UNIVERSITI PUTRA MALAYSIA

EFFECT OF PRETREATED OIL PALM FROND IN ENHANCING THE RUMINAL DEGRADABILITY, GROWTH PERFORMANCE AND MEAT QUALITY OF GOATS

NOR DINI BINTI RUSLI

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By

NOR DINI BINTI RUSLI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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Chairman : Associate Professor Hasliza binti Abu Hassim, PhD
Faculty : Veterinary Medicine

The alternative source of ruminant feed is waste from agricultural by-products such as oil palm fronds (OPF). With an annual production of 13 million tons and low cost, the OPF is of interest in Malaysia as it is abundant. However, OPF contains high neutral detergent fibre with 20% lignin, which can be major constraints to OPF use as livestock feed. It is in line with poor digestibility of OPF in cattle (400 g/kg DM). Consequently, the energy content of OPF ranges between 4.9 and 5.6 MJ of metabolisable energy (ME)/kg DM. There is a need to treat OPF to improve its nutritional value and digestibility to be used as animal feed. Biological pretreatment with enzyme extract from white rot fungi (WRF) is considered a promising technique because of its preferential degradation of lignin with minimum dry matter loss. To make biological pretreatment more effective, a combination of biological with other pretreatments might be necessary. To date, some physical pretreatments have been developed to upgrade the OPF, which include pressing using traditional sugarcane machine. Physical pretreatment may increase particle size, which allows the increase of rumination duration, chewing activity, and ruminal pH, which could offer a favourable environment for rumen microorganisms to thrive. Therefore, the current study was conducted to assess the effect of physical and biological pretreatments of OPF using enzyme extract from WRF on nutritive value and lignocellulosic composition. The study was also investigated to evaluate the potential of physical and biological pretreatments of OPF in enhancing the *in vitro* rumen degradability, growth and health performances, as well as meat quality of goats.

Five different samples of OPF pretreatments were used in this study. The petiole of OPF was subjected to the physical pretreatment (POPF) by pressing using a conventional sugarcane pressing machine and the OPF, which consisted of petiole, leaflet and rachis were biologically pretreated using an enzyme extract of each *Ganoderma lucidum* (*G. lucidum*; BGL) and *Lentinula edodes* (*L. edodes*; BLE), respectively. Another two samples were subjected to a combination of physical and biological pretreatments of *G. lucidum* (CGL) and *L. edodes* (CLE), respectively. The control was non-treated OPF (FOPF). The results revealed that biological pretreatment with enzyme extract of *G. lucidum* and *L. edodes* either alone or combination with physical pretreatment reduced crude fibre and increased crude protein. The BGL, CGL, BLE and CLE reduced crude fibre by 15%, 16.6%, 14% and 13.3% respectively ($p \leq 0.05$). Crude protein contents in BGL, CGL, BLE and CLE were increased by 58.4%, 58.8%, 48.5% and 54.85% respectively ($p \leq 0.05$).

All pretreatments decreased hemicellulose contents significantly as compared to control, but no significant differences were observed among pretreatments. Similarly, the lignin contents (% DM) of all pretreated OPFs showed a significant decrease as compared with the non-treated OPF, but treatments with BGL (10.00) and CGL (10.00) showed the lowest lignin content of OPF ($p \leq 0.05$). However, the physical, biological and combined pretreatments did not change the cellulose content of the OPF ($p \geq 0.05$). The reduction of lignin content of OPF may indicate the cellulose and hemicellulose are converted into simple fermentable sugars such as glucose and xylose. Physical and biological pretreatments may help to reduce the crystallinity of the cellulose fibre in the OPF while also reducing the size of the materials.

For *in vitro* study, two fistulated Katjang goats consuming OPF and commercial pellet daily were used as rumen fluid donors. *In vitro* incubation was carried out at 39°C for 24 hours. Both BGL and CGL showed significantly higher propionate and butyrate concentrations as well as apparent rumen degradable carbohydrate (ARDC) with 6.57mg and 6.54mg, respectively as compared to the FOPF. It appeared that BGL and CGL resulted in higher lignin degradation that increased the *in vitro* rumen degradability, indicating that rumen microbes' access to cellulose was greatly improved. However, comparing these two white rot fungi, *G. lucidum* seemed more promising for improving the *in vitro* rumen degradability. Biological pretreatment with enzyme extract of *G. lucidum* combined with physical pretreatment improved the nutritional values of OPF by decreasing the lignin contents, consequently improving the ruminal degradability along with high total gas production, high VFA and high ARDC. Thus, the biological pretreatment with enzyme extract of *G.lucidum* was selected to pretreat the OPF for feeding trial study in goats.

Twenty, 5-month old male, cross-bred Boer goats with the average initial body weight 21.8±0.5 kg were assigned to treatments in a completely randomised block design of 120-day feeding trial. Five treatment diets containing 20% of OPF with different OPF pretreatments, 50% Napier grass and 30% goat concentrates were used in this study. The T1 consisted of non-treated OPF, T2 contained physically pretreated OPF, T3 with biological pretreated OPF with enzyme extract from WRF, T4 with OPF undergone combination of physical and biological pretreatments and the control (CD) consisted of the basal diet without OPF. During the feeding trial, feed intake and growth performance of goats were measured. After 120-day of the feeding trial, a digestibility trial was carried out on all goats involving total faecal collection. The animals were then slaughtered for carcass traits and meat quality evaluations. The results showed that the intakes of DM, crude protein (CP) and ash were significantly higher in group T3 and T4. However, all pretreatment methods had no adverse effect on body weight (BW) gain. The digestibility of DM, CP, acid detergent fibre and neutral detergent fibre were significantly increased ($p \leq 0.05$) by 14%, 9%, 14% and 13%, respectively in T4 group as compared with control group. Haematological profile was improved based on reference value in all groups. Total faecal egg count in T3 and T4 were significantly reduced ($p \leq 0.05$) by 37.6% and 31.2%, respectively. Besides, hot and cold carcass weights, dressing percentage and meat quality measurements were not affected by the pretreatments. Nevertheless, biological pretreatment of OPF has the potential to promote the accumulation of mono- and polyunsaturated fatty acids in the meat.

In conclusion, this study has demonstrated that physical and biological pretreatment of OPF either alone or in combination improved the quality of OPF by decreasing the lignin contents, consequently improving the *in vitro* ruminal degradability along with high total gas production, high VFA and high ARDC. Biological pretreatment of OPF also increased feed intake and some nutrients' digestibility with no adverse effect on health performance, carcass characteristics, chemical composition and meat quality.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN PELEPAH KELAPA SAWIT DIPRARAWAT DALAM MENINGKATKAN KEBOLEHURAIAN RUMEN, PRESTASI PERTUMBUHAN DAN KUALITI DAGING KAMBING

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Sumber alternatif makanan ruminan adalah sisa dari hasil sampingan pertanian seperti pelepah kelapa sawit (OPF). Dengan pengeluaran tahunan 13 juta tan dan kos rendah, OPF telah menarik minat Malaysia kerana pengeluaran yang banyak. Walau bagaimanapun, OPF mengandungi serat detergen neutral (NDF) yang tinggi dengan 20% lignin yang boleh menjadi kekangan utama penggunaan OPF sebagai makanan ternakan. Ini sejajar dengan pencernaan OPF yang lemah pada lembu (400 g / kg DM). Akibatnya, kandungan tenaga OPF berkisar antara 4.9 dan 5.6 MJ tenaga yang dapat dimetabolisme (ME) / kg DM. Terdapat keperluan untuk merawat OPF untuk meningkatkan nilai nutrien dan pencernaannya untuk dijadikan makanan haiwan. Pra-rawatan biologi dengan ekstrak enzim dari kulat akar putih (WRF) dianggap sebagai teknik yang berpotensi kerana keutamaan penurunan lignin dengan kehilangan bahan kering yang minimum. Untuk menjadikan prarawatan biologi lebih berkesan, kombinasi gabungan biologi dengan prarawatan lain mungkin diperlukan. Setakat ini, beberapa prarawatan fizikal telah dikembangkan untuk meningkatkan OPF, yang termasuk teknik tekanan menggunakan mesin tebu tradisional. Oleh itu, kajian semasa dilakukan untuk menilai kesan prarawatan fizikal dan biologi OPF menggunakan ekstrak enzim dari WRF terhadap nilai pemakanan dan komposisi lignoselulosa. Kajian ini juga diselidiki untuk menilai potensi prarawatan fizikal dan biologi OPF dalam meningkatkan kebolehkurangan *in vitro*, prestasi pertumbuhan dan kesihatan, serta ciri-ciri karkas kambing.

Lima sampel prarawatan OPF yang berbeza digunakan dalam kajian ini; OPF dikenakan prarawatan fizikal (POPF) menggunakan mesin penekan tebu konvensional, OPF ke prarawatan biologi menggunakan ekstrak enzim

masing-masing *Ganoderma lucidum* (*G. lucidum*; BGL) dan *Lentinula edodes* (*L. edodes*; BLE), masing-masing. Dua sampel lain menjalani kombinasi prarawatan fizikal dan biologi *G. lucidum* (CGL) dan *L. edodes* (CLE) masing-masing. Kawalannya adalah OPF (FOPF) yang tidak dirawat. Hasil kajian menunjukkan bahawa prarawatan biologi dengan ekstrak enzim *G. lucidum* dan *L. edodes* secara bersendirian atau gabungan dengan prarawatan fizikal dapat mengurangkan serat kasar tetapi meningkatkan protein kasar. Sampel BGL, CGL, BLE dan CLE dapat mengurangkan serat kasar masing-masing sebanyak 15%, 16.6%, 14% dan 13.3%. Kandungan protein kasar dalam sampel BGL, CGL dan CLE pula masing-masing meningkat sebanyak 58.4%, 58.8%, 48.5% dan 54.85%.

Semua prarawatan menurunkan kandungan hemiselulosa dengan ketara berbanding dengan kawalan, tetapi tidak terdapat perbezaan yang signifikan antara prarawatan. Begitu juga, kandungan lignin dari semua OPF yang telah dirawat menunjukkan penurunan yang ketara berbanding dengan OPF yang tidak dirawat, tetapi rawatan dengan BGL dan CGL menunjukkan kandungan lignin terendah dari OPF. Walau bagaimanapun, prarawatan fizikal, biologi dan gabungan tidak mengubah kandungan selulosa OPF ($p \geq 0.05$). Pengurangan kandungan lignin OPF mungkin menunjukkan selulosa dan hemiselulosa ditukar menjadi gula mudah fermentasi seperti glukosa dan xilosa. Pra-rawatan fizikal dan biologi dapat membantu mengurangkan kristaliniti serat selulosa dalam OPF dan juga mengurangkan ukuran bahan.

Untuk kajian *in vitro*, dua ekor kambing Katjang yang memakan OPF dan pelet komersial setiap hari digunakan sebagai penderma cecair rumen. Inkubasi *in vitro* dilakukan pada suhu 39°C selama 24 jam. Kedua-dua BGL dan CGL menunjukkan kepekatan propionat dan butirat yang jauh lebih tinggi serta karbohidrat yang dapat dikurangkan rumen (ARDC) dengan masing-masing 6.57mg dan 6.54mg berbanding FOPF. Didapati BGL dan CGL menghasilkan degradasi lignin yang lebih tinggi yang meningkatkan kebolehuraian rumen *in vitro*, yang menunjukkan bahawa akses mikroba rumen ke selulosa telah bertambah baik. Walau bagaimanapun, dengan membandingkan kedua-dua kulat reput putih ini, *G. lucidum* nampaknya lebih menjanjikan untuk meningkatkan kebolehuraian rumen *in vitro*. Prarawatan biologi dengan ekstrak enzim *G. lucidum* yang digabungkan dengan prarawatan fizikal meningkatkan nilai pemakanan OPF dengan mengurangkan kandungan lignin, seterusnya meningkatkan pencernaan rumal bersama dengan pengeluaran gas total yang tinggi, VFA tinggi dan ARDC tinggi. Oleh itu, *G. lucidum* dipilih untuk merawat OPF untuk kajian percubaan makan.

Dua puluh ekor kambing Boer jantan berumur 5 bulan dengan berat badan awal rata-rata 21.8±0.5 kg diperuntukkan untuk rawatan dalam reka bentuk blok rawak sepenuhnya dalam percubaan makan 120 hari. Lima diet rawatan yang mengandungi 20% OPF, 50% rumput Napier dan 30% pekat kambing digunakan dalam kajian ini dengan prarawatan OPF yang berbeza. T1 terdiri daripada OPF yang tidak dirawat, T2 mengandungi OPF yang telah dirawat secara fizikal, T3 dengan OPF dirawat secara biologi dengan ekstrak enzim daripada WRF, T4 dengan OPF mengalami kombinasi prarawatan fizikal dan biologi dan kawalan (CD) terdiri daripada diet asas tanpa OPF. Setelah 120 hari percubaan makan, percubaan pencernaan dilakukan pada semua kambing yang melibatkan pengumpulan tahi. Haiwan itu kemudian disembelih untuk penilaian karkas dan penilaian kualiti daging. Hasil kajian menunjukkan bahawa pengambilan bahan kering (DM), protein kasar (CP) dan abu jauh lebih tinggi pada kumpulan T3 dan T4. Walau bagaimanapun, semua kaedah prarawatan tidak memberi kesan buruk terhadap kenaikan berat badan (BW). Cerna DM, CP, serat pencuci asid dan serat pencuci neutral meningkat dengan ketara ($p \leq 0.05$) masing-masing sebanyak 14%, 9%, 14% dan 13% dalam kumpulan T4 berbanding dengan kawalan. Profil hematologi diperbaiki berdasarkan nilai rujukan dalam semua kumpulan. Jumlah telur faecal pada T3 dan T4 dikurangkan dengan ketara ($p \leq 0.05$) masing-masing sebanyak 37.6% dan 31.2%. Selain itu, berat karkas panas dan sejuk, peratusan berpakaian dan pengukuran kualiti daging tidak dipengaruhi oleh perlakuan awal. Walaupun begitu, prarawatan gabungan OPF berpotensi untuk mendorong pengumpulan asid lemak mono dan tak jenuh ganda dalam daging.

Kesimpulannya, kajian ini telah menunjukkan bahawa prarawatan fizikal dan biologi OPF sama ada secara bersendirian atau gabungan telah meningkatkan kualiti OPF dengan menurunkan kandungan lignin, akibatnya meningkatkan pencernaan ruminal bersama dengan pengeluaran gas yang tinggi, VFA tinggi dan ARDC tinggi. Prarawatan gabungan OPF juga meningkatkan pengambilan makanan dan kecernaan beberapa nutrien tanpa kesan buruk terhadap prestasi kesihatan, ciri bangkai, komposisi kimia dan kualiti daging.

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LIST OF ABBREVIATIONS

%	percentage
±	plus-Minus
°C	degree celcius
a*	redness
ADF	acid detergent fibre
ADG	average daily gain
ADL	acid detergent lignin
ALA	linolenic acid
ANOVA	analysis of variance
AOAC	Association of Analytical Chemistry
ARDC	apparent rumen degradable carbohydrate
ATCC	American Type Culture Collection
b*	yellowness
BGL	Biological pretreatment of OPF with enzyme extracts from *G. lucidum*
BLE	Biological pretreatment of OPF with enzyme extracts from *L. edodes*
BW	body weight
CF	crude fibre
CGL	Combination of physical and biological pretreatments of *G. lucidum*
CH_4	methane
CLE	Combination of physical and biological pretreatments of *L. edodes*
cm	centimetres
CO_2	carbon dioxide
CP	crude protein
DM	dry matter
DMI	dry matter intake
EDTA	ehtylenediamine tetra acetic acid-disodium salt
EE	ether extract
EFB	empty fruit bunches

EPG	eggs per gram of faeces
FCE	feed conversion efficiency
FCR	feed conversion ratio
FEC	faecal egg counts
FOPF	non-treated OPF
GE	gross energy
H_2	hydrogen
H_2SO_4	sulphuric acid
H_2SO_4	sulphuric acid
H_3BO_3	boric acid
Hb	haemoglobin
HCT	haematocrit
J	joules
L	litres
L*	lightness
LA	linoleic acid
LiP	lignin peroxidase
LYM	lymphocytes
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
ME	metabolisable energy
MnP	manganese peroxidase
MPV	Mean platelet volume
$Na_2HPO_4 \cdot 12H_2O$	disodium phosphate
$NaH_2PO_4 \cdot H_2O$	monosodium phosphate
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
NDF	neutral detergent fibre
NFE	nitrogen free extract
NH_3	ammonia
NH_4Cl	ammonium chloride
OM	organic matter

OPF	oil palm frond
OPT	oil palm trunk
PCT	plateletcrit
PDA	potato dextrose agar
PDW	Platelet distribution width
PLT	Platelet count
POPF	Physical pretreatment of OPF
PUFA	polyunsaturated fatty acids
PVPP	polyvinyl polypyrrolidone
RBC	Red blood cells
RDW	Red blood cell distribution width
SFA	saturated fatty acids
SPSS	Statistical Package for Social Science
UFA	unsaturated fatty acid
VFA	Volatile fatty acid
WBC	white blood cell
WRF	white rot fungi

CHAPTER 1

GENERAL INTRODUCTION

1.1 Background of the study

The world population is increasing and estimated to reach 9.2 billion in 2050. Hence the demands for meat and milk products are expected to increase as well. Increases in human population, urbanisation and global incomes could affect natural resources, particularly soil and water, resulting from food and feed competition. Furthermore, limited resource availability due to the competition can increase the market price, contributing to the price inflation of feedstuff. It is imperative that ruminant feed needs to be utilised from agricultural by-products rather than grains grown for human nutrition. The utilisation of agricultural by-product needs urgent attention by adopting proper approaches to recycle and reuse it as well as upgrade its potential as animal feed. Malaysia's palm oil industry generates abundant by-products obtained from the cultivation of oil palm trees, including oil palm fronds (OPF). The industry produces approximately 83 million tons of oil palm biomass in 2016, with 13 million tons of OPF (Loh, 2017; Kum and Zahari, 2011). The OPF is of interest as it is abundant, low-cost and commonly fed to beef and dairy animals at 50% and 30% of inclusion level, respectively (Wan Zahari et al., 2003). Most local farmers in Malaysia extensively chopped the OPF and combined it with other feedstuff as total mixed rations (Ng et al., 2011). The OPF is made up of leaflets, rachis, stems, and petioles, with the petiole accounting for 70% of the OPF's dry matter (DM). However, the crude protein (CP) content is higher in leaves than the petioles. Previous reports demonstrated the use of OPF for ruminant feeding and its effect on feed intake and digestion (Rajion *et al*., 2001; Wan Zahari *et al*., 2003). The addition of OPF in beef cattle and dairy cow's diet could support live weight gain between 0.6 and 0.8 kg per day and milk yield of about 20 litres per day (Wan Zahari et al., 2003). Further, Rajion et al. (2001) also reported that the incorporation of OPF might increase the unsaturated fatty acid (UFA) proportion in rumen contents and sheep plasma since the content of UFA in OPF is high. However, the total fat content of OPF is low (Ghani et al., 2017). These reports make OPF an auspicious source of roughage for ruminants, but OPF cannot be fed solely as animal feed due to its poor nutritive values with the energy content of 4.9 to 6.5 MJ kg-1 DM (Hassim et al. 2010).

The key approaches to pretreatment include physical, chemical and biological strategies. These pretreatments have been used to reduce lignocellulose composition in agricultural by-products, increasing the feeding value for ruminants. To date, some physical pretreatments have been developed to upgrade the OPF, which include pressing using a conventional sugarcane machine (Zahari et al., 2012), pelleting (Dahlan et al., 2000), grinding, chopping (Oshibe et al., 2001) and steaming (Paengkoum et al., 2006). A study by Wan Zahari et al. (2003) reported that pelleting, grinding and steaming increased the intake and digestibility of OPF and improved the performance of cattle.

Furthermore, the application of physical pretreatment in OPF is considered cost practical, easily operated, contain no impurities, low risk of health and safety. For chemical pretreatment, OPF would be initiated by chemical reactions using an alkaline solution, commonly sodium hydroxide (NaOH), ammonia (NH3) and urea. This pretreatment needs low temperature and pressure management compared with other pretreatment strategies (Mosier et al. 2005). Besides, treatment with NaOH was reported to cause detriment to the palatability of OPF in cattle (Kawamoto et al., 2001). Some studies have shown that urea pretreated rice and wheat straws are safer, more palatable and cost-effective than NaOH pretreatment (Al-Shami, 2008; Walli, 2010). However, some other factors restrict the effectiveness of urea, such as the toxicity level, moisture content and duration of the pretreatment.

Studies have reported that biological pretreatment with white rot fungi (WRF) can effectively degrade lignin and further enhance digestibility of various lignocellulosic biomass (Hassim et al., 2012; Okano et al., 2005, Taniguchi et al., 2005). Also, biological pretreatment with WRF colonization was also reported to improve ruminal OPF degradability (Rahman et al., 2011). However, the relatively high biomass losses during fungi colonization should be taken into account in biological pretreatment. The approach of WRF colonisation is still time consuming and causing DM losses due to fungal metabolism. Therefore, a proper method to preferentially remove lignin, but not polysaccharides (cellulose and hemicellulose), are needed if biological pretreatment with WRF is to be successful. Study has shown that this limitation can be resolved by minimizing the DM loss in wheat straw when the pretreatment used enzyme extract from WRF as compared with the WRF mass (Rodrigues et al., 2008).

Generally, a better digestible feed or crop residues gives a high feed intake, which is vital for animal production. Physical pretreatment of OPF by pelletising increased the intake and digestibility, improved the feed intake and growth performance of cattle (Wan Zahari et al., 2003), while feeding *Ganoderma* sp. treated wheat straw resulted in a higher DM intake and a higher digestibility of DM, CP, hemicellulose and cellulose (Shrivastava et al., 2012). It was shown that there was a positive relation between biological pretreatment of agricultural by-products with palatability, feed intake, rumen microbial synthesis and digestibility, which could improve animal performance such as weight gain, feed efficiency and health (Alsersy et al. 2015; Elghandour et al. 2015).

Therefore, the first aim of this study was to investigate whether the physical pretreatment and biological pretreatments with enzyme extracts from two WRF; *G. lucidum* and *L. edodes* could improve the nutritive value of OPF as well as the *in vitro* rumen degradability (**Part 1**). The potential of OPF as animal feed following physical and biological pretreatment with enzyme extracts from WRF was characterised by evaluating the nutrients and lignocellulosic profiles (**Chapter 3**). The findings of the study highlight the importance of the biological pretreatment of OPF using an enzyme extract of each *G. lucidum* and *L. edodes* resulted in improving the nutritional contents of OPF by increasing the crude protein content and degrading the lignin content in OPF. Further, the effect of physical and biological pretreatments of OPF on *in vitro* ruminal degradability of goats was evaluated (**Chapter 4**). Biological pretreatment with enzyme extract of *G. lucidum* and combination pretreatment of OPF with enzyme extract of *G. lucidum* are promising to improve the ruminal digestibility along with high total gas production, high volatile fatty acid (VFA) and high apparent rumen degradable carbohydrate (ARDC). For **Part II**, the aim was to evaluate the effect of physical and biological pretreatments of OPF on the intake, growth and health of the goats (**Chapter 5**). All pretreatment groups of goats had a higher total DM intake than those fed non-treated OPF and control diet. Combined pretreatment of OPF significantly improved the apparent DM, organic matter, CP, acid detergent fibre and neutral detergent fibre digestibility. Further, the effectiveness of physical and biological pretreatments to improve carcass characteristics and meat quality was assessed (**Chapter 6**). Figure 1.1 shows the flow chart of the overall PhD study.

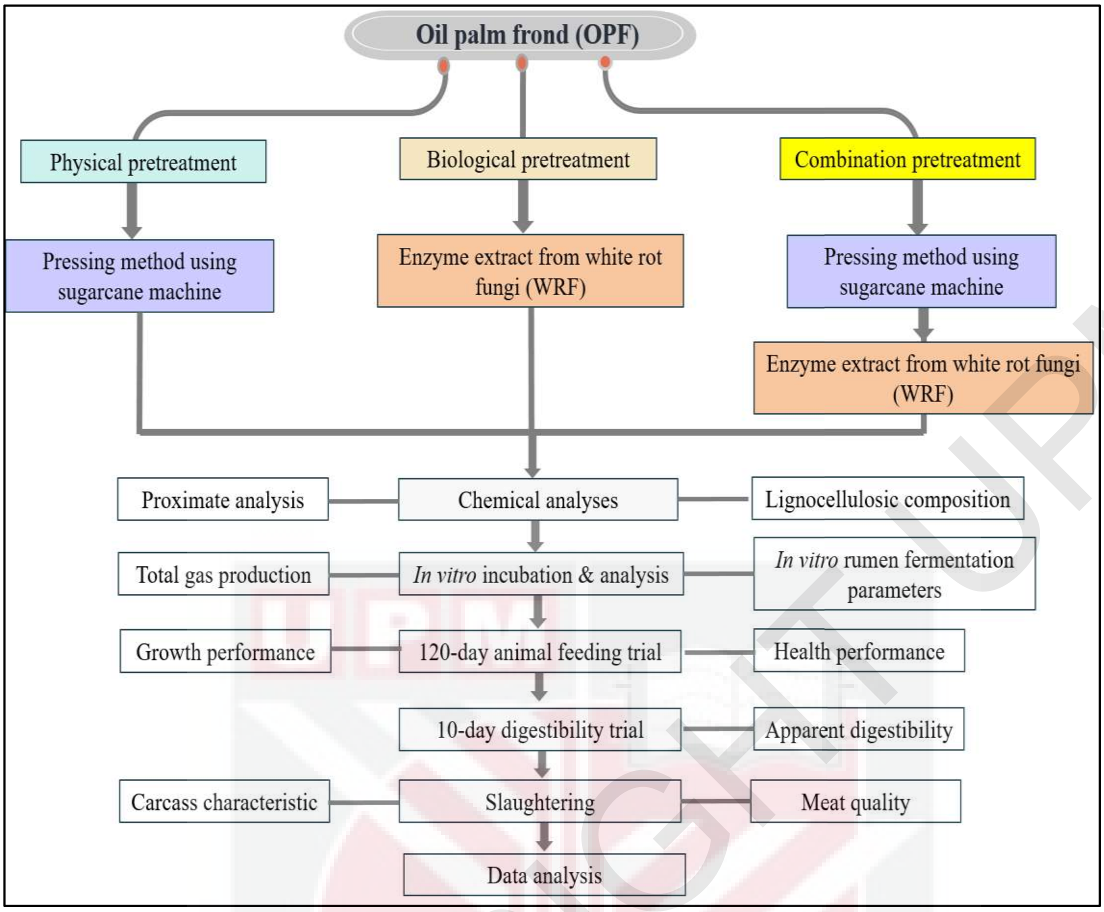

Figure 1.1 : Flow chart of PhD study

1.2 Problem statement

Oil palm frond's lignin content is also high at 205g/kg DM on average (Nordin et al., 2016) and can affect OPF intake and digestibility. Lignin, the most recalcitrant part of the cell wall of plants, serves as a barrier and restricts rumen microorganisms to degrade OPF. In addition, these rumen microorganisms are not prepared for lignin degradation because they do not generate enzymes with ligninolytic activity (Pollegioni et al., 2015), resulting in a reduced degradability (often less than 50%) of the original material, leading to a low metabolisable energy (ME) content (≤ 7.5 MJ/kg DM). In reality, feeding ruminants with only OPF without any additional feed to cover the requirements of all nutritional ruminal microorganisms will result in poor degradability that is further reflected in animal performance including growth and health performances. According to a previous report, OPF increased total n-3 PUFA in sheep plasma, potentially benefiting human health (Rajion et al., 2001). However, another study found that using OPF in the diet had no effect on fatty acid metabolism, and that OPF feeding did not increase ruminant fatty acid composition (Ghani et al., 2017; Hassim et al., 2010). However, there is still little evidence to date on the effects of pretreated OPF supplementation on fatty acid composition and meat quality of goats.

1.3 Hypothesis

Since OPF provides a sustainable feed for the ruminant industry, investigating the best pretreatment strategy for the improvement of the nutritional value of OPF may be a viable option to address these problems. In many practices, physical pretreatment is usually applied as the first method before the biomass is pretreated with other pretreatment strategy. Recently, the pressing method of OPF using a conventional sugarcane pressing machine was used to obtain OPF juice for the potential fermentation substrate (Zahari et al., 2012). The leftover from the pressing method was OPF pressed fibre, which could be a good feed resource for ruminant. Biological pretreatment is also considered a preferable choice among the pretreatments due to its practicality, environmentally sustainable and safer approach, comparing with chemical pretreatment. Biological pretreated OPF with WRF was reported to increase the *in vitro* ruminal degradability (Rahman et al., 2011). The findings of this study will be useful in disclosing details on the pressing method and the use of enzyme extract from WRF for pretreating and upgrading the OPF. Therefore, it is hypothesised that the physical pretreatment by pressing method and biological pretreatment using enzyme extract from WRF could help improve nutritive values of OPF and its ruminal degradability as well as enhance the growth performance, apparent digestibility and health performance of goats. Both pretreatments are also expected to improve the carcass characteristics and meat quality of goats.

1.4 Objectives

1. To assess the effect of physical and biological pretreatment of OPF using enzyme extract from WRF on nutritive value and lignocellulosic composition.

2. To evaluate the effect of physical and biological pretreatment of OPF using enzyme extract from WRF on the *in vitro* ruminal degradability characteristics.

3. To evaluate the effect of physical and biological pretreatments of OPF using enzyme extract from WRF on the growth performance, apparent digestibility and health performance of goats.

4. To assess the effect of physical and biological pretreatments of OPF using enzyme extract from WRF on carcass characteristics and meat quality of goats.

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