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Effect of physical pretreated of oil palm frond on apparent digestibility of
Boer goats

By

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled “**Effect of physical pretreated oil palm frond on apparent digestibility of Boer goats**” by IRIS CHIENG, matric number F14A0089 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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FYP FIAT

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ABSTRACT

Oil palm frond (OPF) was generated the most abundant biomass in palm oil industry. It was widely utilized as animal feed, but OPF cannot be used alone due to its low nutritive value and low digestibility. In order to improve the digestibility of OPF, pretreatment method was applied. Hence, the current study was conducted to determine the digestible nutrients of Boer goats following OPF feeding and to evaluate the effect of physical pretreatment of OPF on apparent digestibility of Boer goat. A trial was conducted at goat farm, Agro Technopark, Universiti Malaysia Kelantan, Jeli campus for 120 days feeding trial and ten (10) days digestibility trial. Twelve (12) male Boer goats were fed with three treatment diets: 88% Napier grass and 12% pellet (Control Group); 84% Napier grass, 12% pellet and 4% chopped OPF (Treatment 1) and 84% Napier grass, 12% pellet and 4% pressed OPF (Treatment 2). All Boer goats were placed in individual pen and free to access water in three months. The results showed that there was no significant difference ($p>0.05$) observed on apparent digestibility of Boer goats among the treatments. Control group presented higher crude protein and organic matter digestibility compare to other treatments. The highest significant value of crude fat is in Control Group. The dry matter digestibility value of Treatment 2 is higher compare to other treatment. It was concluded that physical pretreated OPF did not give significant effect comparing to other treatments but highly digestible to Boer goat.

Keywords: Oil palm frond, pretreated, Boer goat, apparent digestibility, physical.

**Kesan pra-rawatan fizikal pelepah kelapa sawit terhadap kebolehceraan
ketara kambing Boer**

ABSRTAK

Pelepah kelapa sawit (OPF) telah menjana biojisim yang paling banyak di dalam industri kelapa sawit. Pelepah kelapa sawit (OPF) diguna secara meluas sebagai makanan haiwan, tetapi ia tidak boleh digunakan secara berasingan kerana nilai pemakanan dan pencernaan yang rendah. Kaedah pra-rawatan telah digunakan untuk meningkatkan pencernaan terhadap OPF. Oleh itu, kajian dijalankan untuk menyelidik pencernaan nutrisi kambing Boer diikuti dengan pemberian makanan OPF dan kesan pra-rawatan fizikal dinilai dalam kebolehceraan ketara kambing Boer. Percubaan pemberian makanan telah dijalankan selama 120 hari dan 10 hari untuk percubaan pencernaan terhadap kambing Boer di ladang kambing, Agro Technopark, Universiti Malaysia Kelantan, Kampus Jeli. Tiga rawatan permakanan terdiri daripa kawalan: 88 % rumput Napier dan 12 % pellet; Rawatan 1: 84 % rumput Napier, 12 % pelet dan 4 % OPF yang dicincang dan Rawatan 2: 84 % rumput Napier, 12 % pelet and 4 % OPF yang ditekan telah diberi makan kepada 12 ekor kambing Boer. Kambing Boer ditempatkan di dalam kandang yang berasingan dengan bekalan air minuman selama tiga bulan. Hasil kajian menunjukkan bahawa tiada perubahan yang signifikan ($p>0.05$) dalam kebolehceraan ketara kambing Boer. Kawalan menunjukkan kandungan protein dan bahan organik yang lebih tinggi berbanding dengan rawatan yang lain. Kawalan juga menunjukkan nilai signifikan yang paling tinggi dalam kandungan lemak. Rawatan 2 mempunyai nilai pencernaan bahan kering yang paling tinggi berbanding dengan rawatan yang lain. Kesimpulannya, OPF dengan pra-rawatan fizikal tidak memberi kesan perubahan yang ketara tetapi mudah dihadam oleh kambing Boer.

Kata kunci: pelepah kelapa sawit, prarawatan, kambing Boer, kobolehceraan ketara, fizikal.

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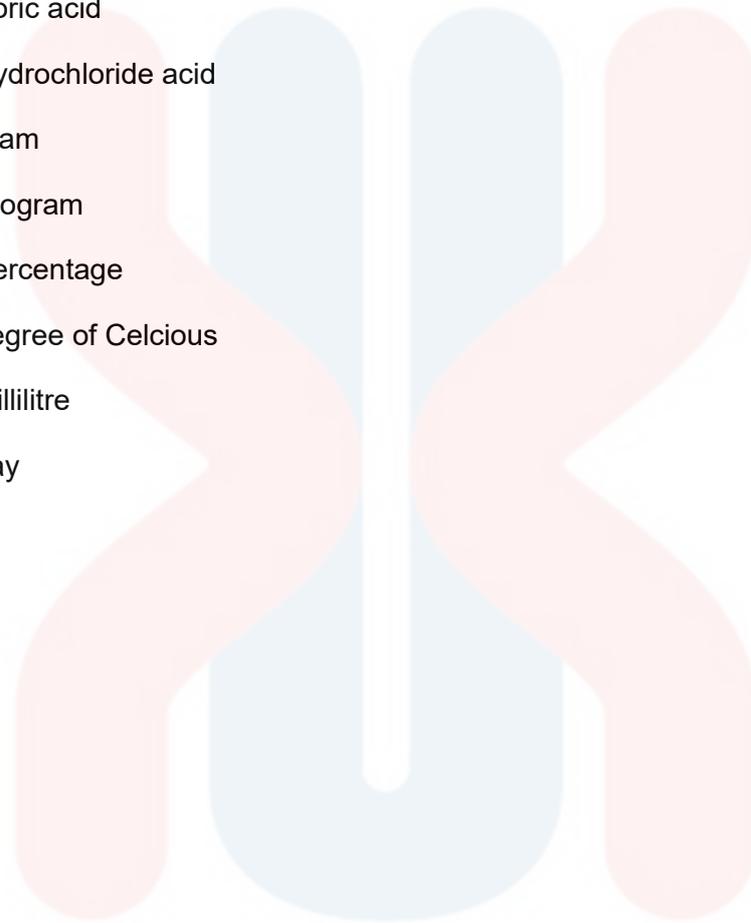


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LIST OF ABBREVIATIONS AND SYMBOLS

OPF	Oil Palm Frond
CP	Crude Protein
CF	Crude Fat
CF	Crude Fibre
EE	Ether Extracts
DM	Dry matter
OM	Organic matter
N ₂ SO ₄	Sulphuric acid
PKS	Palm Kernel Shell
OPL	Oil Palm Leaves
OPT	Oil Palm Trunks
POME	Palm Oil Mill Effluent
MF	Mesocarp Fibre
EFB	Empty Fruit Bunches
GP	Grape Pomace
MARDI	Malaysia Agriculture Research and Development Institute
UFA	Unsaturated Fatty Acid
TEM	Transmission Electron Microscopy
ADF	Acid Detergent Fibre
Ca	Calcium
P	Phosphorus
DMI	Dry Matter Intake
DMD	Dry Matter Digestibility
NDF	Neutral Detergent Fibre
HCW	Hot Compressed Water
SSF	Solid-State Fermentation
PDA	Potato Dextrose Agar

JIS	Japan Industrial Standard
NAOH	Sodium hydroxide
H ₃ BO ₃	Boric acid
HCL	Hydrochloride acid
g	gram
kg	kilogram
%	Percentage
°C	degree of Celcius
ml	millilitre
d	day



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Chapter 1

INTRODUCTION

1.1 Background of the study

According to Wan Zahari et al. (2003), the total population of ruminants in Malaysia are 1132,000 heads. Population of animal increase which means the demand of feed inclines as well. There are some crops applied as feed source in livestock production and edible to human namely, corn, wheat, barley, oats and soybean (Mowrey and Spain 1999; Sapkota et al., 2007; Wilkinson 2011). Therefore, the scenario of competition between feed and food is gradually severe. Hence, researchers are finding another feed source to prevent competing with food production, for example, orange peel (Eleni, Evangelia and Parasekvi, 2015), coffee waste (Habtamu, 2014), oil palm by product, banana leaves (Michael et al., 2016) and sugar cane (Pate et al., 2002).

According to Malaysia Palm Oil Board (2011), production of oil palm (*Elaeis guineensis*) has boosted rapidly and Malaysia becomes second largest producer of oil palm in the world. Plantation area of oil palm in Malaysia is up to 5.6 million in 2015 (MPOB, 2015). After processing the fibre crops, the waste product and by-product are able become alternative feeds (Myer, 2003). Furthermore, lots of biomass waste produced during harvesting oil palm fruits or processing oil palm. Example of the wastes are oil palm fronds (OPF), palm kernel shell (PKS), oil palm leaves (OPL), oil palm trunks (OPT), palm oil mill effluent (POME), mesocarp fibre (MF) and empty fruit bunches (EFB) (Mushtaq et al., 2015). The OPF was reported

to be the most abundant oil palm biomass generated from palm oil industry, thus there are lots of research investigated on the utilization of OPF as animal feed.

Pin et al. (2017) study the effect of oil palm frond by fungal treated on meat quality, carcass traits, performance and muscle chemical composition of finishing goats. However, there was no significant effect observed in the study. The hind leg and chump were the only changes showed in the study by fungal treated (FT) inclusion. Effect of protein and energy on growing Saanen goats by feeding oil palm frond (OPF) as roughages was conducted by Pramote et al (2006). The study indicated 86g/d BW gain could reach by feeding OPF diet for growing dairy goats. OPF has been determined as an unrealized feed for ruminants (Dahlan, 2000). Goat and sheep highly acceptable OPF if dry matter intake (DMI) was 30-45 g/kg live weight (Dahlan, 1992).

OPF contains 4.7% crude protein and 38.5% crude fibre (Wong and Wan Zahari 1992; Wan Zahari et al., 2000) and 38.8% cellulose, 36.4% hemicelluloses and 19.3% lignin (Khor and Lim, 2006). OPF has low nutritive value due to high lignin constraint. Lignin is another form of insoluble fiber which able degrades by many microorganisms (Saritha et al., 2012). In order to loosen up the lignocellulosic contents, the OPF should undergo the pretreatment technique. The current study investigated the physical pretreatment by pressing method of OPF.

The important element in feed nutritive value is digestibility. High amount of certain nutrient absorbed by animal represent that high digestibility of the feedstuff (Coleman and Moore, 2003). On the other hand, low digestibility means that the feedstuff is undesirable as sole feed to animal. The ability to digest the feed is influenced by several factors such as feed composition and palatability. Apparent digestibility is the formula to check the digestibility of feedstuff (Nurhaita

et al., 2010). Studies on the effect of physical pretreatment of OPF on the digestibility of the goats are limiting. Therefore, this study was conducted to evaluate the effect of physical pretreatment of OPF on apparent digestibility.

1.2 Problem statement

Competition between human food and animal feed recently had become an issue in the world. There is some crop is edible by human and animal, for example: wheat, barley, corn and oats.

1.3 Hypothesis

H₀: This study hypothesized that the physical pretreated OPF could not give any significant effect on apparent digestibility of Boer goats. The digestibility of the physical pretreated OPF by goats not higher compared to non-treated OPF.

H₁: This study hypothesized that the physical pretreated OPF could give the significant effect on apparent digestibility of Boer goats. The digestibility of the physical pretreated OPF by goats will be higher compared to non-treated OPF.

1.4 Objective

1. To assess the digestible nutrients of Boer goats following the OPF feeding.
2. To evaluate the effect of physical pretreatment of OPF on apparent digestibility of Boer goats.

1.5 Scope of study

Malaysia produce much agricultural waste in every year. OPF contain low nutrition value with high lignin structure. Hence, physical pre-treated OPF would increase the nutrition value in ruminant feed.

1.6 Significance of study

OPF contain low protein and metabolize energy with high lignin. Nutrition of OPF can be improved by physical pretreatment. Physical pretreatment applied in this study was pressing method.

Chapter 2

LITERATURE REVIEW

2.1 Agriculture by-products as animal feed

Agricultural by-products are available in many countries with large quantity such as rice straw, maize stover, oil palm frond and sugarcane bagasse (Devendra, 2009; Sarnklong et al., 2010; Wan Zahari et al., 2003), tomato seed (Cassinerio et al., 2015) and others. The cattle with fed on urea-treated straw showed increase the feed intake and gained weight than non-treated straw (Nguyen,2004). The cottonseed cake in legume act as supplement to maize stover which increased the digestibility of Ethiopian highland sheep compare with legume supplements alone (Girma et al., 1993). Sugarcane may apply as alternative feed source for beef cattle in subtropical and tropical areas. Starchy concentrate ingredient, cereal grains could improve the performance of cattle which fed by sugarcane-based diets mix with urea (Pate et al., 2002).

According to Cassinerio et al., (2015), productions of lactating dairy cows do not change when whole tomato seeds replaced whole cottonseed in the diet. Besides, urea concentration in milk and plasma both decreased with increasing whole tomato seeds. For broiler breeder and pullets, cottonseed is the primary protein source in growing diets (Lordelo et al., 2004). In addition, digestibility of total fatty acids and crude protein decreased while increasing proportion of whole

tomato seeds. Moreover, fecal concentration of linoleic and α -linolenic acids increased while whole tomato seeds increased (Heguy et al., 2015).

High percentage of soluble non-starch polysaccharides boost the small intestine relative length and reduced carcass yield. The birds consume high level of citrus pulp appeared high polyunsaturated fatty acids content in meat (Mourao et al., 2008). Dairy ewes indicated significant effect on fat and non-fat solid (NFS). The fat of milk increased 17% while 5.4% in NFS (Pantelis et al., 2008). Citrus by-product feedstuff (BPF) can be used as a high energy feed in ruminant rations to support growth and lactation of ruminants. Level of citrus pulp should control between 150 and 200g/kg DM to prevent low palatability of goat (Migwi et al., 2001).

Grape pomace (GP) is a source of polyphenols with great antioxidant capacity. The birds fed with GP showed increase total intake and digestibility of extractable polyphenols compared with birds fed supplemented and unsupplemented vitamin E diets (Goni et al., 2007). Lipid oxidation of meat reduced during refrigerated after grape pomace and vitamin E is included in the diet of chicken (Chamorro, 2014). Birds indicate positive antioxidant effect of GP when the level of GP is 6% (Brenes et al., 2008).

Malaysia Agriculture Research and Development Institute (MARDI) used fungal treatment and specific microbes and enzymes to improve the availability of bio-processed PKC (Wan Zahari and Wong, 2009). The major problems of PKC are inconsistencies of quality. The maximum inclusion level of bioprocessed PKC in poultry diets is 30% with 23% crude protein and 8% fiber. According to Wan

Zahari and Alimon (2006), PKC can be fed at up to 30% in catfish (*Clarias gariepinus*) while 20% in Tilapia *Oreochromis niloticus*. After tilapia had feeding enzyme pre-treated PKC, it showed superior growth and feed efficiency compared to fish fed same levels of untreated PKC (NG et al., 2002). Enzyme treated PKC could up to 30% to incorporated into red tilapia diets. By-products from human food able to apply as livestock diets but by product from livestock diets are not suitable as human food due to safety, quality, cultural, or digestibility considerations (Jude et al., 2013).

The oil palm industry plays a significant role as the backbone of Malaysia's economic development. Plantation area shows inclined from 97000 ha in 1965 to 4.5 million ha in 2008. The plantation area in Sarawak is 0.74 million hectares, Sabah is 1.33 million hacter and Peninsular Malaysia is 2.41 million hectares (MPOB, 2009). The largest area occupied by private-estate sector which around 60 percent of the total area. In the other hand, 28 percent of estates owned by government and smallholder have 12 percent of estates (Wan Zahari et al., 2012).

According to Euis et al. (2013), OPF able acts as mixture for ruminants feed, for pulp, and for making compost. Moreover, production of bio-ethanol is another potential of OPF. Frond fiber from OPF able to applied as cellulose feedstock for bio-ethanol. Fresh OPF can ferment to ethanol directly after pressed by sugarcane machine. Low cost and high cellulose content are the main factor that OPF become highly attractive feedstock (Goh et al., 2010). The inclusion levels of OPF increased while the amount of unsaturated fatty acid (UFA) dropped after incubating (Hassim, 2010).

In Malaysia, over amount of oil palm biomass become a challenge for the industry (Lim et al., 2015; Yacob, 2008). Expansion of oil palm plantation was cause to high expenses of effective waste management (Khalil et al., 2014). In addition, OPF has some limitation during processing and storing. After harvesting OPF, it must be chopped within two days. OPF become moldy during storage due to high content of water. Last but not least, transport cost is the concern of using OPF (Dahlan, 2000).

2.2 Plant cell wall characteristics of oil palm frond (OPF)

OPF fibres have variety sizes of vascular bundles. Each vascular bundle was made up of a fibrous sheath, vessels, fibres, and phloem and parenchyma tissues. Thin-walled parenchyma ground tissue consist much vascular bundle. Palisade and mesophyll cells contain thin-walled parenchyma cells which are significant to absorb sunlight in an optimum manner for photosynthesis process. Besides, some vascular bundles included several protoxylem elements as well. In the other hand, xylem and phloem tissues are easily distinguishable. Each bundle has two separate areas which divide by phloem. Parenchyma cells separated protoxylem and metaxylem vessels in the bundle. Parenchyma cells built a living barrier to the possible transfer of gas bubbles between proto- and metaxylem vessels (Tomlinson, 2001). Image analyzer used to observe thick-walled fiber in OPF. Phloem and parenchyma cells contain unlignified primary wall which resulted in a weak positive reaction with toluidine blue. In a vascular bundle, the fiber contains more lignified than metaxylem vessels. According to Abdul Kahlil et al. (2006), OPF has most of fiber and vessel material except phloem. Transmission electron microscopy (TEM) confirmed that cell wall of OPF consists of primary and secondary layers. The percentage of lignin in OPF is 23.3%, cellulose is 39.5%, hemicellulose is 29.8% and Ash is 5.7% (Masashi et al., 2007).

2.3 OPF as animal feed

OPF can get from oil palm tree when harvesting or pruning for replanting. The total OPF production per year is 5500kg per hectare and the average weight of OPF pruned from a mature plant is 13.3kg. Dahlan (2000) reported dry matter (DM) of OPF is 349 g/kg, crude protein (CP) is 70g/kg, ash is 50 g/kg, ether extract (EE) is 24 g/kg, crude fibre (CF) is 323 g/kg, acid detergent fibre (ADF) is 536 g/kg, lignin is 276 g/kg, calcium (Ca) is 4 g/kg and phosphorus (P) is 9 g/kg. In addition, metabolize energy (MJ/kg DM) of OPF is 6.5 while the gross energy is 17.2 (Dahlan, 2000). Petiole provides 70% of dry matter in the OPF while leaves and rachis provide some dry matter as well. Percentage of crude protein (CP) in the leaves is higher than petioles. Furthermore, constantly available of frond confirm the supply of OPF petioles during fronds pruning for fruit harvesting (Che Mohd et al., 2014). OPF can express in variety form to utilize as feedstuff such as freshly chopped, silage, or processed into pellets and cubes. The percentage of 30% is the optimum inclusion level in beef and dairy animals. OPF can help to improve the feeding value by some processing techniques. For example, urea and molasses treatments, preservation as silage, alkali treatment, enzymatic degradation, pelletizing and steaming under high temperature and high pressure. Urea and molasses assist OPF nearly reach the requirement of ruminants for energy and protein (Wan Zahari et al., 2012). The digestibility appears to improve when the optimum level of urea inclusion in steamed OPF showed 30g/kg ration. Dry matter intake (DMI) and dry matter digestibility (DMD) reduced when increasing the level of urea in steamed OPF. It showed that dry matter is inversely proportional to the level of urea in steamed OPF. OPF can enhance the feeding value after undergoing microbial fermentation by Japanese koji. The feeding value

show improved by increasing the CP content, reducing the neutral detergent fibre (NDF) and improving the DMD of the feed, particularly with *Aspergillus awamori* (Ramli et al., 2010). The optimum length of OPF is 2-3cm for conserved as silage for feeding livestock. OPF must ensile under anaerobic conditions in order to produce good quality silage without applied by any additives. However, the addition of urea with 1 to 2% can prevent mould growth. Moreover, more than 3% urea can reduce the nutritive value of the silage. Kedah-Kelantan (KK) bulls showed a DMD value of about 45% for OPF silage. According to Abu Hassan et al. (1993) and Ishida et al. (1994), growing and finishing beef cattle, and lactating cows involved with further long-term feeding. Abu Hassan et al. (1993) reported that the cows fed with 30% OPF silage indicated that produce more milk than those fed with 50% OPF silage. Hence, it obvious that the feed cost can be reduced by OPF silage.

2.4 Pretreatment method of OPF

a) Physical pretreatment

According Devendra and Ankit (2015), physical pre-treatment had been used as one of method for lignocellulosic materials. Surface area increased by reducing the size of biomass through physical pretreatment. Lignocellulosic biomass includes cellulose, hemicelluloses, lignin, extractives, several inorganic materials and compositions of each vary depending on the origin of the lignocellulosic material (Singla et al., 2012; Saini et al., 2014). Physical pretreatment had been proved able to improve lignocellulosic biomass accessibility to enzymatic hydrolysis (Devendra et al., 2015).

OPF was dried for 48 hours in a universal oven which is 60°C. Then, the dried OPF located in desiccators to lower the temperature. The dried OPF was cut into the smaller size and grind by a manual grinder machine (Siti et al., 2014). Moreover, hot compressed water (HCW) is another physical pre-treatment of OPF. HCW need temperature around 160-200°C, residence time (45-90 minutes), and liquid-solid ratio (8-16 v/w) on OPF. Overall glucose yield improved up to 83.72% with a severity of 3.31 and liquid–solid ratio of 8.0 compared to untreated raw OPF. It may break the strong cellulose-hemicelluloses –lignin associations to raise the accessibility of enzyme to cellulose. HCW also change the structure of cellulose in order to accelerate enzymatic hydrolysis (Goh et al., 2012).

b) Chemical pretreatment

The effective pretreatment method is dilute acid hydrolysis. There are many advantages used for pretreatment. For example, recover the sugar from the hemicelluloses part of the material, improve further lignin separation, and produce partially pure cellulose. Sulphuric acid can apply for pretreatment and hydrolysis (Lavarack and Griffi, 2002). Nevertheless, hydrochloric acid, nitric acid, and other acids can apply as well (Gamez et al., 2006; Rodríguez-Chong et al., 2004). The most cost-effective method is using dilute sulphuric acid (Chen et al., 2012)

c) Biological pre-treatment

Biological pretreatment includes some microorganism, white-rot, brown-rot, or soft-rot fungi to destroy biomass (Canam et al., 2011; Anwar et al., 2014). The main advantages by using biological pretreatment is consuming less energy and less damage to the environment (Chen et al., 2010). One of fungal pre-treatment is solid-state fermentation (SSF) which provides advantages over liquid-state cultivation (Wan and Li, 2012). Condition of lower moisture increases the chance of occurring fermentation (Holke et al., 2004).

SSF provides vital benefit over submerged fermentation, for example, non-aseptic conditions, lower capital costs, low energy expenditure, less water usage, high volumetric productivity and reproducibility (Krishna, 2005; Jain et al., 2013). Moreover, bio-pulping applications is suitable to use fungal pre-treatment (Breen and Singleton, 1999; Singh et al., 2010).

On the other hand, biological pre-treatment can divide into two groups, fungi and without fungi pre-treatment. Species of *Ganoderma lucidum* selected applied for fungi pre-treatment due to highest lignin degradation (Rahman *et al.*,

2011). Potato dextrose agar (PDA) plate was added with a small portion of *Ganoderma lucidum* and chloramphenicol which is 0.05% then incubated at 30 °C for 7 days. After that, the fungal culture was moved to the same medium to get pure colonies. The culture was remaining on PDA agar slants under 4 °C (Zhang et al., 2007). Besides, subculture needs to undergo on the culture once per month. Fungal colony was transferred to a new PDA plate if the agar plug had grown around 7mm diameter (Rahman et al., 2010). The new PDA plate needs to incubate at 30 ± 2 °C and 37 ± 2 °C. Measured the diameter of fungal colony every day and stop it when the colony was distributed across the agar plate (Penpaka et al., 2011). On the other hand, another biological pre-treatment was conducted by adding 60 mL of JIS (Japan Industrial Standard) broth (3 g KH₂PO₄, 2 g MgSO₄.7H₂O, 25 g glucose, 5 g peptone, and 10 g malt extract in 1 L mixture solution) into 30 g of biomass, then the mixture was sterilized for 30 minutes and incubated for 4 weeks (Euis et al., 2013)

2.5 Apparent digestibility in goat

Apparent digestibility is calculated by nutrients contained in the dietary intake minus the nutrients contained in the faeces (Rahman et al., 2013). The measurement of apparent digestibility is less complex than measuring true digestibility and it suited to the demand of diagnostic livestock systems research (FAO Corporate Document Repository). Apparent digestibility includes of neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and organic matter (OM), crude fibre (CF), ether extracts (EE)/ crude fat (CF) and dry matter (DM) (Silva and Queiroz, 2002).

NDF is made up of four main chemical components. Cellulose and hemicelluloses are potentially digestible and able to resist the attack of digesting microorganisms in the rumen of cows due to their complex chemical structures (Robinson, 1999). They become indigestible once pass out of the rumen. Lignin and cutin are the other main components of NDF. Lignin is indigestible in the rumen and not digested over time depending on linkage type (Raffrenato et al., 2016). ADF is a component which similar to NDF but only contains cellulose, lignin and cutin (Hans and Kenneth, 1992). ADF can remove by boiling the hay (or NDF) in a detergent solution at an acid pH. ADF was acquired as a preparatory step for lignin analysis

The undigested microorganism, unspent enzymes, mucosal debris and others are found in the faeces. This makes the digestibility coefficient define as an apparent digestibility but not a true digestibility (Ranjhan, 2001; McDonald et al., 1988). The digestibility of organic matter and net energy in the food are affected by fibre. In addition, the gross energy of feed and apparent digestibility of organic

matter is negatively correlated with the content of the fibre fraction (Kay et al., 1998).

Determination content of crude protein content in the feed is very significant because most feeds are classified according to protein content. It also gives indirect data about the digestible energy of the feed. Where protein content is high, crude fiber is usually low and digestibility of fodder is high. Protein is the vital source of enzyme and hormone synthesis which regulates the body functions (Ranjhan, 1999). Animal applied protein to build new cells and replace the worn out tissues. The method to calculate the crude protein from the nitrogen content of the food is Kjeldahl (McDonald et al., 1988).

Providing fibre could substrate for microbial fermentation in the rumen of ruminants (Ahamefule and Udo, 2010). When crude fibre is up to 77.89%, the apparent digestibility coefficient of dietary will be improved (Aye and Adegun, 2010). Interaction among ingredient components could reduce in the digestibility coefficient of ash in diet (Okoruwa and Bamigboye, 2015). Good digestibility feed could enhancement of growth performance in goats. Total digestible nutrient plays a vital role for estimation of nutrient from animal performance. (Okoruwa et al., 2012). Besides, digestible and metabolizable energy requirement of animals are the main factor to determine growth performance of animal (Johnson et al., 2003)

Chapter 3

MATERIALS AND METHODS

3.1 Preparation of feed

Napier grass and OPF were collected from Tanah Merah, Kelantan and delivered every day to the goat house at Agro Technopark, Universiti Malaysia Kelantan (UMK), Jeli Campus. Commercial goat pellet was bought from Ternak Tani, Bukit Bunga, Kelantan.

3.1.1 Physical pretreatment

Fresh OPF was pressed using sugarcane machine every morning. The pressed OPF was given to the goats in the afternoon.

3.2 Feeding trial

The feeding trial was carried out for 120 days at goat farm, Agro Technopark, Universiti Malaysia Kelantan, Jeli Campus. Twelve (12) male Boer goats were selected and randomly assigned to three different dietary treatments which is 88% Napier grass and 12% pellet (Control Group); 84% Napier grass, 12% pellet and 4% chopped OPF (Treatment 1) and 84% Napier grass, 12% pellet and 4% pressed OPF (Treatment 2). The animals were housed individually in the pen 2 m above the ground. The commercial pellet was offered to animals in the

morning meal throughout the feeding period, while the Napier grass and OPF were offered in the afternoon.

3.3 Apparent digestibility of faeces

Following 120 days of feeding trial, three animals per treatment were adjusted to the digestibility trial. Feces was collected for 10 days. Feces voided by each goat was weighed and recorded every day, mixed and 10% of representative samples were taken and stored in plastic bag separately. The dried samples of feces were ground through 1 mm sieve and stored until analyzed. The fecal samples were analysed its proximate composition. Nutrient digestibility (%) was calculated as a difference between nutrient intake and nutrient voided in the feces divided by nutrient intake and the quotient multiplied by 100 (Rahman et al., 2013). The formula to calculate apparent digestibility:

$$\text{Apparent digestibility} = \left(\frac{\text{Nutrient in feeds} - \text{Nutrient in faeces}}{\text{Nutrient in feeds}} \right) \times 100\%$$

3.4 Proximate analysis of faeces sample

3.4.1 Determination of dry matter and moisture content

Feed samples been weighed by using electronic balancer. This step must do speedily to prevent evaporation. After that, samples been putted into oven for drying at 60⁰c for 2 days (AOAC, 1995). Dry weight been measured on the next day to determine DM content and loss of moisture in the feed samples. According to Shareef (2015), the formula used to determine the DM and moisture content:

$$\%DM = W_f / W_i \times 100\%$$

Where,

%DM=percentage of DM,

W_i = initial weight of sample

W_f= final weight of sample

3.4.2 Determination of crude fibre (CF) content

According to Shareef (2015), Gerhardt Fibretherm used to determine CF content in the samples. First, FibreBags were dried 1 hour in Memmert oven and cooled in the desiccators for 30 minutes. Then, the FibreBags were weighed to get value of m₁. FibreBags with glass spacers were inserted into the carousel. One gram of samples were weighed and put into FibreBags along with glass spacers to get the m₂ value. Next, glass spacers, carousel and samples were washed by 40mL petroleum ether in three times. Then, the FibreBags were dried for 2 minutes before washing the two phases. For first phase, after solution start to boil, FibreBags and samples were boiling in 260mL of 0.13M sulphuric acid (H₂SO₄) for 30 minutes. Then, the FibreBags were rinsed by hot water to remove the acid. For second phase, boil the sample with 330mL of 0.11M sodium hydroxide (NAOH) solution for 30 minutes after the solution started to boil. FibreBags and samples were rinsed with hot water again. Then, FibreBags were separated from carousel. In the other hand, glass spacers removed carefully from the FibreBags without bringing out any samples.

Memmert oven applied to dry the FibreBags for 105°C at 4 hours and put into desiccators with 30 minutes for cooling. Crucibles were incinerated for 600°C inside the Carbolite furnace for 30 minutes. Each crucible was inserted by FibreBags and weighed to get m3 values. The crucible with blank FibreBags were weighed to get the m6 value. Next, all crucibles with FibreBags were incinerated for 4 hours at 600°C.

Besides, the crucibles were leaved overnight for cooling. The crucibles along with the ash were weighed to get m4 values. The blank FibreBags ash and the blank crucible were weighed to get m7 value. The values of ash from the blank FibreBags (m5) were determined from the value of m6 and m7. The following formula was used to determine the %CF of the samples,

$$M5 = m7 - m6 \quad \% \text{ CF} = (m3 - m1 - m4 - m5) \times 100 / m2$$

Where,

% CF= percentage of CF

M1= weight of Fibre bag (g)

M2= Initial sample weight (g)

M3= Incinerating crucible and dried FibreBag after digestion (g)

M4= Incinerating crucible and ash (g)

M5= Blank value of the empty FibreBag (g)

M6= Incinerating crucible (g)

M7= Incinerating crucible and ash of the empty FibreBag (g)

3.4.3 Determine crude protein (CP) content

According to Shareef (2015), Kjeldahl method was applied to analyse CP content in sample. Gerhardt Kjeldatherm and Gerhardt Vapodest were the important equipment for analysis. Digestion, distillation and titration were undergone for Kjeldahl methods. One hour was spent for digestion part in condition of 400°C. Switch off air condition when weigh the 1gram sample by analytical balance. After that, the samples were into digestion tube. Distilled water, Kjeldahl tablet and sulphuric acid were put into the digestion tube which was 10mL, 2 tablet, and 12mL respectively. Before inserting the digestion rack, turn on the digestion block of Gerhardt Kjeldatherm and heated to reach 400°C for pre-heating. Vaporisation can prevent by attach tightly between the fume manifold and digestion tube before turning the H₂SO₄ aspirator completely. Reset the pre-heated digestion block from 40°C to 250°C for 30 minutes. Next, reset back the temperature to 400°C for another 30 minutes. After 60 minutes, the digestion rack was removed and moved into rack holder which locates inside the fume chamber for cooling.

For distillation, the system run for 3 times for cleaning. Next, 40% of NAOH was placed in alkali tank of Gerhardt Vapodest distillation unit. Then, 80mL of distilled water and 50mL of 45% NAOH utilized to dilute the digested sample. Add 30mL of receiver solution to receiver flask. The 250mL Erlenmeyer titration flask was placed on receiving platform while filled in with 4% boric acid (H₃BO₃). Then, the indicator was added into receiver solution tank. The digestion tube attached to distillation unit and the samples were distilled for 5 minutes. The receiving flask was removed from the unit for titration process.

For titration, H_3BO_3 was titrated with standard 0.1M Hydrochloride acid (HCL) to reach pink colorization end point. The volume of HCl used for titration was recorded. The following formula was used for determination of CP content in the sample,

$$\%N = \frac{(T-B) \times N \times 14,007 \times 100}{\text{Weight of sample (mg)}} \quad \%CP = \%N \times F$$

Where,

%N= Percentage of nitrogen in the sample

T= Volume of titrant used for feed sample

B= Volume of titrant used for blank sample

N= Normality of titrant

%CP= percentage of CP

F= Conversion factor for nitrogen to protein

3.4.4 Determine ether extracts (EE)/ crude fat (CF) content

According to Shareef (2015), aluminium cups were heated for 30 minutes at 103°C and dry in desiccators for 20 minutes to cool off before start to determine. Only 4 decimal places taken for the weight reading when recording. Next, the “MAINS” button was pressed while make sure switch on the lamp. The temperature was set according to suitability of solvent used which can achieve 3-5 drops per second. The water tap was opened for reflux condensers. Thimbles were prepared to attach on adapters. The percentage of the crude fat was calculated using the following formula,

$$\%EE = \frac{W_f - W_i}{W_s} \times 100$$

Where,

%EE= Percentage of EE/ crude fat

W_i= Initial weight of the aluminium cup

W_f= Final weight of the aluminium cup

W_s= Weight of the sample

3.4.5 Determine Ash content

According to Shareef (2015), empty crucible was weighed and recorded. Next, the sample along with crucible were weighed and recorded as well. Next, the crucibles which including sample were put in the furnace at 500°C for 4 hours. After that, the crucibles were removed from furnace and cool off before put into desiccators 20 minutes. Then, all crucibles were weighed and recorded. Lastly,

weigh of crucible were calculated. The following formula was used to determine the total ash content in the samples,

$$\%Ash = \frac{W_i - W_f}{W_s} \times 100$$

Where,

%Ash= Percentage of Ash

W_i = Weight of the crucible with sample

W_f = Weight of the crucible with ash

W_s = Weight of the sample

3.5 Statistical analysis

Significance difference of apparent digestibility between 3 groups were determined by ANOVA. One ways ANOVA method was calculated by post hoc test. The data of average apparent digestibility from each group were applied for ANOVA calculation. Result from ANOVA was calculated by IBM SPSS software.



Chapter 4

RESULT

By using IBM SPSS, the analysis apparent digestibility of Boer goats was presented. The result of apparent digestibility of Boer goats is shown in Table 1. EE and CP showed no significant effect ($p>0.05$) in apparent digestibility of Boer goats. However, EE showed highest mean value in CG (87.11%) followed by Treatment 1 (81.64%) and Treatment 2 (78.95%). Similarly, CP of Boer goats showed decreased linearly from CG to T2 when physical pretreated OPF was added. The CP in CG, T1 and T2 are 76.96%, 71.18%, 67.21% respectively.

In addition, there is no significant effect ($p>0.05$) in CF as well. Nevertheless, CF digestibility showed highest in T1 (99.19%) and T2 (99.10%) when physical pre-treated OPF was added. CG showed lowest percentage (96.51%) in CF digestibility. The digestibility of DM and OM also indicated no significant effect ($p>0.05$) in Boer goats. Nonetheless, the percentage of DM in CG, T1 and T2 were similar which is 99.67%, 99.62% and 99.50%. OM digestibility in T1 (94.94%) represented lowest value compare to CG (97.4%) and T2 (95.98%).

OM digestibility showed the highest value in CG and the lowest value found in CP digestibility. Next, DM digestibly showed the highest value in CG and CP digestibility showed lowest value in the group as well. Last but not least, Treatment 1 showed highest value in OM digestibility and lowest value in CP digestibility.

Table 1: Apparent digestibility of Boer goats with different treatment.

Digestibility parameter	Control Group (Mean±SEM)	Treatment 1 (Mean±SEM)	Treatment 2 (Mean±SEM)
EE	87.11±4.85 ^a	81.64±4.82 ^a	78.95±3.80 ^a
CF	96.51±1.37 ^a	99.19±0.14 ^a	99.10±0.19 ^a
CP	76.96±7.90 ^a	71.18±0.70 ^a	67.21±3.04 ^a
DM	99.67±0.10 ^a	99.62±0.13 ^a	99.50±0.05 ^a
OM	97.4±0.62 ^a	94.94±1.22 ^a	95.98±0.35 ^a

CF (Crude fibre); EE (Ether Extract/ Crude fat); CP (Crude Protein); DM (Dry matter); OM (Organic matter); SEM (standard error mean) followed by same superscripts in same row means no significant difference.

Chapter 5

DISCUSSION

All the experimental goats showed moderate growth performances throughout the feeding trial, which indicate physical pretreated OPF did not showed adverse effect to Boer goats. In this present study, the result showed that there was no significant difference in EE ($p>0.05$) between the three groups. Reduction of EE percentage from 87.11% (CG) to 78.95% (T2). Besides, T2 showed lowest EE intake in grass (20.75g) and pellet (47663.47g) but highest EE in feces (10046.08g). Changes in EE confirms that reports of Okoruwa et al. (2013) and Okoruwa and Bamigboye (2015) that ether extract content of a diet is essential for the assessment of its digestibility. Low EE intake may probably due to lignin in the OPF.

Moreover, there is no significant difference in organic matter ($p>0.05$) between the three groups. CG showed highest percentage (97.4%) in organic matter. This is because CG showed lowest value of OM in feces which is 31018.31g in 10 days and lowest OM intake in grass (7183.54g). In this present study, range of OM digestibility indicated from 78 to 87% which is higher than study of Islam et al. (2000) reported that OM digestibility of whole OPF was 56%. On the other hand, T1 showed highest percentage of crude fibre (99.19%). However, T1 showed no significant difference ($p>0.05$) for the apparent digestibility of Boer goats. Highest percentage of crude fibre in T1 and T2 may bring adverse effect to digestibility of Boer goats (Hamdi Mayulu, 2014). In addition, the fiber digestion

efficiency reduced by high silica content and slow fermentation rate of fiber in OPF (Dahlan, 2000).

The crude protein indicated lowest value of digestibility percentage in the 5 nutrients. Crude protein digestibility showed decline linearly through the 3 groups which were 76.96%, 71.18% and 67.21%. T2 showed lowest value of CP intake in grass and pellet but highest value in feces. Hence, T2 showed lowest CP digestibility compare with CG and T1. The result of present study showed difference with study of Islam et al. (2000) which stated that CP digestibility of whole OPF was 43%. This may due to cell wall of OPF which lead to slowly degraded in the rumen. Okoruwa et al. (2012) stated that fermentation process of OPF taken long time. The cell wall of Napier grass was more resistant to rumen microbial fermentation. (Kariuki et al., 2001 and Widawati and Thalib, 2009).

According to NRC (2007), the CP intake of a goat should be 94.6g/d to meet the nutrient requirements. Ration of low level of protein to carbohydrate reduced the digestibility of goat. (McDonald et al., 2011). In addition, Rashid et al. (2005) stated CP 82g/d could increase the body weight of Boer goats. CP showed no significant effect ($p>0.05$) might be explained by poor total mixed rations for Boer goats. Tan et al. (2001) reported that satisfied crude protein concentration in the diet could slightly influence the fiber digestion. T1 showed highest percentage of CF digestibility (99.19%). In present study, CP difference might explain as drying which could change physical and chemical structure of the fibre in the mulberry in order to reduce the ability of the rumen microbes to attack and digest the fibre (Vu et al., 2011).

The crude protein and dry matter digestibility can increase by ammonization (Alexandre et al., 2015). High digestibility indicates the high percentage of nutrient absorbed by cattle. In the other hand, low digestibility presented the feedstuffs have less ability to supply nutrient for primary needs or production purposes. The number and activity of microorganisms increased when digestibility is increasing. Enzyme work will be increased when the digestibility is increasing. Example of microorganisms are proteolytic, cellulolytic, hemicellulolytic, amylolytic, lipolytic and others (Hamdi, 2014). The amount of nutrient degraded and digested in gastrointestinal tract can determine by measuring the digestibility of feedstuff (Coleman and Moore, 2003; Nurhaita et al., 2010).

High CP digestibility and non-structural carbohydrate of mulberry leaves could increase the digestibility of DM and OM (Vu et al., 2011). CP digestibility of buffalo grass (67.8%) is similar to treatment 3 which recorded as 67.21%. (Vu et al., 2011). Moreover, optimum ratio of nitrogen (N) to sulphur (S) play an important role for efficient ruminal microbial growth for diets based on fibrous materials like OPF (Wan Zahari et al., 2003).

Adequate protein supply could increase the microorganism activity, process of digestion and consumption. The undegraded feed in rumen is the protein source of ruminant. The high yield of production could form from low degradation of feedstuff in the sheep performance (Haddad et al., 2005; Rubianti et al., 2007; Silva et al., 2007). Proteolysis undergo when the level of CP is high which indicates as buffering effect that hinder to reduce the pH to the levels that are optimum for fermentation (Napasirth et al., 2015). Average daily DMI increased

from 448 to 608g when increasing the CP level (Negesse et al., 2001). However, Hwangbo et al. (2009) reported that no significant effect on DMI when the CP level increased from 14 to 20%. Wiese et al. (2003) reported that higher DMI might be due to a better availability of nutrients which are readily been degraded by rumen microbes. In this present study, the diet without physical pretreated OPF is more palatable and degradation easily.

Cellulolytic bacterium which lives inside the rumen plays an important role to digest fiber-feed. The digestibility of fiber-feed can be maximized through fermentation when the rumen bacteria is offered with enough nutrient. Ammonia is a significant source of nitrogen which needed by rumen bacteria to improve the growth. Moreover, the digestibility of fiber-feed can be improved by adding amino acid and peptide (Zain, 2007). Low quality of feedstuff could affect the performance of fiber-digested rumen microbe (Hamdi, 2014).

According to Abu Hassan et al. (1993), 50% OPF and 50% grass based diet were no significant difference for the goats. This is because function of rumen can be improved when percentage of OPF is more than 50%. However, ruminants can produce more milk when the ratio change to 30% OPF and 70% concentrate. This ratio was not implied in this present study which show no effective to improve the digestibility of Boer goats.

In the other hand, Zahari and Alimon (2003) stated 30% is a recommended inclusion level of PKC in sheep. The recommended inclusion level of OPF for beef and dairy animals is 30% (Wan Zahari et al., 2003). Cu toxicity could happen for sheep when the inclusion level is more than 80% with long-term feeding of PKC.

However, the Cu toxicity does not happen in cattle, buffaloes, goats and other animals. OPF is a good source of fiber which contain 45% crude fibre (Dahlan, 2000).

There was no significant effect ($p>0.05$) between goats fed with low energy diet which containing 3 and 4% urea (Pramote et al., 2006). Islam et al. (2000) reported the 40%OPF: 60%concentrate could improve degradation values and digestion of cattle. The previous study also showed higher degradation in cattle when the ratio of OPF: concentrate alter to 50%OPF:50%concentrate. This study clearly shows that attachment of fibrolytic bacteria was a pivotal process in the substrata surface phenomenon for fiber digestion and that mechanisms of both attachment and digestion are apparently influenced by pH as an environmental factor in the ruminal microbial ecosystem.

The dry matter digestibility decrease linearly through the three groups which were CG (99.67%), T1 (99.62%) and T2 (99.50%). DM digestibility of whole OPF in goats showed 52% (Islam et al., 2000). This agree with reported by Faciola and Broderick (2013) stated the ruminal protozoa numbers decreased as the apparent digestibility of DM decreased. The feed additives from plants can vary the abundances of protozoa and proteolytic bacteria and change the digestibility of nutrients reported by Patra and Yu (2014). It can be noticed the digestibility of the 3 groups were recorded in a similar result. Pin et al. (2016) reported that fungal treated oil palm frond (FTOPF) had no significant effect ($p>0.05$) on DM and OM digestibility.

According to previous study Abdul Kahlil et al. (2006), the major obstacle for applied OPF as livestock feed is lignin. Garry and Warren (2003) stated the

resilient covalent bonding with hemicellulose and cellulose cause to slow physical and microbial degradation of ingested feed. Dahlan (2000) stated OPF had high percentage water (more than 55%). The chopped fresh OPF contain 58.6% (Oshibe et al., 2001). Hence, high moisture may cause a decrease in palatability which leads to a decrease in roughage DM intake (Rahman, 2013). Islam et al., (2000) reported OPF can be used as a maintenance feed for ruminants. The study also mentioned maintenance could be more efficient if leaflet separated from OPF. From previous study, Islam et al. (2000) reported that the high level of OPF in diet resulted in a lower NH₃-N concentration. The NH₃-N concentration might be the primary limitation for the growth of rumen microorganism on most fibrous feed.

According to previous study, Luginbuhl et al. (2000) stated increased percentage of WCS (white cottonseed) in diet could decrease the DMI of goats linearly ($P < 0.05$). The result obtained from this study is different with the study of Shahjalal et al. (2000) who reported that high protein diet for goats had significant effect for the digestibility of CP and EE when compared with the goats who received low protein diets. The small changes between the three groups showed that physical pre-treated OPF did not depress the feed intake.

Chapter 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The physical pretreated OPF did not show any significant effect on apparent digestibility of Boer goats. There is no significant effect on apparent digestible nutrient in Control group, Treatment 1 and Treatment 2. Pressed OPF showed less efficient digestible than chopped OPF.

6.2 Recommendation

Biological pre-treatment suggested to apply for OPF to remove the lignin and bring significant effect for Boer goats. *Trametes versicolor* is one of type of white rot fungi which had the highest lignin degradation.

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APPENDIX A



Figure 1 Setting net



Figure 2 Setting banner



Figure 3 Feces before filter



Figure 4 Filter the feces



Figure 5 Forceps



Figure 6 Electronic Weighing Balance



Figure 7 Feces ready for drying



Figure 8 Gerhardt fat extraction

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Figure 9 Gerhardt Fibretherm



Figure 10 Dessicator



Figure 11 Gerhardt Vapodest

APPENDIX B

Table 1 Result of proximate analysis of Napier grass, Napier grass and chopped OPF, Napier grass and pressed OPF and pellet

Nutrient	Napier grass (Mean±SEM)	Napier grass and chopped OPF (Mean±SEM)	Napier grass and pressed OPF (Mean±SEM)	Pellet (Mean±SEM)
EE	1.53±0.10	0.82±0.19	0.19±0.03	3.96±0.02
CF	35.50±0.23	22.60 ±1.11	27.71±0.57	16.98±0.41
CP	13.91±0.52	9.23±0.19	6.14±0.02	15.21±0.33
DM	26.41±0.71	24.27±0.70	33.03±0.42	89.05±0.11
OM	96.84±0.35	98.64±0.06	99.79±0.24	95.00±0.20

Table 2 Result of proximate analysis of feces for T1, T2 and T3.

Nutrient	Control Group (Mean±SEM)	Treatment 1 (Mean±SEM)	Treatment 2 (Mean±SEM)
EE	1.56±0.15	1.65±0.18	1.77±0.20
CF	2.15±0.97	0.36±0.11	0.33±0.05
CP	11.25±1.40	10.74±1.38	10.88±1.04
DM	0.75±0.21	0.75±0.21	0.96±0.00
OM	8.18±0.44	11.58±3.00	8.31±0.19

Table 3 Total CP intake of grass, pellet and feces in 10 days.

Treatment	Grass (g)	Pellet (g)	Feces (g)
CG	1031.47	189867.30	43990.05
T1	1129.88	189777.24	55019.23
T2	670.74	183061.79	60326.47

Table 4 Total OM intake of grass, pellet and feces in 10 days.

Treatment	Grass (g)	Pellet (g)	Feces (g)
CG	7183.54	1185825.60	31018.31
T1	12068.87	1185263.16	60626.88
T2	10900.06	1143321.43	46404.52

Table 5 Total EE intake of grass, pellet and feces in 10 days.

Treatment	Grass (g)	Pellet (g)	Feces (g)
CG	113.49	49435.41	6385.25
T1	97.48	49411.96	9093.30
T2	20.75	47663.47	10046.08

Table 6 Total CF intake of grass, pellet and feces in 10 days.

Treatment	Grass (g)	Pellet (g)	Feces (g)
CG	2633.37	211973.04	7493.48
T1	2765.17	211872.50	1746.18
T2	3026.76	204375.18	1857.88

Table 7 Total DM intake of grass, pellet and feces 10 days.

Treatment	Grass (g)	Pellet (g)	Feces (g)
CG	1959.08	1111672.49	3721.21
T1	2969.5	1111145.22	4208.96
T2	3608.96	1071826.23	5357.66

Table 8 Total DMI of T1, T2 and T3 in 10 days.

Treatment	Grass (g)	Pellet (g)
CG	74.18	12483.69
T1	122.35	12477.77
T2	109.23	12036.23

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Table 9 Total weight of feces of T1, T2 and T3 in 10 days.

Treatment	Weight of feces (g)
CG	3880.05
T1	5327.77
T2	5594.62