}	0.011
Oleic, C18:1 n-9 cis	0.14±0.02^{a}	0.09±0.04^{a}	0.12±0.01^{a}	0.60±0.01^{b}	0.59±0.01^{b}	<0.0001

Linoleic, C18:2 n-6	0.13±0.01	0.14±0.03	0.11±0	0.13±0.02	0.15±0.02	0.811
Arachidonic, C20:4 n-6	0.12±0	0.12±0.01	0.16±0.01	0.15±0.02	0.12±0.01	0.229
SFA (% of total FA)	66.87±0.82^{b}	71.23±3.41^{c}	61.09±1.74^{b}	42.69±0.94^{a}	44.64±2.71^{a}	<0.0001
UFA (% of total FA)	33.13±0.82^{a}	28.76±3.42^{a}	38.91±1.75^{a}	55.35±2.70^{b}	57.31±0.93^{b}	<0.0001
PUFA n-6	0.25±0.01	0.26±0.04	0.27±0.02	0.29±0.02	0.27±0.01	0.849
UFA:SFA	0.50±0.02	0.41±0.06	0.64±0.05	1.34±0.05	1.25±0.13	<0.0001
PUFA:SFA	0.32±0.03^{a}	0.30±0.03^{a}	0.40±0.04^{b}	0.44±0.03^{b}	0.44±0.02^{b}	0.028
TOTAL FA	1.19±0.06^{a}	1.22±0.13^{a}	1.03±0.05^{a}	1.56±0.06^{b}	1.57±0.05^{b}	0.002

Notes: CON: control diet or basal diet (70% Napier grass and 30% goat pellet); NON: 20% Non-treated OPF, 50% Napier grass and 30% goat pellet; PHY: 20% Physical pre-treated OPF, 50% Napier grass and 30% goat pellet; BIO: 20% Biological pre-treated OPF, 50% Napier grass and 30% goat pellet; COM: 20% combination pre-treated OPF, 50% Napier grass and 30% goat pellet;
^{abc} values with dissimilar superscripts within a row show significant difference at P<0.05
Saturated fatty acids (SFA) = Palmitic (C16:0) + Stearic (C18:0)
Unsaturated fatty acids (UFA) = Oleic acid (C18:1n-9 cis)
PUFA n-6 = Arachidonic (C20:4 n-6) + Linoleic (C18:2 n-6)

Table 4. Fatty acid composition of the subcutaneous fat of goats fed diets containing pre-treated oil palm frond.

Fatty Acids	Control	NON	PHY	BIO	COM	p-value
	g/100 g of total fatty acids (Mean ± SE)					
Lauric, C12:0	0.14±0.03^{a}	0.45±0.02^{b}	0.82±0.03^{b}	0.1±0.05^{a}	0.14±0.03^{a}	<0.0001
Myristic, C14:0	1.90±0.05^{a}	2.77±0.05^{ab}	13.04±0.10	3.4±0.08^{c}	0.26±0.02^{b}	<0.0001
Pentadecanoic, C15:0	0.40±0.05	0.43±0.02	0.52±0.02^{b}	1.55±0.02^{ab}	0.2±0.11^{a}	0.02
Palmitic, C16:0	12.53±0.78^{ab}	16.06±0.17^{ab}	21.5±0.76^{b}	9.86±4.93^{a}	11.16±0.44^{a}	0.03
Palmitoleic, C16:1	1.00±0.11^{a}	1.73±0.03^{b}	2.16±0.08^{c}	1.1±0.05^{a}	0.83±0.16^{a}	<0.0001
Heptadecanoic, C17:0	0.90±0.05	0.73±0.03^{a}	1.5±0.28^{b}	0.6±0.1^{ab}	0.23±0.14^{ab}	0.002
Stearic, C18:0	11.46±0.29^{c}	9.80±0.15^{b}	18.86±0.24^{c}	9.6±0.20^{b}	6.5±3.25^{a}	0.001
Oleic, C18:1 n-9 cis	16.76±0.14^{a}	17.50±0.26^{a}	25.93±0.06^{b}	29.16±0.18^{c}	20.93±0.47^{b}	<0.0001
Linoleic, C18:2 n-6	0.9±0.30^{a}	1.00±0.05^{a}	1.10±0.05^{a}	4.3±0.17^{c}	1.89±0.00^{ab}	0
Oleic acid, C18:1 n-9 trans	1.06±0.04^{b}	1.16±0.08^{c}	1.9±0.05^{ab}	0.46±0.08^{a}	0.13±0.08	0
SFA (% of total FA)	62.73±0.34^{c}	55.39±1.10^{b}	54.55±0.31^{b}	46.03±0.31^{a}	49.13±4.30^{a}	0.001
UFA (% of total FA)	37.27±0.34^{a}	44.61±1.10^{b}	45.44±0.31^{b}	50.86±4.3^{c}	53.96±0.31^{c}	0.001
PUFA n-6	0.9±0.30^{a}	1.00±0.05^{a}	1.10±0.05^{a}	4.3±0.17^{c}	1.89±0.00^{ab}	0
UFA:SFA	0.59±0.008^{a}	0.81±0.03^{a}	0.83±0.01^{a}	1.17±0.01^{b}	1.48±0.31^{b}	0.01

PUFA:SFA	0.04±0.02 [a]	0.03±0[a]	0.04±0[a]	0.14±0[c]	0.05±0[b]	<0.0001
TOTAL FA	53.2±0.36[b]	48.22±0.76[b]	70.19±0.57[c]	64.91±0.39[ab]	39.83±4.80[a]	<0.0001

Notes: CON: control diet or basal diet (70% Napier grass and 30% goat pellet); NON: 20% Non-treated OPF, 50% Napier grass and 30% goat pellet; PHY: 20% Physical pre-treated OPF, 50% Napier grass and 30% goat pellet; BIO: 20% Biological pre-treated OPF, 50% Napier grass and 30% goat pellet; COM: 20% combination pre-treated OPF, 50% Napier grass and 30% goat pellet

[abc] values with dissimilar superscripts within a row show significant difference at $P<0.05$

Saturated fatty acids (SFA) = Lauric (C12:0) + Myristic (C14:0) + Pentadecanoic (C15:0) + Palmitic (C16:0) + Heptadecanoic (C17:0) + (Stearic) C18:0.

Unsaturated fatty acids (UFA) = Palmitoleic (C16:1) +Oleic acid (C18:1n-9) + Oleic (C18:1n-9 trans)

PUFA n-6 = C20:4 n-6 + C18:2 n-6

4. Conclusion

This experiment demonstrated that biological pre-treated OPF could promote the accumulation of MUFA and PUFA in meat and could therefore be considered healthy for humans.

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