UNIVERSITI PUTRA MALAYSIA

NUTRITIONAL COMPOSITION OF MEAT AND FAT OF GOAT FED WITH OIL PALM FROND TREATED WITH ENZYME EXTRACT FROM FUNGI

RAJA NUR AISYAH BT RAJA ZAHAR SHAH

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FACULTY OF VETERINARY MEDICINE
UNIVERSITI PUTRA MALAYSIA
SERDANG, SELANGOR
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RAJA NUR AISYAH BT RAJA ZAHAR SHAH

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Nutritional composition of meat and fat of goat fed with oil palm frond treated with enzyme extract from fungi", by Raja Nur Aisyah Bt Raja Zahar Shah, and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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DEDICATION

This project paper is dedicated to Allah S.W.T., who had created me and made all circumstances possible throughout this project,

To my family,

My father, Raja Zahar Shah Bin Raja Azie,

My mother, Mazni Binti Zainuddin

My siblings; Raja Zal Hazmi Shah and Raja Nur Sarah

My friends

And to all my teachers who have committed themselves towards the noble cause of education. I sincerely thank you for your endless support and care.

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Praise to Allah the Almighty for being granted physical and mental strength throughout my life, to be for wanting to learn in order to better oneself and help my country in both the academic and economic fields be more successful in the future.

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CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
°C	Degree Celsius
OPF	Oil Palm Frond
LD	*Longissimus dorsi*
BF	*Bicep femoris*
EFB	Empty Fruit Bunch
PKS	Palm Kernel Shell
MF	Mesocarp Fiber
OPT	Oil Palm Trunk
OPL	Oil Palm Leaves
WRF	White Rot Fungi
cm	Centimetre
DM	Dry matter
IVDMD	*In vitro* dry matter digestability
NCWFE	Nitrogen cell wall free extract
ME	Metabolisable energy
MnP	Manganese-dependant peroxidase
LiP	Lignin peroxidase
g	Gram
M	Mol
VFA	Volatile fatty acids
RBF	Round bottle flask

ml	Millilitre
mol/L	Molar per litre
SEM	Standard error of mean

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian keperluan kursus VPD4999 – Projek Tahun Akhir

KOMPOSISI NUTRISI DAGING DAN LEMAK KAMBING YANG DIBERI OIL PALM FROND SEBAGAI SUMBER MAKANAN SETELAH DIRAWAT OLEH ENZIM EKSTRAK DARIPADA KULAT

Oleh

Raja Nur Aisyah Bt Raja Zahar Shah

2022

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Prof. Madya. Dr. Hafandi Ahmad

Oil palm frond (OPF) merupakan salah satu produk sampingan yang dihasilkan semasa pemprosesan kelapa sawit yang juga mempunyai kadar serat yang tinggi menyebabkan ia sesuai dijadikan sumber makanan utama kepada haiwan. Tetapi kadar lignin yang tinggi menghadkan penggunaan nutrisi OPF. Ekstrak enzim daripada kulat boleh berfungsi sebagai kaedah pra-rawatan untuk merendahkan lignin dan meningkatkan penghadaman serat. Walau bagaimanapun, kajian berkenaan tajuk ini kekal terhad dalam menilai potensi kebolehcernaan OPF dan kesannya terhadap prestasi haiwan. Oleh itu, kajian ini dilaksanakan untuk menilai komposisi nutrisi daging

dan lemak kambing yang diberi pelepah kelapa sawit sebagai sumber makanan setelah dirawat oleh enzim ekstrak daripada kulat. Sebanyak 16 ekor kambing Boer dibahagikan 4 kumpulan (n=4) (Kawalan: Diet asas, Kumpulan 1: 30% OPF dirawat penimbal, Kumpulan 2: 30% OPF dirawat enzim individu, Kumpulan 3: 30% OPF dirawat campuran enzim). *Longissimus dorsi* (LD) dan *Bicep femoris* (BF) telah diambil sampel untuk analisis skor warna, kelembapan, abu, kadar lemak, dan kadar protein. Lemak lunak dan dalaman telah diambil sampel untuk analisis skorwarna dan kadar lemak. Kadar protein untuk kedua-dua daging (13.50% ; 13.12%) menunjukkan signifikan ($p<0.05$) dengan kumpulan 3. Hanya abu untuk BF (0.67%) menunjukkan signifikan ($p<0.05$) berbanding LD (1.06%) ($p>0.05$). OPF yang dirawat dengan enzim tidak mempengaruhi kadar lemak dan warna daging. Peningkatan ketara dilihat ($p<0.05$) bagi kadar lemak (96.18%) untuk lemak dalaman dengan kumpulan 3. Warna lemak dan kadar lemak LD tidak menunjukkan perbezaan yang ketara. Oleh itu, kumpulan 3 disimpulkan mempunyai rawatan terbaik kerana komposisi protein dan lemak yang tinggi dalam daging dan lemak.

***Kata Kunci**: Oil palm frond; enzim, kambing, daging, lemak*

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD4999 – Final Year Project

NUTRITIONAL COMPOSITION OF MEAT AND FAT OF GOAT FED WITH OIL PALM FROND TREATED WITH ENZYME EXTRACT FROM FUNGI

By

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2022

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Oil palm fronds (OPF), the main oil palm by-products have potential to become source of feed for livestock due to high crude fibre but lignin may result inefficiency in utilising the OPF due to limitations on nutrient utilisation. Enzymes extract from fungi can function as pre-treatment methods to degrade lignin and improve digestibility of fibrous by-products. However, limited studies are available to assess its potential on OPF digestibility and animal performance. Thus, this study was performed to assess the nutritional composition of meat and fat when given OPF treated with enzymes extract from fungi as feed. A total of 16 Boer goats was divided into 4 groups (n=4) (Control: Basal diet, Treatment 1: 30% treated OPF buffer pre-treatment, Treatment 2: 30% treated OPF treated with individual enzyme, Treatment 3: 30% treated OPF with cocktail enzyme).

Longissimus dorsi (LD) and *Bicep femoris* (BF) were sampled for colour score, moisture, ash, crude fat, and crude protein analysis. Subcutaneous and internal fat were sampled for colour score and crude fat analysis. Crude protein for both muscles (13.50% ; 13.12%) shows significant ($p<0.05$) with treatment 3. Only ash for BF (0.67%) shows significance ($p<0.05$) compared to LD (1.06%) ($p>0.05$). Meat's crude fat and colour were not affected by OPF treated with enzymes. Significant increase is seen ($p<0.05$) for crude fat (96.18%) of internal fat with treatment 3. Colour of fat and crude fat of LD does not show significant differences. Thus, treatment 3 is concluded having the best treatment due to its high protein and fat composition in meat and fat.

***Keywords**: Oil Palm Frond; enzymes; goat; meat; fat*

1.0 INTRODUCTION

1.1 BACKGROUND

Oil palm tree (*Elaeis guineensis*) is a tropical palm plant which is native to Africa. In less than 100 years, the cultivation of oil palm trees has evolved from being a relatively small-scale crop in Africa to one of the world′s most profitable agricultural commodities. Oil palm trees can grow well in the Malaysian climate. Without a doubt, it has become the most important agricultural crop in Malaysia and has been the key to the national economic expansion (Awalludin et al., 2015).

With its growing expansion, this has led to production of oil palm frond (OPF) which is one of its by-products. According to Ishida and Abu Hassan (1997), it was estimated that annually 19 million OPF is being produced on a dry matter basis during the pruning operation in 1995 in the plantation. At the moment, OPF is not being utilised and is left in the field. But there is much potential for it to become one of the feed sources for ruminants due to its high sugar and carbohydrate rich residue. The OPFs are composed of 58% cellulose, 24% hemicelluloses, 5% lignin, 8% extractives and 5% ashes.

The amount of cellulose in the OPF is higher than other waste parts (leaves and empty fruit bunches) of oil palm trees. (Mohd Rasli et al., 2017). Its high amount of lignin will result in poor ruminal degradability that prevents microbes from their desired cellulose and hemicellulose.

According to Dashtban et al. (2010), fungi are able to degrade lignin by producing an enzyme term as “ligninases”. This will result in a more efficient ruminal degradation of the feed. Thus, this study is conducted to study the effect of oil palm fronds treated with enzymes extract from fungi on goat’s meat and fat nutritional composition.

1.2 JUSTIFICATIONS

1. There is potential that pre-treated OPF can enhance rumen degradability and improve animal performance
2. Not many studies have been done on pre-treated OPF on assessing animal performance (meat and fat quality)

1.3 OBJECTIVES OF STUDY

The study aimed to assess the nutritional composition of meat and fat from goats fed with OPF treated with enzyme extract from fungi

1.4 HYPOTHESIS

H_0: Goat fed with OPF treated with enzyme extract from fungi will have no effects on its meat and fat nutritional composition

H_A: Goat fed with OPF treated with enzyme extract from fungi will have effects on its meat and fat nutritional composition

2.0 LITERATURE REVIEW

2.1 Oil Palm Waste

The Malaysian oil palm industry has seen rapid growth in development since its first introduction by the British in 1875 as an ornamental plant. It was only in the early 20th century that the oil palm tree was commercially planted in the state of Selangor by Henri Fauconnier (Teoh and C.H., 2002). Now, in the 21st century Malaysia is recognised as the second largest palm oil exporter after Indonesia overtook Malaysia's title on becoming the largest oil palm producer. The PO industry employs approximately 2.3 million people in Malaysia (Mahat 2012), contributing to Malaysia's low unemployment rate of ca. 3.4%, which only recently increased to 4.6% due to the COVID-19 pandemic. (DOSM 2021).

The main problem in the oil palm tree cultivation and its related industries is its substantial amount of biomass wastes (Awalludin et al., 2015). The Malaysian oil palm industry has been one of the largest contributors of lignocellulosic biomass, with more than 90% of the country's total biomass deriving from 5.4 million ha of oil palms. (Loh, 2017).

The wastes such as empty fruit bunches (EFB), palm kernel shell (PKS), mesocarp fiber (MF), palm oil mill effluent (POME), oil palm trunks (OPT), oil palm leaves (OPL) and oil palm fronds (OPF) are generated after the oil palm fruits harvesting, palm oil processing or during oil palm trees replantation. Except POME, the rest wastes are higher in fibre content (Awalludin et al., 2015).

Sundram and Basiron (2011) showed that about 77.2% of the solid waste comes from the upstream sector while 22.8% comes from the downstream. Upstream sector comprises oil palm fronds with an estimated biomass of 46.37 million tonnes dry (58.8%) and oil palm trunk having an estimated biomass of 6.73 million tonnes dry (18.4%). Next, the downstream sector consists of

empty fruit bunch with an estimated biomass of 14.45 million tonnes (8.6%) and fiber and shell with its estimated biomass of 11.17 million tonnes dry (14.2%). The palm fronds generated from pruning make up the highest percentage (58.8%) of solid waste generated in the palm oil industry. (MPOB 2014).

2.2 Oil Palm Frond Utilisation as Animal Feed

Oil palm frond (OPF) is one of the main by-products obtained from the oil palm industry. It is obtained during the process of felling, pruning, and harvesting. Due to its abundance and availability, feeding freshly chopped OPF to ruminants has been widely practised by farmers in Malaysia (Wong & Zahari, 2011).

Research was done by Ishida and Abu Hassan (1997) on the chemical composition and *in vitro* dry matter digestibility (IVDMD) of OPF. The methods that were used in the process are feed analysis method based on neutral detergent system and pepsin-cellulase digestion method. From the study it is shown OPF consisted of 70% fibre and contained about 20% nitrogen cell wall free extract (NCWFE) which indicates the soluble carbohydrate in feed. It was also estimated that the *in vivo* dry matter digestibility of OPF is about 45% which was determined by the regression *of in vivo* dry matter digestibility on IVDMD.

Proximate chemical composition and metabolisable energy (ME) that were retrieved from Ishida and Abu Hassan (1992), Hong et al. (2012) and Mathius (2008) demonstrates that on a dry matter basis, OPF is comprised of 70% fibre and 22% soluble carbohydrate. OPF also displays a low ME value which varies between 4.9 MJ kg^{-1} and 5.6 MJ kg^{-1} DM. Furthermore, the level of crude fibre which ranges between 48.25% - 50.20% further demonstrated the potential of OPF as ruminant roughage.

Oil palm frond has also shown strong potential as animal feed in regard to its positive effect in animal performance. This is proven in a study conducted by Ishida and Abu Hassan (1992) on the effect of oil palm frond silage feeding on utilisation of diet and meat production in fattening cattle. In the study, it has been reported all bulls in the feeding trial show an increase in weight gain with no detrimental effect observed when 30% OPF on a dry matter basis-based diet was given.

In addition, a 30% oil palm frond-based diet has also been seen to increase milk yield in comparison to 50% oil palm frond-based diet which shows a much lower milk yield and no adverse effects on flavour of milk for both diets is observed (Abu Hassan et al. 1993).

2.3 Limitation using Oil Palm Frond as Animal Feed

Despite being able to be used as a roughage, the effectiveness of OPF as roughage is limited. This is due to the oil palm frond also containing lignocellulosic organic material that comprises cellulose, hemicellulose, and lignin. Its cell wall's fibrous components render OPF less digestible for ruminants. (Zakaria et al., 2014).

According to Siti Sabrina et al. (2013), lignocelluloses from fresh OPF contains hemicellulose (23.8%), lignin (20.5%) and α-cellulose (58.27%). The raw oil palm frond was examined for its lignocellulose based on Ehman while its extractives and other composition is in accordance with Teramoto et al. (2009)

Lignin is a complex biopolymer that gives plant strength. It is highly insoluble and also the most recalcitrant component in plant cell walls in which the rate of breakdown of carbohydrates is inversely correlated with lignin content. Lignin will also bind itself to cellulose and hemicellulose resulting in a barrier between cells becoming unbreakable that ultimately affects cellulose and

hemicellulose bioavailability for microbes. Thus, nutritive value received by animal, digestibility, rate and degree of microbial degradation will be affected (Rusli et al., 2020)

Previous studies showed that the ester linkages between lignin, hemicellulose, and cellulose prevent the decomposition of rice straw (Dinh Vu et al., 2017) wheat straw (Van Kuijk et al., 2017) and OPF (Rasli et al., 2017). Ruminants' capability to process organic material is due to their symbiotic interaction with rumen bacteria. Types of bacterial species are also important in evaluating rate of decomposition of feed for fermentation end-product such as synthesis of volatile fatty acid and microbial protein (Wang et al., 2020).

2.4 Pre-treatment of Oil Palm Frond

Pre-treatment is a natural and secure method of treating and successfully removing lignin to improve the digestibility of lignocellulosic biomass. With its primary objective of loosening lignin's complicated structure and interrupting the crystalline structure of cellulose it will also minimise cellulose degradation and the development of inhibitory compounds like phenolic, which crosslinks with hemicelluloses (Rusli et al., 2020).

There are 3 types of pre-treatments which are physical, chemical and biological pre-treatment. Physical pre-treatment involved the process of grinding, chopping, pressing and milling large feed material into smaller sizes and decreasing its crystallinity (Zhang et al., 2010). Large particle size and high concentration of fibre in feed material will increase chewing time, rumination time and rumen's pH. This will provide an ideal environment to increase the population of rumens bacteria but will not influence animal's feed intake (Zhao et al., 2009).

Based on research done by Dahlan et al. (2000) pelletising is one of the successful strategies to increase OPF's feeding value. Pelletising includes conditioning, milling, drying, pelletising and biomass cooling. It is also observed by pelletising OPF this increase goat's digestibility of nutrients

with increased production of volatile fatty acids. Despite its benefits, this process is not appropriate to be used by small-scale farms as the process requires high energy.

Chemical pre-treatment typically entails the application of acidic, alkaline and oxidative agents to enhance nutritive value of agriculture by-products (Sarnklong et al., 2010). Alkaline types of solution are the most commonly used agent in chemical pre-treatment, especially ammonia (NH_3), urea and sodium hydroxide (NaOH). Alkali pre-treatment has been shown to result in degeneration of ester and glycosidic side chains, causing enzyme saccharification of hemicellulose and cellulose during rumen's fermentation process (Kucharska et al., 2018).

A study by Suksombat. (2004) reported there is an increase in feed intake, ruminal digestibility and degradability in ruminant when rice straw and bagasse was pre-treated with NaOH. Several other studies have observed when NaOH were given to rice straw, barley straw, and sugarcane bagasse, dry matter degradability increases.

According to Kawamoto et al. (2001), while having low palatability, NaOH treated OPF showed greater DM digestibility and digestible DM intake (DMI) in cattle compared to OPF pellets and silage. Despite its benefits, its high cost limits the use of NaOH in chemical pre-treatment of agricultural byproducts, making it uneconomical in some nations, particularly developing and transformational nations (Ooi et al., 2017). Safety on using NaOH also needs to be considered as it is hazardous and corrosive.

Compared to physical and chemical pre-treatment, biological pre-treatment is a much more green, safe, and economical strategy. This is due to moderate operating conditions and little energy consumption.

There are 4 different types of biological pre-treatment which are enzymes, yeast, white rot fungi (WRF) and ensilation. Several studies have been conducted on white rot fungi (WRF) which

is renowned for their capacity to break down the lignocellulosic linkages by secreting extracellular ligninolytic enzymes. In the studies it can be deduced that it is the most efficient basidiomycetes for biological pre-treatment of agricultural byproducts like sugarcane bagasse (Okano et al., 2006; Tuyen et al., 2013), maize straw (Akinfemi et al., 2009), OPF (Rahman et al., 2011; Tuyen et al., 2013; Chanjula et al., 2017). The WRF ability to convert lignin, cellulose, and hemicellulose into simple carbohydrates is mainly due to extracellular extracellular lignolytic enzymes include laccase, manganese-dependent peroxidase (MnP), and lignin peroxidase (LiP) (Vrsanska et al., 2016).

A study conducted by Rahman et al. (2011) describes C. *subvermispora* (3 wks) and *L. edodes* and *P. brevispora* (9 wks) to be the most effective for OPF pre-treatment. This results however contraindicates with the report of high biomass losses during fungi colonisation. Apart from time-consuming, the WRF method results in DM losses because of fungus metabolism. According to research, this limitation can be overcome by minimising the amount of dry matter (DM) lost in wheat straw when pre-treatment uses enzyme extract from WRF as opposed to the WRF mass (Rodrigues et al., 2008).

Researches have been conducted in utilising exogenous fibrolytic enzymes to improve the effectiveness of animal performance and the quality of fodder. (Peters et al., 2015). Another study on pre-treatment of corn stower with cellulase and xylanase displayed an increase in organic matter digestibility, metabolisable energy and VFA. (Vallejo et al., 2016). To increase feeding efficiency, these enzymes can target crop residue lignocellulose structure through cellulolytic and hemicellulolytic action. (Abdel-Aziz et al., 2015).

Following the addition of enzymes to the ration, there is a subsequent rise in feed consumption, which may be partially ascribed to the enhancement of nutritional palatability

brought on by the sugars created by the proactive hydrolysis of fibre. In addition, the post-ingestive enzyme's effects, such as an increase in digestion rate and degree, can also promote hydrolysis in the rumen, which lowers stomach fullness and boosts feed intake (Rusli et al., 2020).

3.0 Methodology

3.1 Application for IACUC Approval

Four months prior to the start of the experiment, a request for IACUC (Institutional Animal Care and Use Committee) approval was requested. The approval was granted with IACUC reference code UPM/IACUC/AUP-R010/2022.

3.2 Experimental Design

A total of 16 male *Capra aegagrus hircus* (Boer Cross) were randomly chosen and bought from a farm in Seri Kembangan, Selangor. The goats were then brought to Institusi Pertanian Tropika dan Sekuriti Makanan, UPM by lorry in the morning at 7 am. After 2 weeks of acclimatisation, the goats were divided into 4 treatment groups (n=4). The group were labelled as Control group, Treatment 1, Treatment 2 and Treatment 3.

Control group were given basal diet (30 % non-treated OPF, 40 % Napier and 30 % concentrate), Treatment 1 (30 % of treated OPF (buffer pre-treatment), 40 % Napier and 30 % concentrate), Treatment 2 (30% treated OPF (individual enzyme pre-treatment), 40% Napier and 30% concentrate) and Treatment 3 (30% treated OPF (cocktail enzyme pre-treatment), 40% Napier and 30% concentrate). After 16 weeks, all goats were slaughtered for meat and fat analysis. *Longissimus dorsi* and *Bicep femoris* was taken for meat analysis while subcutaneous fat and internal fat was taken for fat analysis.

3.3 Analysis of Meat and Fat

3.3.1 Meat Colour Score

Meat colour score was done according to the guideline from Meat goat: Selection, carcass evaluation & fabrication guide (2006). The guideline scores lean meat based on their colour with a scoring range of 1 (pale red), 2 (dark red) and 3 (very dark red).

3.3.2 Fat Colour Score

Fat colour score was done based on Guideline for Meat Color Evaluation (1991). The guidelines provide a fat colour score ranging 1 (white), 2 (creamy white), 3 (slightly yellow), 4 (moderately yellow) and 5 (yellow).

3.3.3 Proximate Analysis of Meat and Fat

Proximate analysis is a variety of analytical techniques carried on sample on feed sample to determine its nutritional composition. The elements that were determined are dry matter, moisture, crude protein, crude fat and ash content. All analyses were done according to the AOAC International protocol (Association of Official Agricultural Chemists, 2007).

3.3.3.1 Dry Matter (DM) and moisture content

Porcelain crucibles were labelled and dried in the oven (Memmert Universal Oven UF110, Germany) at 105°C for 30 minutes. The dried crucible is then allowed to cool down inside dessicator for 20 minutes and weighed. About 3.0 g of sample was placed inside the weighed crucibles according to their labels and weight of sample and crucible was recorded. The crucible containing the sample was then dried at 105°C overnight as parts of its drying process. After the

drying process, the crucible is cooled inside the dessicator which then will be weighed again. Dry matter and moisture content were calculated using the formula below:

$$Moisture\ (\%) = \frac{Sample\ weight\ before\ drying - Sample\ weight\ after\ drying}{Sample\ weight\ before\ drying} \times 100$$

$$Dry\ matter\ \% = 100\% - Moisture\ \%$$

3.3.3.2 Ash

The same crucible that was used for the previous dry matter procedure was used for ash procedure. The weight of crucible containing the sample was recorded and placed into the muffle furnace (Carbolite ELF 11/14B Furnace, United Kingdom) at 550°C for 4 hours. Cooling process is done by placing the crucible in the dessicator and weighed again. The percentage of ash was calculated using the following equation:

$$Ash\ \% = \left(\frac{Crucible\ weight\ after\ ashing\ -\ Empty\ crucible\ weight}{Sample\ weight}\right) \times 100$$

3.3.3.3 Crude Fat

Empty round bottle flasks (RBF) were labelled and dried in the oven at 105°C for 1 hour. After being cooled in dessicator for 20 minutes , the flask is weighed. About 3.0 g of sample was weighed and transferred into an extraction thimble and covered with fat free cotton wool. The thimble is then placed into Soxhlet apparatus (Gerhardt Classic Soxhlet Apparatus, Germany). 230 ml of petroleum benzene is poured into RBF which then will be fixed to the Soxhlet apparatus. Water tap is opened and allowed to flow gently through the apparatus throughout the procedure. Heating plate is switched on and put at lowest heat which then will be gradually increased at an interval of 15 minutes until it reaches a stable boiling point. The procedure will run for 4 hours.

After 4 hours, RBF is detached from Soxhlet apparatus and solvent from Soxhlet apparatus is drained into RBF. RBF is then placed in the oven at 80°C overnight to ensure petroleum benzene is completely evaporated. RBF is taken out and cooled down in dessicator and weighed. Percentage of crude fat is calculated using the equation:

$$Crude\ fat\ (\%) = \left(\frac{Dried\ RBF\ weight\ after\ evaporation\ -\ empty\ RBF\ weight}{Sample\ weight}\right) \times 100$$

3.3.3.4 Crude Protein

About 1 g of sample is weighed and put into Kjedahl's flask and one Kjeldahl catalyzer tablet is put inside the flask. 20 ml of Sulphuric Acid (3.5 g K_2SO_4 + 0.4 g $CuSO_4$ x $5H_2O$) is poured into Kjeldahl's flask and shaken gently for 1 minute. Kjldahl's flask is then fixed to Kjeldahl digestion set (Gerhardt Classic Digestion Set, Germany) and the heating plate is switched on. Gradually increase the temperature at an interval of 5 minutes until maximum heat is achieved.The sample was boiled until solution turns bluish or greenish in colour. The chemical reaction occurring during the digestion is as follows:

$$Sample = H_2SO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2$$

As the process is completed, the flask is cooled down which then will proceed with the distillation process using (Gerhardt Vapodest® VAP20s, Malaysia).

Next, 75 ml of 2% Boric acid is added into Erlenmeyer flask along with 8 drops of methyl red and bromocresol green indicator were dropped into each flask. About 25 ml of distilled water is poured into the flask containing the digestion solution while the remaining 25 ml is used to rinse remaining digestion solution in the flask. The remaining digestion solution is poured into the distillation tube. The distillation tube and Erlenmeyer flask is fixed onto distillation and distillate platforms respectively. The process took around 3 minutes. The process involved releasing the entrapped

sulphate salts of ammonium, thus producing ammonia. The chemical reaction that occurred during the distillation is as follows:

$$(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO + 2NH_3 + H_2O$$

Achieving the process above, the ammonia was collected by the 2% boric acid at the distillation set, as shown by the chemical reaction below:

$$2NH_3 + H_2O \rightarrow NH_4OH\ (collected\ by\ boric\ acid)$$

The mixture in the Erlenmeyer flask was then titrated with 0.1M hydrochloric acid in order to determine the nitrogen content, as shown by the formula below:

$$Nitrogen\ (\%) = \left[\frac{(Titrant\ volume - Blank\ value) \times Acid\ normality \times 1.4007}{Sample\ weight}\right] \times 100$$

$$Crude\ protein\ (\%) = Nitrogen\ (\%) \times 6.25$$

Where,

Blank value = 0.2ml

Acid normality = 0.1 ml

The value of nitrogen is then further multiplied by protein factor, which is 6.25, to obtain the crude protein value.

3.4 Analysis of Data

For the statistical analysis, all data obtained were tabulated first in Microsoft Excel 2020 and the data were all expressed as mean ± standard error of mean (SEM). Data was then analysed using IBM SPSS version 28. One-way analysis of variance (ANOVA) was performed for normally

distributed data and Kruskal-Wallis Test for non-normally distributed data. Dunnett's test was carried out to test significance between control and treatment groups at $p<0.05$.

4.0 Results

4.1 Meat Colour Score

The graph in Figure 1 and Figure 2 shows average meat colour score on *Longissimus dorsi* and *Bicep femoris* of all goats in their respective group. All groups for both muscle types displayed having the same meat colour score of 2 which is very dark red. Statistical analyses showed that there were no significant differences between all groups at $p<0.05$.

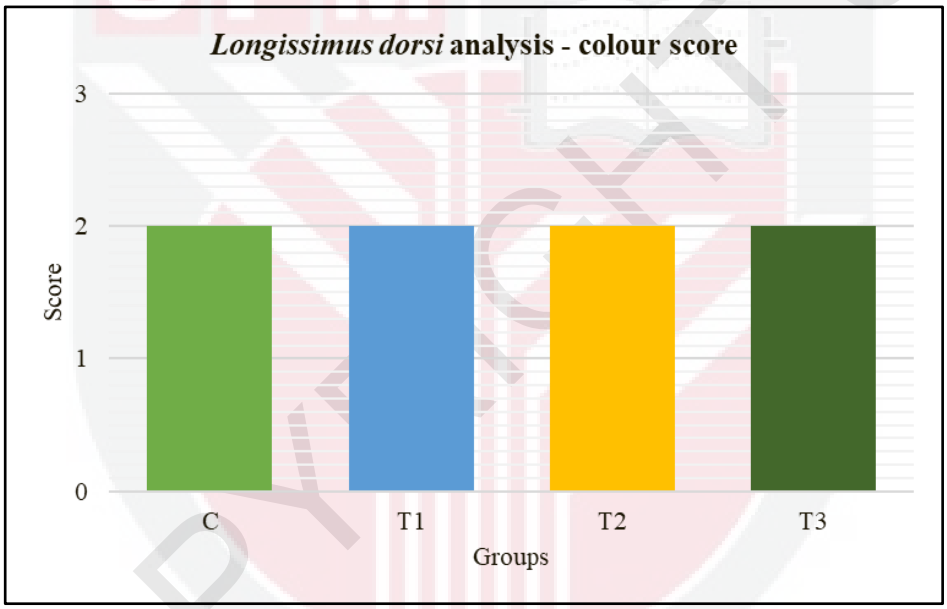

Figure 1: The meat colour score of *Longissimus dorsi* for each experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

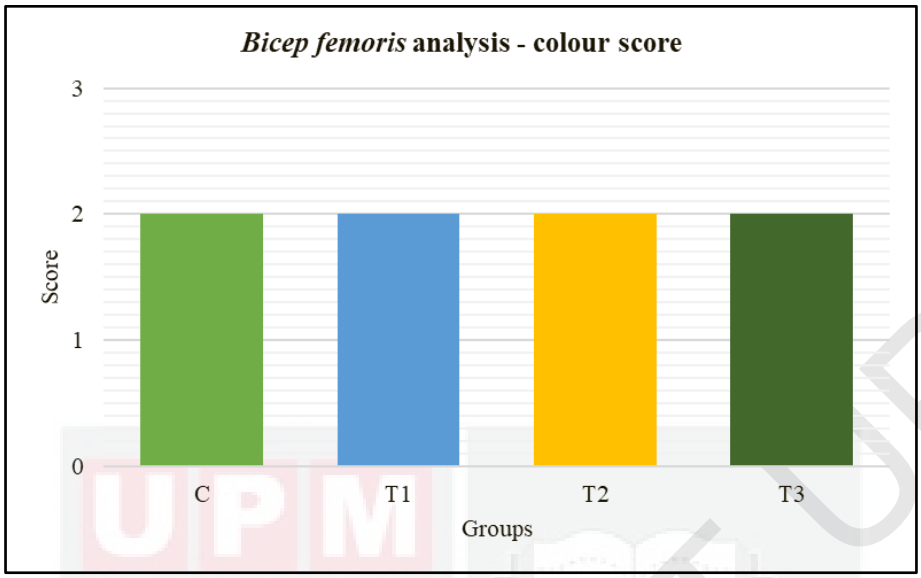

Figure 2: The meat colour score of *Bicep femoris* for each experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

4.2 Fat Colour Score

The graph in Figure 3 shows average fat colour score on subcutaneous fat of all goats in their respective group. For subcutaneous fat, Treatment 1 showed having the same colour score as the control group which is 2 (creamy white) while Treatment 2 and 3 have a lower fat colour score of 1 (white). However, statistical analysis showed no significant differences between all groups at $p<0.05$.

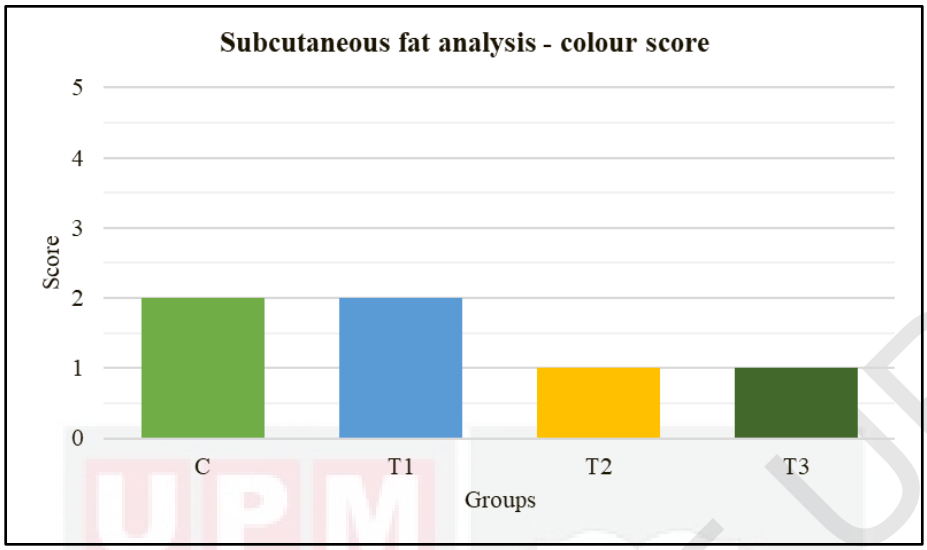

Figure 3: The fat colour score of subcutaneous fat for each experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

The graph in Figure 4 shows average fat colour score on internal fat of all goats in their respective group. For internal fat, Treatment 1, Treatment 2 and Treatment 3 displayed a lower fat colour score of 1 (white) than Control group which showed a fat colour score of 2 (creamy white). Nevertheless, statistical analysis showed no significant differences between all groups at $p<0.05$.

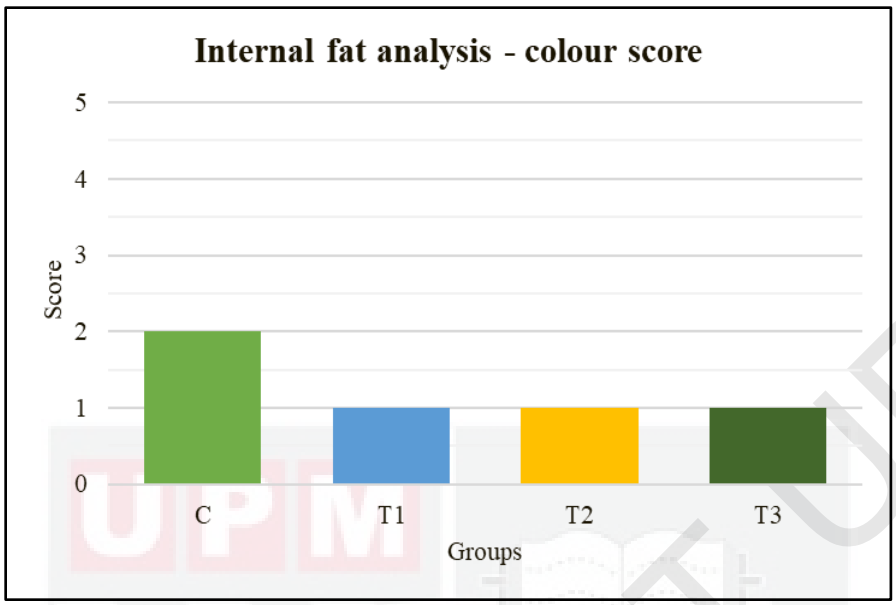

Figure 4: The fat colour score of internal fat for each experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

4.3 Proximate Analysis

4.3.1 Nutritional Composition of Meat

The results of the proximate analyses of *Longissimus dorsi* and *Bicep femoris* of each group were tabulated in Appendix 1 and Appendix 2 respectively. The value used was mean ± standard error of mean of each group.

Figure 5 shows ash content for *Longissimus dorsi* of each group. The result showed Control has the highest ash content at 1.28 - 1.36% followed by Treatment 1, Treatment 2 and Treatment 3 at 1.27 - 1.29%, 1.10 - 1.28% and 1.07 - 1.12% respectively. However, statistical analyses showed that there were no significant differences ($p<0.05$) for ash content between all groups at ($p>0.05$).

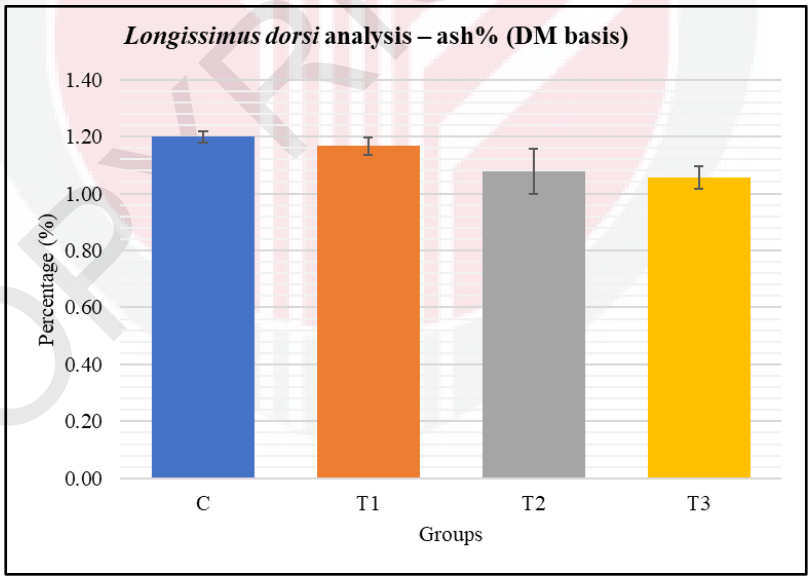

Figure 5: The percentage of ash content in *Longissimus dorsi* of each experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

Figure 6 shows the ash content of *Bicep femoris* of each group. The result of ash content in *Bicep femoris* shows Treatment 3 having the lowest ash content at 0.57 - 0.77%. This is followed by Treatment 2 having ash content at 1.07 - 1.09%, Treatment 1 having ash content at 1.09 - 1.11% and Control having ash content at 1.04 - 1.20%. Statistical analyses showed that there were significant differences ($p<0.05$) for crude protein for Treatment 3.

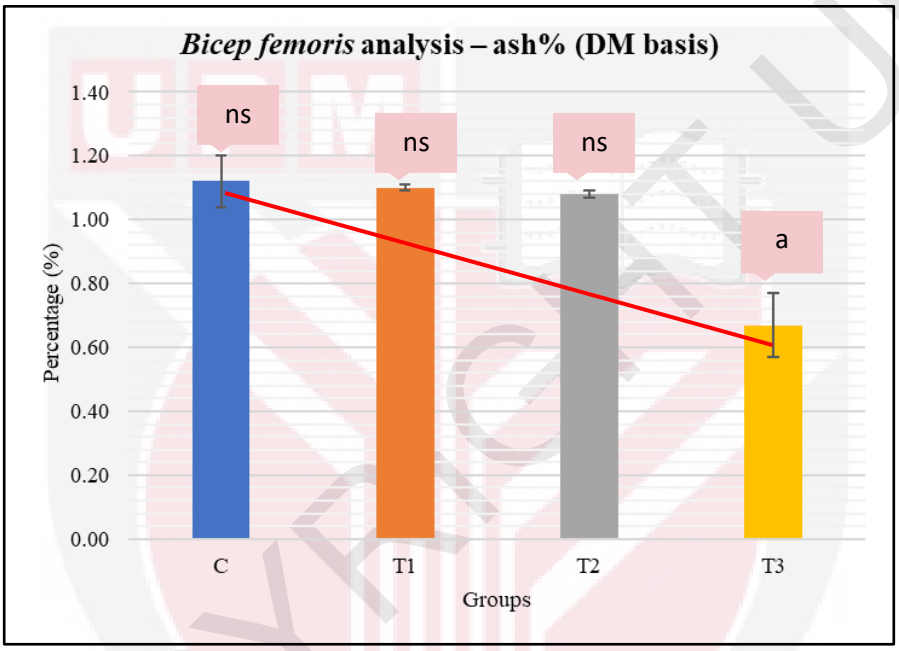

Figure 6: The percentage of ash content in *Bicep femoris* of each experimental group. Groups with different superscripts are significantly different at $p<0.05$

Figure 7 showed crude fat composition in *Longissimus dorsi* of each group. Treatment 3 achieved having the highest crude fat content at 11.62 - 16.42%, followed by Treatment 2 with crude fat content at 7.20 - 12.80%, Treatment 1 with crude fat content at 8.16 -8.64% and Control with crude fat content at 11.62 - 16.42%. Statistical analyses showed no significant differences between all groups at $p<0.05$.

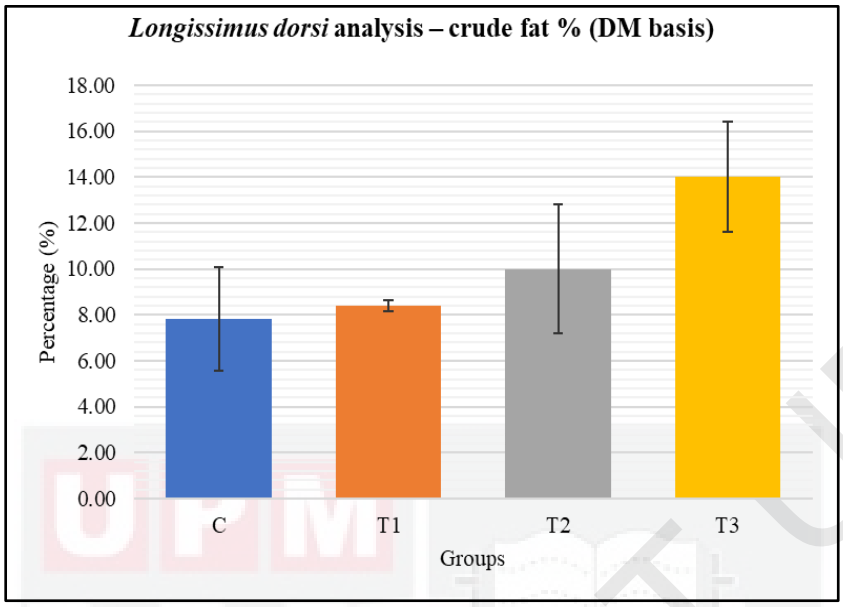

Figure 7: The percentage of crude fat in *Longissimus dorsi* according to the experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

Figure 8 showed crude fat composition in *Bicep femoris* of each group. It has been shown that Treatment 3 has the highest crude fat content at 10.12 - 11.52%, followed by Treatment 1 with crude fat content at 5.84 - 7.02% and Control at 4.80 - 5.36%. Whereas, Treatment 2, has crude fat content at 5.84 - 7.02%. Nevertheless, statistical analyses showed no significant differences between all groups at $p<0.05$

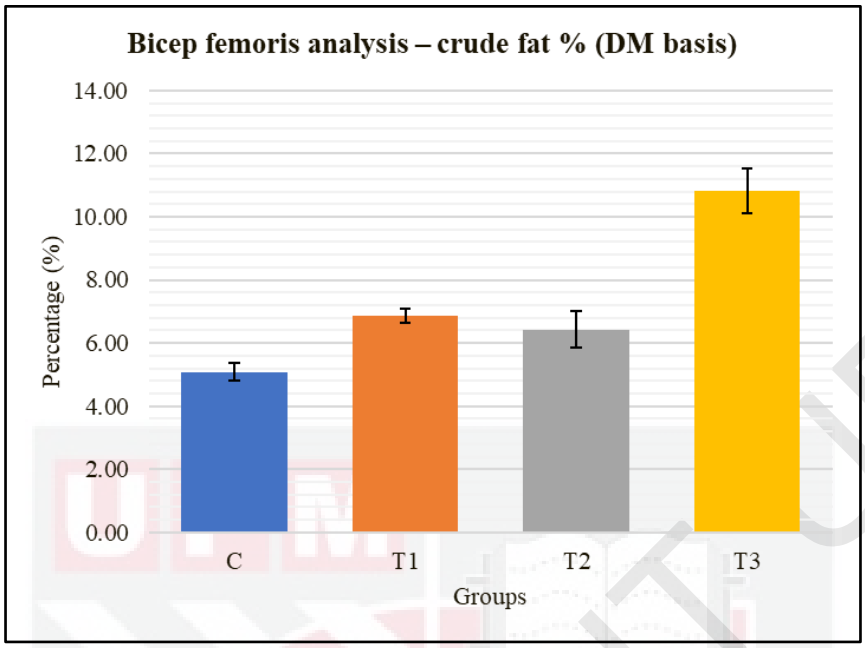

Figure 8: The percentage of crude fat in *Bicep femoris* according to the experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

Figure 9 showed crude protein composition in *Longissimus dorsi* of each group. Based on the result, Treatment 3 has been seen to record the highest crude protein content at 13.44 - 13.56% while Treatment 2 records the second highest crude protein content at 13.21 - 13.23%. As for Control and Treatment 1, crude protein content records at 11.15 - 11.27% and 13.02 - 13.06% respectively. Statistical analyses showed that there were significant differences ($p<0.05$) for crude protein for Treatment 2 and Treatment 3.

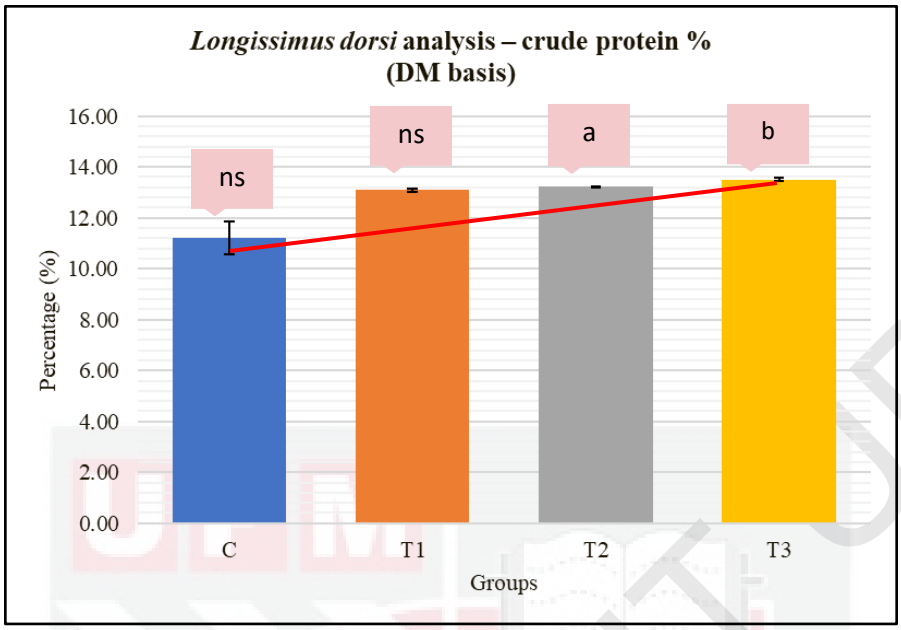

Figure 9: The percentage of crude protein in *Longissimus dorsi* according to the experimental group. Groups with different superscripts are significantly different at $p<0.05$.

Figure 10 showed crude protein composition in *Bicep femoris* of each group. From the result, Treatment 3 records having the highest crude protein content at 12.83 - 13.41% while Control has the lowest crude protein content at 10.56 - 11.26%. As for Treatment 1 and Treatment 2, it records crude protein content at 11.44 - 12.24% and 12.22 - 12.40% respectively. Statistical analyses showed that there were significant differences ($p<0.05$) for crude protein for Treatment 3.

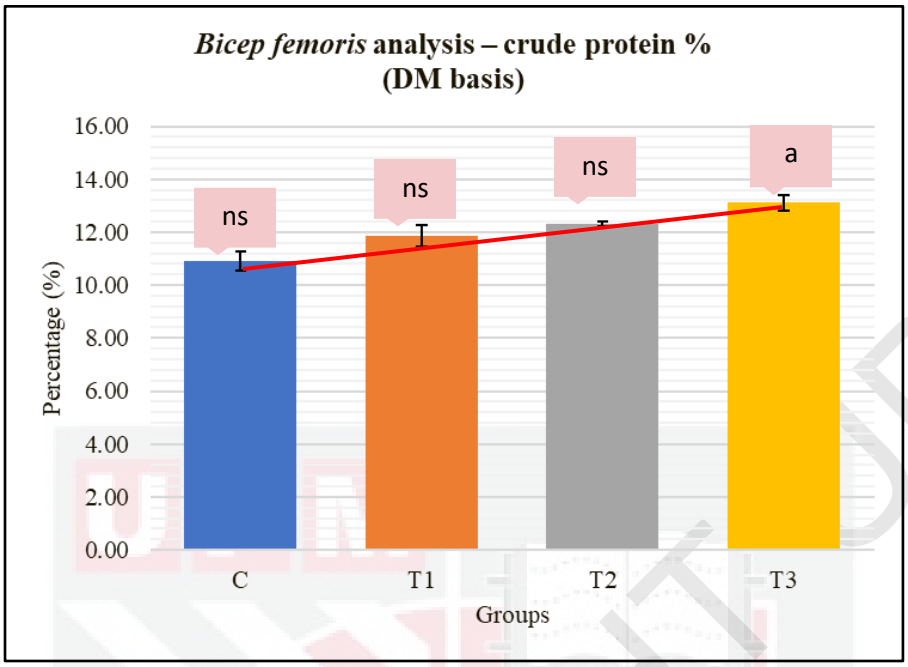

Figure 10: The percentage of crude protein in *Bicep femoris* according to the experimental group. Groups with different superscripts are significantly different at $p<0.05$.

Figure 11 showed the percentage of moisture in *Longissimus dorsi* of each group. Based on the figure, Control recorded having the highest moisture content at 12.80 - 13.60%. This is followed by Treatment 1 with moisture content at 1.27 - 1.29%, Treatment 2 with moisture content at 1.10 - 1.28% and Treatment 3 with moisture content at 1.06 - 1.12%. Statistical analyses showed no significant differences between all groups at $p<0.05$

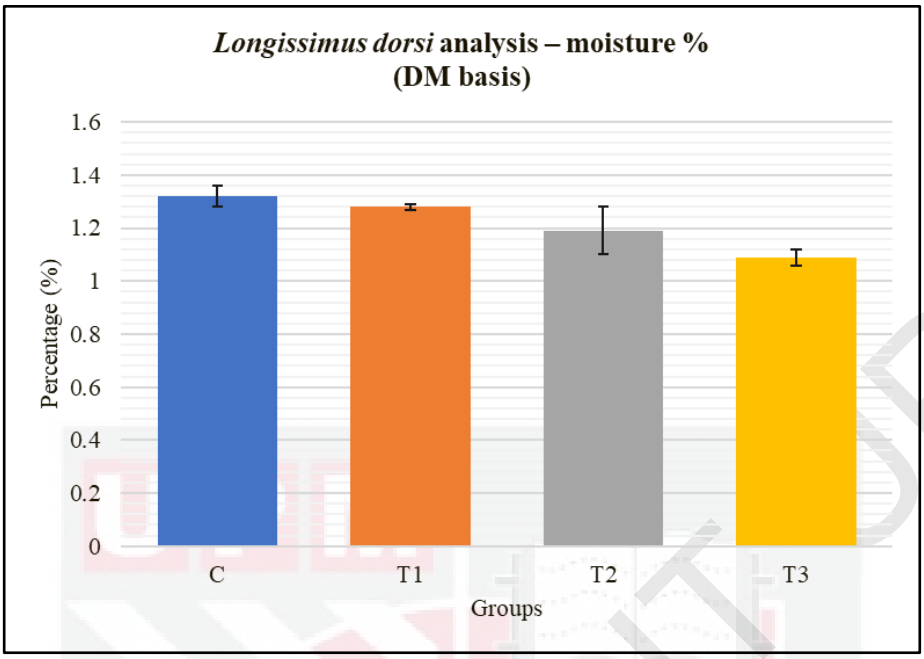

Figure 11: The percentage of moisture in *Longissimus dorsi* according to the experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

Figure 12 showed the percentage of moisture in *Bicep femoris* of each group. Trend of moisture for *Bicep femoris* follows the same trend as *Longissimus dorsi* with Control having the highest moisture percentage at 1.27 - 1.53% and Treatment 3 having the lowest moisture percentage at 1.09 - 1.21%. While for Treatment 1 and Treatment 2 it has moisture content at 1.22 - 1.24% and 1.14 - 1.16% respectively. Statistical analyses showed no significant differences between all groups at $p<0.05$.

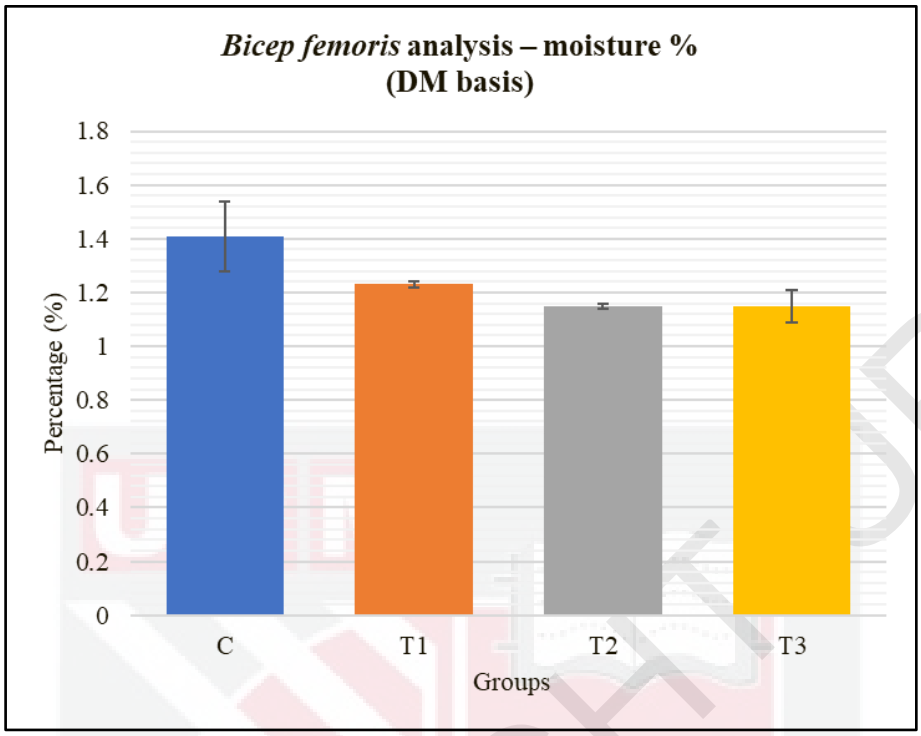

Figure 12: The percentage of moisture in *Bicep femoris* according to the experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

4.3.2 Nutritional Composition of Fat

The results of crude fat composition of subcutaneous fat and internal fat were tabulated in Appendix 3 and Appendix 4 respectively. The value used was mean ± standard error of mean of each group.

Figure 13 showed crude fat composition in subcutaneous fat for all groups. Treatment 3 has the highest crude fat at 72.11 - 80.97%, followed by Treatment 2 at 68.46 - 77.00%, Treatment 1 at 61.63 - 70.45% and Control at 57.57 - 67.57%. Statistical analyses showed no significant differences between all groups at $p<0.05$.

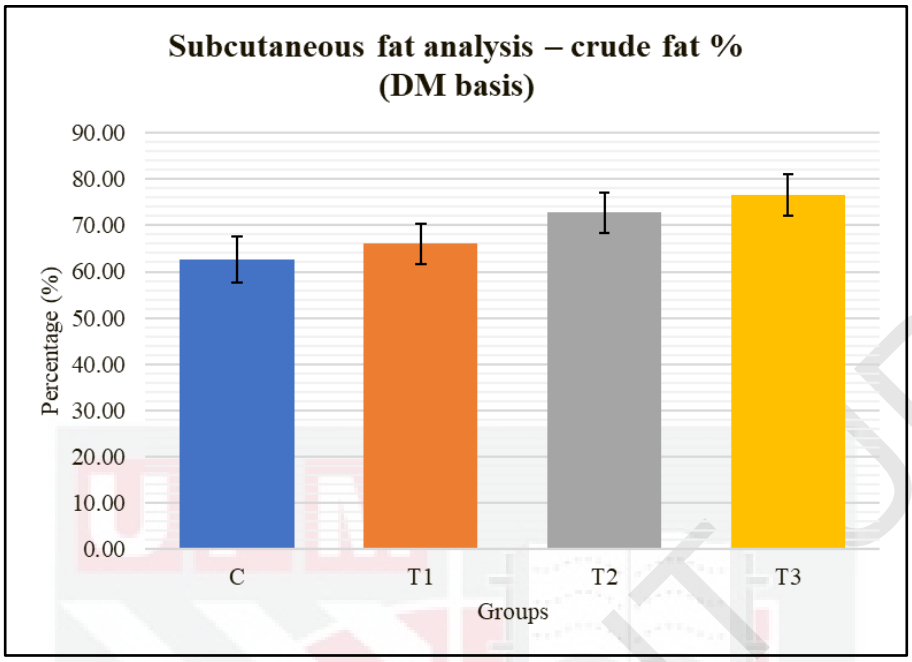

Figure 13: The percentage of crude fat in subcutaneous fat according to the experimental group. There were no significant differences between control and treatment groups ($p>0.05$)

Figure 14 showed crude fat composition of internal fat in all groups. Treatment 3 has the highest crude fat composition at 95.70 - 96.66% followed by Treatment 2 with crude fat composition at 94.78 - 96.90%. As for Treatment 1 and Control has crude fat composition at 81.10 - 89.10 and 78.68 - 86.34% respectively. Statistical analyses showed that there were significant differences ($p<0.05$) for crude protein for Treatment 2 and Treatment 3.

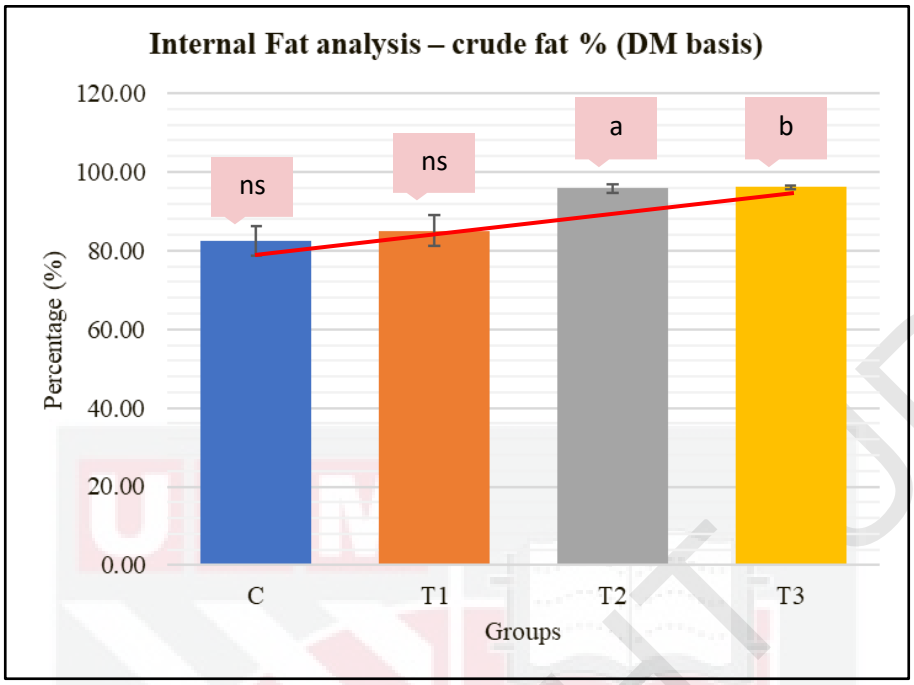

Figure 14: The percentage of crude fat in internal fat according to the experimental group. Groups with different superscripts are significantly different at $p<0.05$.

5.0 DISCUSSION

According to Young and West (2001) lean meat colour plays an important role to consumers as it is an indicator to the meat quality. When exposed to air, red meat has a tendency to turn brown and meat also tends to degrade with time due to microbial activity, thus the browning of meat with time is a poor indicator of spoiling. In this study, the colour of *Longissimus dorsi* and *Bicep femoris* for all groups has the same score of 2 which is very dark red. This correlates with findings that generally, goat meat has a meat colour of dark red with coarse texture (Meat Speciation., 2012). Research from Dransfield et al. (2004) and Craddock et al. (2008) state dietary energy level from the treatment group does not contribute to meat colour. In contrast, meat colour is more likely to be affected by breed, slaughter weight, nutritional composition of feed and ageing (Kadim et al., 2003; Beriain et al., 2000; Kannan et al., 2006).

Fat colour is also another indicator on freshness and quality for consumers as they prefer white colour fat in contrast with yellow colour fat (Liu et al., 2022). The fat colour for both subcutaneous fat and internal fat show on having a colour score that mostly ranges between 1 (white) and 2 (creamy white). From this study, decreased yellowness in fat has been associated with high fat content in the fat sample. In a study conducted by Dunne et al. (2009) a decrease in colour in adipose tissue is due to dilution of carotenoid by high triacylglycerol in it.

Ash content for both *Longissimus dorsi* and *Bicep femoris* shows a decrease in trend from Control group to Treatment 3. Based on study by McClements (2022) ash content is affected by protein and carbohydrate content in the sample. Mineral analysis needs to be done on the sample as a specific amount of inorganic compound needs to be measured to correctly evaluate the quality

and nutrition of the sample whereas the ash content only measures the total amount of mineral within the sample.

Regarding crude fat and crude protein content for both *Longissimus dorsi* and *Bicep femoris*, it both shows an increase in trend from Control to Treatment 3. According to Atti et al. (2004) the increase for both crude fat and crude protein is influenced by dry matter intake of the animal itself. When given enzyme supplementation in ration this will improve dietary palatability due to production of sugar from hydrolysis of fiber (Rusli et al., 2020).

The decrease in trend for moisture content for both *Longissimus dorsi* and *Bicep femoris* from Control to Treatment 3 is consistent with findings from Saturno et al. (2020) that as fat content increases in muscle its moisture content will decrease. This is mostly due to the inability of adipose tissue to hold water content (Stankov et al., 2002). In this study, the fat content of *Longissimus dorsi* and *Bicep femoris* is inversely proportional to its corresponding moisture content.

Despite composition of crude fat in subcutaneous fat shows non-significance while internal fat shows significance, it both have the same result which result shows increase in trend from Control to Treatment 3. Study by Dahlan. (2000) reported increased digestibility of feed will result in higher nutrient utilisation by animals which influence fat content in goats. This is further supported with a study from Hermiati et al., (2013) regarding use of enzyme from *P. chrysosporium* on OPF that reported reduction of lignin and increase in α-cellulose in OPF treated with the enzyme will increase nutrient utilisation by animal.

6.0 CONCLUSION

As indicated by crude protein and crude fat content of *Longissimus dorsi* and *Bicep femoris,* it is concluded Treatment 3 shows better results compared to other treatment groups based on its high crude protein and crude fat of meat and fat. In regard to crude fat content of subcutaneous fat and internal fat, the result still remains unclear as further research needs to be done regarding fatty acid composition to determine and measure the amount of specific fatty acid in lipids from the sample. Thus, it is beneficial to use enzyme extract from fungi to treat OPF as it can increase digestibility, nutrient utilisation, and animal performance such as meat and fat quality.

7.0 RECOMMENDATIONS

For future studies it is recommended to increase sample size to minimise error margin and decrease variability. Furthermore, more procedures such as mineral analysis and fatty acid composition need to be done to further assess effectiveness of pre-treated OPF on meat and fat quality.

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APPENDICES

Appendix 1. Proximate analysis of *Longissimus dorsi* of each group in terms of moisture, ash, crude fat, and protein

Nutritional component (%)	Groups			
	Control	Treatment 1	Treatment 2	Treatment 3
Moisture ^{ns}	1.32 ± 0.04	1.28 ± 0.01	1.19 ± 0.09	1.09 ± 0.03
Ash ^{ns}	1.20 ± 0.02	1.17 ± 0.03	1.08 ± 0.08	1.06 ± 0.04
Crude Fat ^{ns}	7.82 ± 2.24	8.40 ± 0.24	10.00 ± 2.80	14.02 ± 2.40
Crude Protein	11.21 ± 0.06 ^{ns}	13.09 ± 0.07 ^{ns}	13.22 ± 0.01 ^{a}	13.50 ± 0.06 ^{b}

Values are means ± standard error. ^{ns} Not statistically significant at $p<0.05$. ^{abcd} Values with different superscripts at the same row differed significantly at $p<0.05$. Control: basal diet (30 % non-treated OPF, 40 % Napier and 30 % concentrate), Treatment 1 (30 % of treated OPF (buffer pre-treatment), 40 % Napier and 30 % concentrate), Treatment 2 (30% treated OPF (individual enzyme pre-treatment), 40% Napier and 30% concentrate) and Treatment 3 (30% treated OPF (cocktail enzyme pre-treatment), 40% Napier and 30% concentrate)

Appendix 2. Proximate analysis of *Bicep femoris* of each group in terms of moisture, ash, crude fat and protein

Nutritional component (%)	Groups			
	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**
Moisture ^{ns}	1.40 ± 0.13	1.23 ± 0.01	1.15 ± 0.01	1.15 ± 0.06
Ash	1.12 ± 0.08	1.10 ± 0.01	1.08 ± 0.01	0.67 ± 0.10
Crude Fat ^{ns}	5.08 ± 0.28	6.86 ± 0.22	6.43 ± 0.59	10.82 ± 0.70
Crude Protein	10.91 ± 0.35 ^{ns}	11.84 ± 0.40 ^{ns}	12.31 ± 0.09 ^{ns}	13.12 ± 0.29 ^{a}

Values are means ± standard error. ^{ns} Not statistically significant at $p<0.05$. ^{abcd} Values with different superscripts at the same row differed significantly at $p<0.05$. Control: basal diet (30 % non-treated OPF, 40 % Napier and 30 % concentrate), Treatment 1 (30 % of treated OPF (buffer pre-treatment), 40 % Napier and 30 % concentrate), Treatment 2 (30% treated OPF (individual enzyme pre-treatment), 40% Napier and 30% concentrate) and Treatment 3 (30% treated OPF (cocktail enzyme pre-treatment), 40% Napier and 30% concentrate)

Appendix 3. Proximate analysis of subcutaneous fat of each group in terms of crude fat

Nutritional component (%)	Groups			
	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**
Crude Fat [ns]	62.57 ± 5.00	66.04 ± 4.41	72.73 ± 4.27	76.54 ± 4.43

Values are means ± standard error. [ns] Not statistically significant at $p<0.05$. [abcd] Values with different superscripts at the same row differed significantly at $p<0.05$. Control: basal diet (30 % non-treated OPF, 40 % Napier and 30 % concentrate), Treatment 1 (30 % of treated OPF (buffer pre-treatment), 40 % Napier and 30 % concentrate), Treatment 2 (30% treated OPF (individual enzyme pre-treatment), 40% Napier and 30% concentrate) and Treatment 3 (30% treated OPF (cocktail enzyme pre-treatment), 40% Napier and 30% concentrate)

Appendix 4. Proximate analysis of internal fat of each group in terms of crude fat

Nutritional component (%)	Groups			
	Control	**Treatment 1**	**Treatment 2**	**Treatment 3**
Crude Fat	82.51 ± 3.83 [ns]	85.10 ± 4.00 [ns]	95.84 ± 1.06 [a]	96.18 ± 0.48 [b]

Values are means ± standard error. [ns] Not statistically significant at $p<0.05$. [abcd] Values with different superscripts at the same row differed significantly at $p<0.05$. Control: basal diet (30 % non-treated OPF, 40 % Napier and 30 % concentrate), Treatment 1 (30 % of treated OPF (buffer pre-treatment), 40 % Napier and 30 % concentrate), Treatment 2 (30% treated OPF (individual enzyme pre-treatment), 40% Napier and 30% concentrate) and Treatment 3 (30% treated OPF (cocktail enzyme pre-treatment), 40% Napier and 30% concentrate)

Appendix 5: Crude fat content obtained by using Soxhlet fat analysis method. The samples were added with petroleum benzene to extract the fat.

Appendix 6: Crude Protein content obtained by using the Kjeldahl method. The samples were digested, distilled, and then titrated.