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Evaluation on Serum Mineral Profile in Boer Goats Feed with Physical Pretreatment of Oil Palm Frond

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 "**Evaluation of Physical Pretreatment of Oil Palm Frond on Serum Profile in Goats"** by **Zafirah Binti Muhd**, matric number **F14A0455** has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agriculture Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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Evaluation on Serum Mineral Profile in Boer Goats Feed with Physical Pretreatment of Oil Palm Frond

ABSTRACT

Common nutritional problem in ruminants is usually caused by mineral deficiency. Minerals are important for productive and reproductive physiology of goats. Since oil palm frond (OPF) is abundant and cheap, it is widely used as ruminant feed in Malaysia. However, OPF is high in lignin content and cause the low digestibility. In order to upgrade the use of OPF, physical pretreatment was applied in the current study. Hence, the present study aims to evaluate the effect of OPF feeding on the serum mineral profile of goats as well as to investigate the efficacy of physical pretreatment of OPF on the serum mineral profile of goats. In animal feed trial, fifteen (15) goats of 4 months old were allocated to three different dietary groups which are; 20% fresh OPF + 50% Napier grass + 30% commercial pellet (treatment 1), 20% OPF pressed fibre + 50% Napier grass + 30% pellet (treatment 2), and one of control treatment which only fed with 50% Napier grass and 30% commercial goat pellet. The blood was drawn at jugular vein using 21 gauge needles and blood was collected into the red top tube for mineral analysis by Atomic Absorption Spectrometry. Mineral concentrations of Ca, Cu, Fe, Mg and Zn shows no significantly difference between the treatment. Control treatment shows 4205.57 of Ca; 0.91 of Cu; 3.25 of Fe; 1845.90 of Mg; 1.54 of Zn (mg/L), while group 1 shows 354.20 of Ca , 1.03 of Cu, 3.73 of Fe, 123.83 of Mg, 1.49 of Zn (mg/L). Lastly, mineral concentration in Group 2 are 334.40 of Ca; 1.46 of Cu; 3.04 of Fe; 182.43 of Mg; 1.30 of Zn (mg/L). In conclusion, the mineral contents are vary between treatment. Physical pretreatment of OPF did not improve the level of mineral contents in goats' serum.

Key word: *Oil Palm Frond (OPF), Physical Pretreatment, mineral serum, Boer goat, Atomic Absorption Spectroscopy(AAS).*

ABSTRAK

kata kunci: *Pelepah Kelapa Sawit (PKS), Pretreatment Fizikal, Serum Mineral, Kambing Boer, Spektroskopi Penyerapan Atom (SPA)*

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LIST OF ABBREVIATIONS

OPF	Oil Plam Frond
Ca	Calcium
Cu	Copper
Fe	Iron
Mg	Magnesium
Zn	Zinc
SPSS	Statistical Package For Social Science
AAS	Atomic Absorption Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma Optical Emission Spectroscopy
HCL	Hollow Cathode Lamp
EDL	Electrodeless Discharge Lamp
RBC	Red Blood Cell
TEM	Transmission electron microscopy
PKC	Palm Kernel
POME	Palm Oil Mill Effluent

OPT	Oil palm trunk
EFB	Empty Fruit Bunches
PPF	Palm Press Fibre

LIST OF SYMBOL

Kg	Kilogram
mL	Millilitre
°C	Celsius
%	Percentage
mm	Millimetre
w/v	Weight / volume
ppm	Part per million
Rpm	Revolution per minute
µm	Micrometer

CHAPTER 1

INTRODUCTION

1.1 Background of study

Oil palm tree is an ornamental plant in Malaysia before it is developed in agriculture crop. Oil palm is a sustainable source in Malaysia and normally available all the year around. On annual basis, the scientific name of oil palm is *Elaeis guineensis* were belong to subfamily of Arecoideae (Awalludin et al., 2015). It is a tropical palm plant with no branches and originates from West Africa before introduce in Malaysia. In the facts, this oil palm tree can reach up 30 meter and their life expectancy about 25 - 30 years before the production of fresh fruit bunch declined (Ng et al., 2013). The oil palm industry is classified as third largest industry in Malaysia after electric, oil and gas. Malaysia is categories as one of the largest exporter in the world. According to the Ferdous Alam et al. (2015), reported 4.49 million hectares land in Malaysia was used for oil palm plantation with 17.73 million tons of palm oil.

Oil palm leaves physically is “feather like” shape and it can achieve 5 meters long. Normally, the oil palm fronds are left rotting on soil. There about 24 branches of fronds for each of matured oil palm frond and average weight of fronds is 82.5kg per palm each year depend on it age (Zahari et al., 2003). Dahlan (2000) reported that average weight of OPF pruned from a mature plant is 13.3 kg with 5500 kg/ha/year production of OPF. The average number of weights for oil palm frond has varied each year, depending on to production of oil palm plantation.

Oil palm waste is obtained after harvesting that include oil palm frond (OPF), oil palm trunk (OPT), empty fruit bunches (EFB), palm press fibre (PPF), palm kernel (PKC) and palm oil mill effluent (POME) are studied. The large amount of sustainable oil palm waste has gained research interest in their potential used for livestock feed.

The OPF is high in lignin content and cause the low digestibility. To overcome the problem, there are several pretreatment strategies which include biological, physical and chemical pretreatment. These pretreatment methods can be used to break down the lignin then allow the rumen microbes to access to cellulose and hemicelluloses. While in this research there only focus on physical pretreatment. Goat performance can be assessed by body weight gain, body condition score as well as determination of blood level in order to evaluate the health status of goats. In addition, blood mineral profile is also important to check the health condition of the goats. Common nutritional problem in ruminants is usually caused by mineral deficiency. Hence, this present study aims to evaluate the effect of OPF feeding on the serum mineral profile of goats as well as to investigate the efficacy of physical pretreatment of OPF on the serum mineral profile of goats.

1.2 Problem Statement

The major problem is higher production of oil palm would increase the waste production, especially frond and it will cause the environmental problem. On the other hand, utilize of OPF by-product as animal feed whereby it is already known to be a good roughage source and suggested OPF can replace the Napier grass according to Hassim et al., (2010). Beside that, interest in enhanced of utilizing the indigenous feed resource such as OPF was increased in developing countries including Malaysia. On the other hand, the insufficient mineral in feed will cause deficiency that leads to low productivity and growth. Due to this the research in finding the mineral content in goat serum was analysed where most problem is because the performance is below expectation which related deficiency of certain mineral in Goat.

1.3 Hypotheses

- To determine either the different pretreatment from OPF may affect the mineral in goat serum.

1.4 Objectives of Study

- To determine the mineral status in goat following OPF feeding.
- To evaluate the effect of physical pretreatment of OPF on serum mineral profile of goats

1.5 Scope of Study

This research was conducted in Malaysia University Kelantan (UMK). OPF was sent by the supplier from Tanah Merah before pressing using sugarcane machine. After that, OPF was then mixed with different formulation according to pretreatment and given to all goat which were placed in individual pens. After that, serum was collected from goat blood was centrifuged and stored in freezer until ready to analyse using AAS which dilution was done before analyse process.

1.6 Significance of Study .

The research conducted to study on mineral analysis in goat serum was carried out. This is because the interest to investigate the efficiency of by-product such oil palm frond as animal feed and also to determine the mineral status in goat blood after applied OPF in their dietary. For this reason the Atomic Absorption Spectroscopy is found to be more effective to analyse mineral content in serum. Thus, the findings that will be collected at the end of the research and it can give an idea to farmer to find alternative for cost effective sources that have the potential to enhanced animal performance. Besides that, OPF may help to meet sufficient mineral requirement in goat.

1.7 Limitation of Study

1. Ambient temperature is required during handling the blood serum to prevent haemolysis that can interfere the result. Long distance between goat house and laboratory may be slightly because the haemolysis to be occurs.
2. The deionised water are not available during experiment as the machine is not functioning. So it may effect to mineral content and distilled water was used to replace deionised water that contained other ions.

CHAPTER 2

LITERATURE REVIEW

2.1 Current Issues in the Use of Agriculture By-Products as Animal Feed

The numbers of agro-industrial by-products are extensible and practically have been used as animal feed around the world for a few years ago. Present study found that around 11 company use agro-industrial by-product to feed their livestock (Onyeonagu & Njoku, 2010). Effect biomass in the environment becomes massive problem to most of the country. So many studies have been done to solve this problem and it includes to utilization of waste material like oil palm frond which used in animal feed due to their nutrient content. By-product or wasted is defined as any substance that holder, required or intend to discard (Ramírez, 2007). This agriculture by-product is usually preserved for a certain period of time either by artificial or natural method and undergoes processing acidification before use as animal feed. Nowadays, the animal feed is costly, that's the reason why so many farmers use by-product as other alternative to feed the animal. All agriculture by product contain varies nutritive value and depend on type of material used. There are various types of agriculture by-product that can used in the diet of animals. As an example, is peels of cassava, rice bran, coffee pulp, plantain, cocoyam and yam, sun dried poultry manure, cocoa pod husk, legume straws, cereal and corncob, but for legume straws, cereal and corncob are used in small amount as it is low digestible which reported by Dzowela, (1990).

2.2 Reason used Agriculture by Product in Animal Diet

The population of human increase every year, as impact the demand of agriculture product is increasing then it is a cause limitation of land area. Consequently, competition demand between human and animal lead to feed shortage which mean the availability of animal feed such as forages is limited. Due to limited forages the prices will be more costly. Besides that, seasonal fluctuation can cause the quality and quantity of forage decreases. In view, by product has high potential to be used as animal feed because of low cost, content high nutritive level and less competition between human and animal. From the study conclude that used of agriculture by product can reduce the total feed cost about 70% (Sindhu et al., 2002).

2.3 Importance Used Agriculture by Product as Animal Feed

Based on previous study, there are some benefits from used agriculture by product as animal feed. In some country the livestock are underfeeding by product is alternative way because it can help to reduce feed cost especially for poor country (Sindhu et al., 2002). Another benefit is to upgraded the quality of feed, to ensure the availability of feed thus reduce the shortage of feed resource and farmer can increase their profit margin. Even though, the agriculture by product are content toxic but it is important protein source and to improve the nutritive value or detoxify by use variety type of method and technology.

The agriculture by product with high moisture content is difficult to handle thus lead to growth of mould and cause feed to be contaminated so animal will get disease if those feed are given to them. From previous study suggest to used feed block technology for by product that consist of high amount of moisture content (Amata, 2014). Besides that, in his article also state that most of agriculture by-product are not really known the nutritive value like content of fiber, vitamin, mineral, energy and most importance is crude protein as we know that protein is needed by animal for production.

2.4 Plant Cell Wall Characteristics of Oil Palm Frond (OPF)

Malaysia is a second large production of palm oil after Indonesia. Oil palm frond was obtained during harvesting or replanting of oil palm plantation, it used as by-product and was good sources as roughage for animal feed (Wan Zahari et al., 2003). The main component in plant cell wall is fiber (cellulose) (Syamani, 2015) and study state that cellulose were high content in oil palm(Saurabh et al., 2016). Referring to figure 2.1 is shown the physical and also structure of cellulose, hemicelulose and lignin component in lignocellulosic biomass.

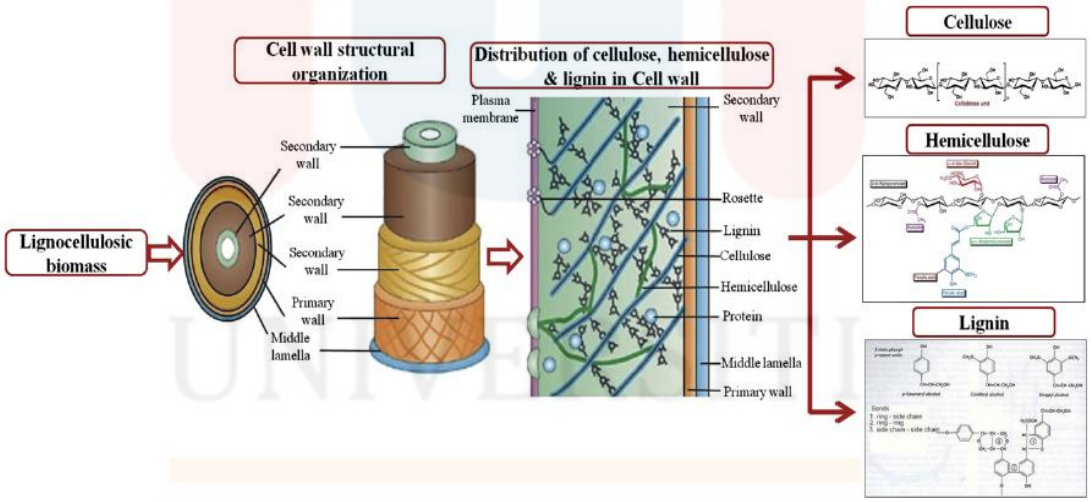

Figure 2.1. Diagrammatic illustration of the framework of lignocellulose (Menon & Rao, 2012).

2.4.1 Lignocellulose of OPF

Oil palm frond is categorized under lignocellulosic biomass. Lignocelluloses is known as primary building block of cell wall (Kumar et.al.,2009). According to Shrestha (2008) define lignocellulose as composed of carbohydrate polymer which is cellulose and hemicellulose and also aromatic polymer which is lignin.

2.4.2 Cellulose

Cellulose is a non branched chain of 1-4 linked β-D-anhydroglucopyranose units or a polysaccharide carbohydrate derived from beta glucose with formula ($C_6H_{10}O_5$) (Ciolacu, Ciolacu, & Popa, 2011).

2.4.3 Hemicellulose

Hemicellulose is composed of various 5- and 6- carbon sugar such as arabinose, galactose, glucose, mannose and xylose.

2.4.4 Lignin

Lignin is composed three component of phenolic which is p-coumaryl alcohol (H), coniferyl alcohol (G) and sinapyl alcohol (Rubin, 2008).

2.5 Primary and Secondary Cell Wall

The cell wall of OPF is divided into two parts, primary cell wall and secondary cell wall. Primary cell wall is a look as solid boundary cell whereby the process of cell division is happened and increase the xylem cell divided. Abdul Khalil et al. (2007) reported that thickness of primary wall is 0.11~0.47μm. Secondary cell wall is placed in between primary wall and membrane and it differentiates by S1, S2, and S3 layer. S1 is known as brighter of the wall so it can see clearly in Transmission electron microscopy (TEM) migrographer, it has the lowest lignin concentration content compare to other layer and study found that OPF fiber have thickest S1 layer thus show that OPF are resistance to transwall fracture. S2 layer is strengthening by micro fibrils with forty time thicker compare to other layer. OPF has thickest S2 layer with 3.43μm. S3 layer get a less attention because it hard to study and like other layer S3 has the thickest layer in OPF with 2.37μm in comparison another plant fiber (Abdul Khalil et al., 2007).

Middle lamella is a gluey pectin layer which placed in the middle of plant cell with another plant cell to adjoining cell together. OPF comes out various shape, size and structure of vascular bundle. The shape of vascular bundle in oil palm frond is almost round and made up of xylem and phloem which were the main component besides vessel, parenchyma and fibrous sheath (Khalil et al.,2006).

Several studies has revealed that lignified of metaxylem vessel in vascular bundle were less than fibers. In general, chemical composition in OPF is high holocellulose and Ash.

2.6 OPF as Animal Feed

Malaysia produced large production of frond from oil palm plantation per year and due to its high in crude fiber content makes these as a promising source of roughage feed for animal especially ruminant. OPF were used to feed animal as other alternative in order to reduce feed cost besides it was used as replacement of grasses in case limiting of forage or fodder but in the same time the handling of OPF require more cost (Dahlan, 2000). Previous study conduct by (Ishida & Abu Hassan, 1997) was reported that level need of OPF in their mix feed ration for goat and dairy cattle was 30% and 50% for cattle. Usually, local farmer feed OPF to their livestock in form chopped into small pieces, or either preserves as silage or undergoes some process to form pellet or cubes before mixed into feed ration. However, pelleted is less palatability and less digestibility as compare to OPF silage (Ishida & Abu Hassan, 1997).

Recent research are done with variable method and silage is one of method that used to make some improve OPF feeding value as nutrient composition of OPF are low content of crude protein, high amount of crude fiber and lignin. The used of OPF as feed for livestock such as cattle, goat, sheep, buffalo, and deer.

Experiment conducted by (Ishida & Abu Hassan, 1997)on cattle and dairy cattle were feed with OPF was carried out and result showed that OPF are could be utilize to formulate a complete feed for fattening bull with around 30-40% of it in the diet. By using OPF to feed dairy cattle, can increase the production of milk and also improve the quality of milk. Therefore, the report suggest a combination of 70% concentrate based diet and 30% OPF silage for produce efficiently. From research study by Ebrahimi et al., (2013) where OPF were fed to local Kacang crossbred male goat revealed that it not give bad effect to growth performance

2.7 Pretreatment Strategies of OPF

Pretreatment strategies of OPF are categorized into three main groups which is physical pre-treatment, chemical pretreatment and biological pretreatment. Other than that, there have many other pretreatment method such as physicochemical and electrical (Kumar et al., 2009). In general, some of pretreatment above are used to increase the digestibility of OPF in ruminants as OPF consists low digestibility. Pretreatment is actually the process or action before to separate lignocellulose content such as lignin, hemicellulose and cellulose. Pretreatment in OPF will help in increasing total surface area and porosity of the plant thus, help the ruminants to digest well.

2.7.1 Physical Pretreatment

Physical pretreatment refer to action to reducing material of lignocellulosic from large particle into small particle i.e. powder. It usually involved process of pressing, milling, chipping, grinding or any combination of these pretreatments. The size of lignocellulosic material after chipping about 10 – 30 mm and after grinding is around 0.2-2 mm was reported by Kumar et al., (2009). In this experiment, the OPF was cut the petiole into half by using cleaver and pressed using sugarcane machine.

2.7.2 Chemical Pretreatment

Chemical pretreatment method for lignocellulosic biomass has many type i.e. Liquid hot water, Acid hydrolysis (weak acid hydrolysis and strong acid hydrolysis), Alkaline hydrolysis (calcium or sodium hydroxide and ammonia), Oxidative delignification (hydrogen peroxide, ozonolysis and wet oxidation), Room temperature ionic liquid (RTIL) and Organosolv process (Kumar, 2009) & (Harmsen et al., 2010). Several studies of OPF reported by using RTIL and sodium hydroxide of alkaline pretreatment (Sukri et al.,2014, Aainaa et al., 2014). From the report mention above showed that alkaline pretreatment process will cause reducing of lignin barrier, cellulose become swelling and cellulose decrystalization happen partially (Cheng et al., 2010). In the other hand, the lignin solubility and proficiency of the pretreatment process can be increase by used large quantity of ionic liquid. The advantages and disadvantages of chemical pre-treatment is varied depend on type of method used. Some research suggests liquid hot water as the most efficient pretreatment (Hermiati et al., 2013).

2.7.3 Biological Pretreatment

Biological pretreatment is treatment that uses various type of fungi such as white brown or soft rot fungi and bacteria (Harmsen et.al.,2010). Specifically, white rot fungi and soft rot fungi will degrade the cellulose and lignin while brown rot fungi only degraded the cellulose (Sindhu et al., 2016). This pretreatment showed a great benefit as it is safe to be used, environmental friendly and economically viable strategy for intensify rate of enzymatic saccharification. Basically, advantages of biological pretreatment need less energy and degrades both lignin and hemicellulose. The disadvantage is very low rate of biological hydrolysis (Kumar, 2009) & (Harmsen et al., 2010).

2.8 OPF Mineral Content

Table 2.1. Macro mineral contents (g/100 g DM) of different fraction and whole oil palm frond.(ISLAM et al., 2000)

Fractions	Ca	P	Mg	Na	K	S	Cu	Zn	Mn	Fe	S
Petiole	0.466^{b}	0.09^{b}	0.093^{c}	0.018^{b}	0.644^{c}	0.094^{a}	2.51^{c}	10.00^{c}	40.00^{b}	100.33^{b}	0.094^{a}
Leaflet	0.529^{a}	0.183^{a}	0.168^{a}	0.039^{a}	0.876^{a}	0.080^{a}	3.04^{a}	13.20^{a}	45.00^{a}	121.67^{a}	0.080^{a}
Midrib	0.370^{c}	0.188^{a}	0.117^{b}	0.043^{a}	0.726^{b}	0.076^{a}	1.569^{d}	9.733^{c}	40.00^{b}	85.33^{c}	0.076^{a}
OPF	0.530^{a}	0.108^{b}	0.180^{a}	0.049^{a}	0.697^{bc}	0.096^{a}	2.71^{b}	11.17^{b}	44.66^{a}	106.67^{b}	0.096^{a}
LSD	0.04	0.02	0.010	0.02	0.06	0.05	0.01	0.02	1.32	5.05	0.01
Sig. level	0.001	0.001	0.001	0.02	0.001	NS	0.01	0.01	0.01	0.01	0.01

Means with different superscripts within a column differ significantly. lsd=Least significance difference.

Basically small ruminant depends on grazing and small amount of concentrated is fed at a certain time in a year especially during the raining season. At that time the animal will totally depend on concentrates (eg. Pellet) due to shortage of plant biomass supply.

The mineral content in plants may vary based on their species, yield, soil, plant maturity, pasture management, different area and paucity of information, elucidate the season changes is one of the factors that affect the mineral status of the plant (Hajer et al., 2014). Table 2.1 showed the amount of mineral in OPF where Ca is 0.530 g/100g DM, Cu 2.71 g/100g DM, Fe 106.67 g/100g DM, Mg 0.180 g/100g DM and Zn 11.17 g/100g DM. Thus, it showed that the highest mineral content in OPF is Fe followed by Zn, Cu, Ca and Mg.

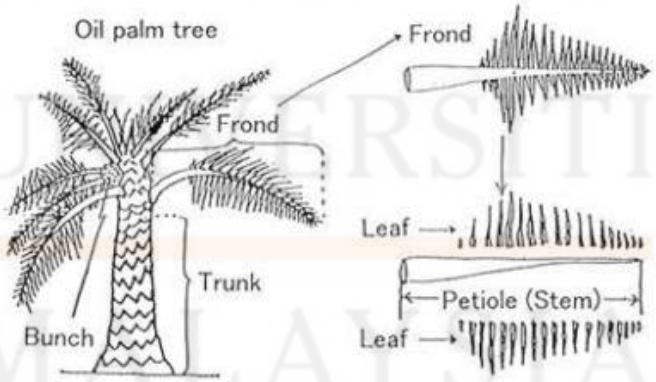

Figure 2.2. Oil palm and oil palm frond (Ishida & Abu Hassan, 1997).

2.9 Mineral

Generally, we already know that in blood contain white, red blood cell and plasma. However there also content mineral and other substances. Mineral is define as inorganic element that need for growth, physiological function and productivity in animal.

There are few function of mineral which is (i) structural component of body, organ and tissue, (ii) constituents of body fluid and tissue as electrolytes concern with maintenance of osmotic pressure, acid-base balance, membrane permeability and tissue irritability, (iii) catalysts in enzyme and hormonal system as an integral and specific component of structure of metallic –enzyme or as activators within those systems (Pandey & Ranjith, 2013).

The mineral is classified into two groups which are macro mineral considered as major mineral and micro mineral or known as trace element. Mineral that need at large amount in diet is macro mineral comprise calcium, phophorus, sodium, chlorine, magnesium and sulphur and this essential mineral is usually exist in the cell and tissue in the animal body. Macro mineral play important roles for growth, health and reproduction while mineral that needed in small quantities (ppb) in diet is micro mineral which is iron, iodine, copper, zinc, molybdenum, manganese, cobalt, selenium and fluorine (Schweinzer et al., 2017) (Hajer et al., 2014) (Pandey & Ranjith, 2013) (Poppenga et al., 2012) (Fraga, 2005). The classification of the macro- and micro mineral are as shown in table below.

Table 2.2. Macromineral and micromineral (Machen, n.d.).

Macro	Micro
Calcium	Iron
Phosphorus	Iodine
Sodium	Copper
Chlorine	Zinc
Magnesium	Molybdenum
Potassium	Manganese
Sulfur	Cobalt
	Selenium
	Fluorine

2.9.1 Factor Requirement of Mineral

The facts are scanty of literature in the requirement of mineral in goat meat breed such as Boer. Some of mineral are produced in the ruminant body and most of the mineral are obtained from the feeds and forages that the animal consumed. In addition, their mineral intake affected by the mineral content in the plant and seed also the concentration of all mineral in plant based on the genotype, soil, climate and stage of maturity (Meschy, 2000). This showed that the nutrient requirement of specific mineral are different and influence by a few factor such as age, weight, stage of production, lactation status, breed, stress and bioavailability.

2.9.2 Macromineral

2.9.2.1 Calcium : Ca

Based on study reported by Brzeziñska & Krawczyk (2009) approximately 11-17g of calcium is needed by goat daily. This mineral are rich in the animal body and around 98% (Radwińska & Żarczyńska, 2014) to 99% (Machen, n.d.). Ca can be found in the bone and teeth. The Ca play vital roles in blood clotting, cell membrane permeability, strengthening the bone, muscle and nerve contraction, cardiac regulation and enzyme activation. However, the vitamin D is necessary for calcium absorption and most of forages are lack of calcium. Thus, better feed formulation is required to meet the calcium requirements. Besides that, calcium sources can be obtained from legumes forages.

2.9.2.2 Magnesium : Mg

Magnesium can be found in muscle of the ruminant animal and beside calcium, magnesium also can be found in bone. The vital function of magnesium is required in enzymatic reactions and for normal operation nervous and muscular system. Usually, magnesium can be obtained from bran and oil seed meals and cakes.

2.9.3 Micromineral

2.9.3.1 Iron : Fe

The major role of iron is as a component of haemoglobin that getting involve in respiration and oxygen transport through haemoglobin. Concentrates such as pellet has low amount of iron. The sources of iron can be obtain from green laefy material, legumes and seed coats.

2.9.3.2 Copper : Cu

The mineral content of Cu is different between animal species and breed (Haenlein & Anke, 2011). Thus, goats sensitivity toward copper toxicity are less depending on age and they are able to endure more elevated amounts of Cu in their diet (Meschy, 2000). The concentration of copper in ruminant animals is affected by age as adult animal has lower Cu concentration compared to young animal. Copper is crucial in animal body as it involved in the formation of red blood cell (haemoglobin) and it is required for activity enzymes.

2.9.3.3 Zinc : Zn

The importance of zinc in animal is to maintaining the reproductive rthym which are necessary in animal reproduction system. Zinc helps in the maturation of spermatozoa in male reproductive organ. Besides, the other functions of zinc are involving in hormonal function, immune function and growth. Usually, zinc used to treat the skin problem. In general Zn sources can be obtained from bran. Table 2.2 shows the recommended trace element allowances for meat production goats.

Table 2.3. Recommended trace element allowances for goats (Meschy, 2000).

Element	Recommended dietary level (mg/kg DM)
Copper	8–10
Cobalt	0.1
Iodine	0.4–0.6
Manganese	40–50
Zinc	50
Selenium	0.1
Molybdenum	0.1

2.9.4 Deficiency of Mineral

Insufficient mineral in ruminant dietary can affect the growth performance on the animals as it can cause poor growth rate, affect the health, poor milk formation and it may lead to birth of abnormal kids (Hajeret al., 2014). In addition, from journal written by Haenlein & Anke (2011) the other effect of deficiency is decrease the milk production or cause toxicity and even worse it can cause high mortality.

2.9.4.1 Calcium

Deficient of Ca show the symptom in the skeletal system where the bone softening and deforming which will result in lameness and rickets in young animal while osteomalacia in adult animal. Thus, this insufficient intake of calcium will give high impact to doe and dairy animal (Bagasse, 2000) (Vázquez-Armijo et al., 2011).

2.9.4.2 Copper

The imbalance amount of Cu in goat will result the shorten life span of red blood cell (RBC) and lower Cu amount (<0.2 mg/L) in the blood will cause the normal harmatopoiesis and anaemia. Other symptoms that are obviously can be detected are decreasing in body weight and deterioration formation of collagen on the animals.

Exceeding amount of Cu from the necessary level will result in Cu aggregation in the liver cell where liver will be damaged. Then, it will show symptoms of haemolyticcrisis that can cause mortality to the animal. This is because the animals suffer from stress, jaundice and tissue necrosis. However, it only implies more obviously in sheep rather than in goat. Due to this problem, the goat is more tolerant to Cu deficiency. Besides that, the other symptoms that be observed by naked eyes are the hair coat of the animal is rough, looks pale in colour and also can cause diarrhea in the animal.

2.9.4.3 Iron

Major deficiency in Fe can cause an anaemia which the concentration of heamoglobin in animal's blood is reduced and it may cause loss of blood. On the other hand, exceeding intake of Fe will lower the animal's feed intake and it may cause the animal more resistances to disease (Hart & Schauer, 2015).

2.9.4.5 Magnesium

Grass tetany is one of the symptoms of inadequate Mg in animal's body. This clinical sign is usually affect the animal that grazing on fast growing pasture and they become excitable, reduced feed efficiency, staggering and falling (Bagasse, 2000).

2.9.4.6 Zinc

Zn is a mineral that necessary in animal's body. A form of deficiency in Zn will lead in reduced weight gain, reduced feed intake, impairment hair growth, bone deformities and can cause infertility for both male and females (Vázquez-Armijo et al., 2011) (Larson, 2005).

2.10 Determination of Mineral in Goat

The health and productivity of the ruminant animals are reflects from the importance of mineral analysis as the concentration level of minerals play a crucial role to the animals (Hajer et al, 2014). There are various ways to analyse the micro mineral content the goat's blood serum. One of them are using blood sample, serum, blood plasma, hair or fur internal organ such as liver and also can be analysed from their milk (Schweinzer et al., 2017).

Based on Kincaid (2000) review on assessment of trace element status of ruminants mention that liver is the best endogenous store of many trace elements and indicator for detection deficient of micro mineral such as As, Cu, I, Mn, Mo and Pb. But this sample method is persistent and seldom to be practiced.

Next, hairs are easy to be collected, stored for some time and also contain high concentration of micro mineral than blood. This type of sample are preferred to analyse Mo and I but it has the weakness, including hair growth not continual which influenced on breed, age, colour of hair and contamination. Thus, this sample is respond too slow to changes in the concentration of trace minerals Kincaid (2000).

Common samples that used to analyse the mineral content in the animals are blood serum and milk where drawing blood is a minimally invasive procedure. It is recommended to analyse the micro mineral by blood sample as it has been used in many studies. This will be more cost effective while running this study. It has been reported by Poppenga et al., (2012) that serum that obtains from the blood sample is more preferable as diagnostic tool in identifying the essential minerals contents in goat.

However, some of the minerals are not able to be detected by using blood serum such as selenium and it requires blood sample in order to analyse the minerals content. Besides that, blood plasma also can be used to analyse mineral content in the ruminant animals. There are many advantages of analysing using blood plasma as mineral concentration in both serum and plasma able to identify the productive status, showing their health (Hajer et al., 2014) and diagnose variety of disease (Khan et al., 2008)

2.11 Serum vs plasma

Generally, serum and plasma are the fluid part of the blood and they are differ based on the clotting factor that contain fibrinogen which responsible to clot the blood. Serum is a colourless fluid that formed after the coagulation of the blood where fibrinogen is being removed. In consequences, plasma is yellowish and clear fluid which separate from unclotted blood (anticoagulant) and clotting factor (fibrinogen) present in the plasma (Hrubec et al., 2002) (Issaq, Xiao, & Veenstra, 2007).

2.12 Normal dietary level in serum

There are many type of mineral that can be analysed but we are more focusing on these 5 minerals which calcium, copper, iron, magnesium and zinc because these five minerals that usually insufficient or deficient in the ruminant's diet.

The normal mineral level in goat value from Schweinzer et al., (2017) were used as references which is the range of Calcium (Ca) is 88.2 - 120 mg/l, copper (Cu) is 0.8 - 1.111 mg/l, iron (Fe) is 1280- 1410 mg/l, magnesium (Mg) is 26.7 - 36.5 mg/l and zinc (Zn) is 654 - 1243 mg/l.

2.13 Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectroscopy (AAS) is a technique for the detection of concentrations of particular chemical elements present in the samples by using the absorption of light and measured in part per billion. Usually, samples are either in liquid or solid, then it will vaporize in a flame or graphite furnace.

There are three types of technique of AAS which is; flame atomic absorption spectroscopy, graphite furnace atomic absorption spectroscopy and inductively coupled plasma optical emission spectroscopy (ICP-OES) and inductively coupled plasma optical emission spectroscopy (ICP-MS).

In this research the flame atomic absorption spectroscopy was used due to the intended for determination of higher concentration to analyse the mineral content which is calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), zinc (Zn) in serum. This technique can only analyse the solution.

The perform in atomic absorption spectroscopy requires a main light source which is a hollow cathode lamp (HCL) and the electrodeless discharge lamp (EDL), an atom source, a monochromator to isolate the particular wavelength of light to be measured, a detector to measure the light accurately, electronics to process the data signal and a data display or reporting system to show the results.

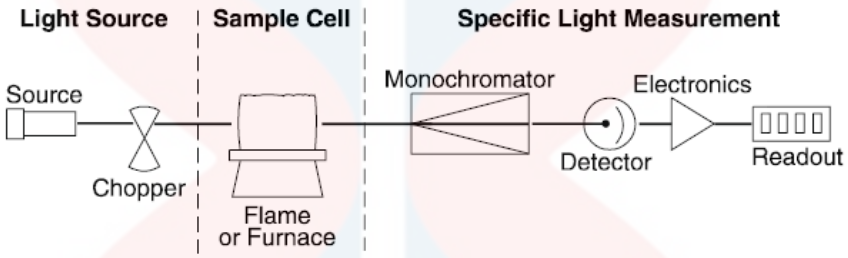

Figure 2.3. Single- Beam Atomic Absorption Spectrometer (Elmer, P.,1996).

The sample was taken into the nebulizer where it is vaporized and conditioned to provide the best sample for the flame. A lamp provides a source of light where the light with specific wavelength will go through sample cell into wavelength selector or know as monochromator then hit the detector that usually a photomultiplier tube to measure the transmittance of the sample. Transmittance refers to the amount of light that passes completely through the sample cell and strikes the detector then the electronic will convert the reading to data or result will put out on a tv monitor.

By using this technique there are few advantages; analysis in short time which it only take 10-15 second per sample per element, has a very good precision, easy automation of the measurement and the most important thing is it is low cost (Kopl, 2013).

2.14 Uses AAS Method

The reason used of AAS to analyse the mineral in serum because it is has high accuracy compare to other method and because of this AAS was widely used to analyse mineral (Bagasse, 2000).

The red tubes was used for serum collection and this method can save time as it required short time where at least need about 30 minutes in room temperature of serum formation. In some cases the clinical chemistry are best performed using plasma, other would best performed using serum thus both can be used but depend on analyte to determine. Basically, serum is preferred because it has higher metabolite concentration that more sensitive and give effective result compare to plasma it has anticoagulant which may be interference the analysis particularly trace element.

Factor that can effect the concentration of mineral in serum

CHAPTER 3

METHODOLOGY

3.1 Experimental Design

Below show the flow chart of experimental design that carried out in this research.

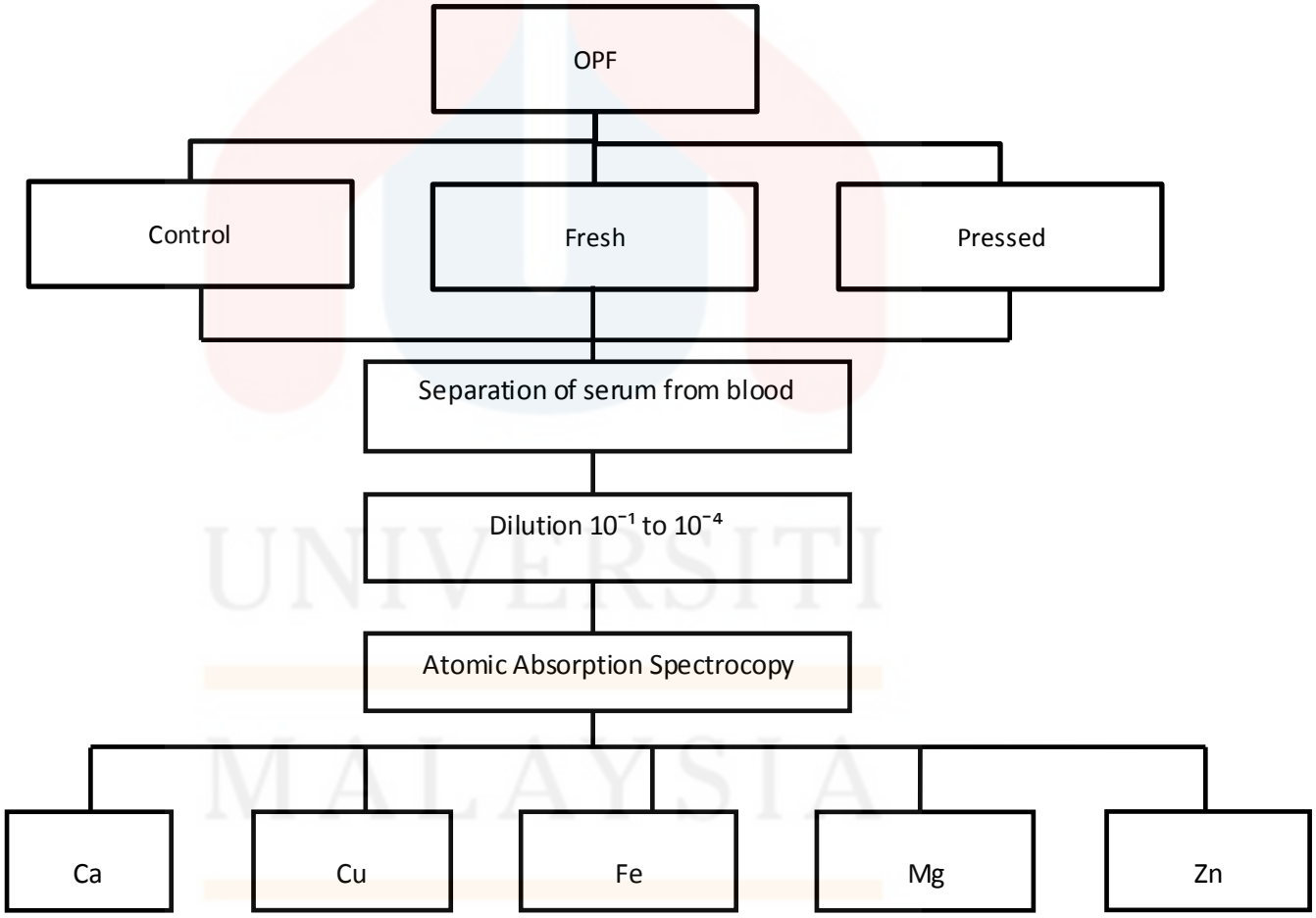

Figure 3.1. Flow Chart of Experimental Design.

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3.1.1 Animal and Diet

Twenty heads of cross breed of Boer goat kids that were about 4 month years old with an average live weight of 20 to 25 kg. They were allocated into five different groups and assigned different feed formulations. The group were labelled control group, treatment 1, treatment 2, treatment 3 and the last one is treatment 4 whereby the treatment 1 and 3 is given OPF fresh and treatment 2 and 4 is given OPF pressed. All goat was fed with local feed sources such as Napier grass, and pellet with *ad libitum* of clean water. The goat kids were reared including adaptation period with adequate facilities. After 4 months, about 15 heads of goat were selected according good performed such as weight for blood drawn.

3.1.2 Feed Material

The major material used is OPF, 20 heads of buck crossed breed Boer goat, Napier grass and goat pellet.

3.1.3 Feed Equipment

Equipment that will be used for feed preparations are chopper machine, cleaver and sugar cane machine.

3.1.4 Preparation of Feed

All was prepared before the feeding process was start.

3.1.4.1 Diet for Control Group

The Napier grass was sent to Agropark's UMK by the supplier and cut into small pieces by using a chopper machine.

These 50% of Napier grass will be served with 30% of commercial goat pellets was provided for feeding process.

3.1.4.2 Fresh OPF

Fresh OPF was sent to Agropark by the supplier and chopped using machine chopper. These 20% OPF with 50% of Napier grass and 30% of commercial pellet was provided for feeding process.

3.1.4.3 Physical Pretreated OPF

3.1.4.3.1 Pressed of OPF

Fresh OPF was sent by supplier to Agropark and was pressed with the sugarcane press machine to remove excess water. Thus, the OPF fibre will be collected. The 20% of OPF fibre with 50% of Napier grass and 30% of commercial goat pellet was provided for feeding process.

Table 3.1 Feed formulation of control group, treatment 1, treatment 2, treatment 3 and treatment 4.

Groups	Dietary treatment			
	Napier grass (50%)	Goat pellet (30%)	Oil palm frond (20%)	Total feed (kg)
Control	1.75kg	0.75kg	None	2.5kg
Treatment 1& 3	1.75kg	0.75kg	Fresh OPF/ 0.5kg	2.5kg
Treatment 2 & 4	1.75kg	0.75kg	Physical pretreated OPF / 0.5kg	2.5kg

3.2 Sample Preparation

Figure 3.2. Material and equipment used blood serum sample.

Figure 3.3. Material and equipment used in dilution process.

Figure 3.4. Flame Atomic Absorption Spectroscopy machine.

3.2.1 Sample Material

Material for blood samples are 21 gauge needle, vacutainer holder, red top tube, where each of it need about 30 pieces,1 ice box, and ice pack,

In addition, materials used for mineral serum analysis are 10% w/v containing Lanthanum Chloride solution that needs about 0.5ml OF each of stock solution and 0.15ml of each of dilution, distilled water and 3ml blood serum of each sample. 1.5ml Microcentrifuge tube, 95 pieces of 90mm filter paper, 100 pieces of 10ml Syringe, 2 pieces of 5 ml syringe, 0.45mm Syringe filter, and blue tip, a lot of distilled water

3.2.2 Sample Equipment

Equipment for mineral serum analysis are, Microcentrifuge holder,15ml and 50ml Falcon tube, Micropipette, falcon tube Holder ,5 pieces of 75mm filter funnel, Flame Atomic Absorption Spectroscopy machine and 1000ml Schott bottle.

3.2.3 Procedure of Experiment

3.2.3.1 Blood Collecting Samples

The blood drawn was done on 14 December. The research comprised only 15 samples of normal goat blood were selected to analyse. The goats were properly restrained and blood was drawn via jugular veni puncture using 21gauge into a red top vacutainer tube before feeding. Blood sample were transport to laboratory in the ice box.

The blood are allowed let to be clotted at least 1 hour at room temperature for serum formation. Most of report let until 2 hour (Eldaw & Ahmed, 2016) before the serum were harvested. Beside that, the minimum time used for serum formation can be between 30 – 60 minutes which showed in journal written by (Samardzija, Vince, & Duricic, 2013)(Tuck et al., 2009) and some report say that it can be 3 hour (Akinrinmade & Akinrinde, 2012). The right time os from 30 up to 5 hour if more than this the blood will haemolysis as reported by (Tuck et al., 2009).

All the serum was collected using micropipette and transfer into 1.5 ml sterile microcentrifuge tube and was centrifuge at 3000 rpm, 25°C for 15 minute. After that, each supernatant serum was transferred into the news sterile microcentrifuge tube and stored at -20 °C in freezer until ready for analysis.

3.2.4 Mineral Serum Analysis

The frozen serum was thawed at 4 °C before the stock solution was prepared. Then, 90mm filter paper in the filter funnel was readily placed on the falcon tube. 3ml serum was inserted into syringe and syringe filter was loaded. Serum has been passing through filter paper and filter funnel into 50ml of falcon tube. Then, 10% concentration of lanthanum of solution was added which about 0.5ml used 5ml syringe. Last but not least, around 46.5 ml of the distilled water was filled with a falcon tube up to 50ml. After all solution was added the falcon tube were inverted.

Dilution 10^{-1} has been prepared where the filter paper and filter funnel was placed in the 15ml of falcon tube with 1.5ml of the stock solution and 0.15ml of lanthanum was added then the distilled water was filled up to 15ml. After that, the dilution was continued until dilution 10^{-4}. The entire sample was placed in the chilled until the analysis process. However, the analysis process can not be longer than 1 to 2 weeks.

Then the zinc (Zn), copper (Cu), magnesium (Mg), sodium (Na) and calcium (Ca) was determined using Atomic Absorption Spectrometer (AAS) and data was represented at computer were collected.

3.3 Statistical Analysis

All parameters were triplicated while the result of the mean and standard error were calculated using Statistical Package for Social Science (SPSS) version 25.0. The data by comparing pretreatment that had a good level value of mineral (Ca,Cu,Fe,Mg and Zn) in goat serum was determined using Duncan multiple range test from one-way analysis of varience (ANOVA). Thus, expected significance is defined at $P<0.05$.

CHAPTER 4

RESULTS

Table 4.1. Mean value of mineral in serum in ppm.

Treatment		Goat ID	Mean (ppm)				
			Ca	Cu	Fe	Mg	Zn
Control		151	5013	0.548	2.81	1152	1.583
		156	167.7	1.426	3.098	97.71	1.852
		158	7436	0.767	3.837	4288	1.187
Napier + pellet + OPF **fresh**	Treatment 1	152	361.9	0.772	3.452	139.6	1.424
		150	255.4	0.808	5.071	88.29	1.563
		030	445.3	1.498	2.653	143.6	1.485
	Treatment 3	159	238000	0.931	3.588	21680	1.085
		145	325.2	1.812	3.756	108.4	1.587
		147	435.4	1.434	3.006	106.5	1.59
Napier + pellet +OPF **pressed**	Treatment 2	143	340.3	1.665	2.865	210.4	1.455
		149	322.2	1.745	2.917	100.7	1.548
		160	340.7	0.965	3.332	236.2	0.883
	Treatment 4	153	364.6	0.915	2.681	215	1.49
		144	504.7	1.317	2.608	153.7	1.636
		155	7256	1.121	3.065	1722	1.277

Table 4.2. The mean mineral of Ca,Cu, Fe,Mg and Zn are no significantly different. This means that feeding the OPF fresh and

pressed give no effect to mineral content in goat.

	Ca	Cu	Fe	Mg	Zn
Normal goat value	88.2 – 120.2	0.8 – 1.11	1.28 – 1.41	26.7 – 36.5	0.65 – 1.24
Control	4205.57 ± 2136.66	0.91±0.26	3.25±0.31	1845.90±1258.41	1.54±0.19
Treatment 1	354.20± 54.95	1.03±0.24	3.73±0.71	123.83±17.81	1.49±0.04
Treatment 2	334.40±6.10	1.46±0.25	3.04±0.15	182.43±41.54	1.30±0.21

4.1 Ca

Control group consists of Napier and pellet only while treatment 1 and 3 consist of OPF fresh. The treatment 2 and 4 are consisting of OPF pressed.

The highest values of Ca between treatments are treatment 2 with

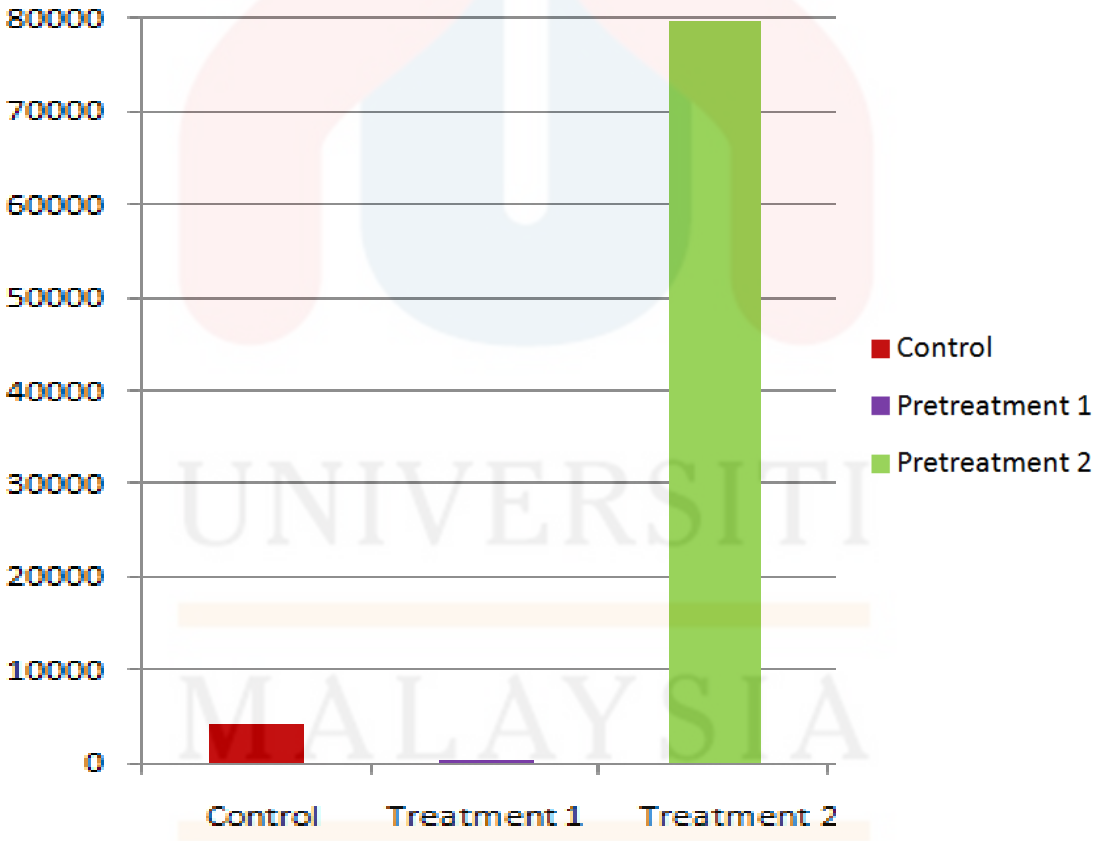

Figure 4.3. Comparison Ca in different treatment.

4.2 Cu

The mineral value of Cu is higher in treatment 2 with (1.46±0.25) while the lowest treatment is a control group (0.91±0.26).

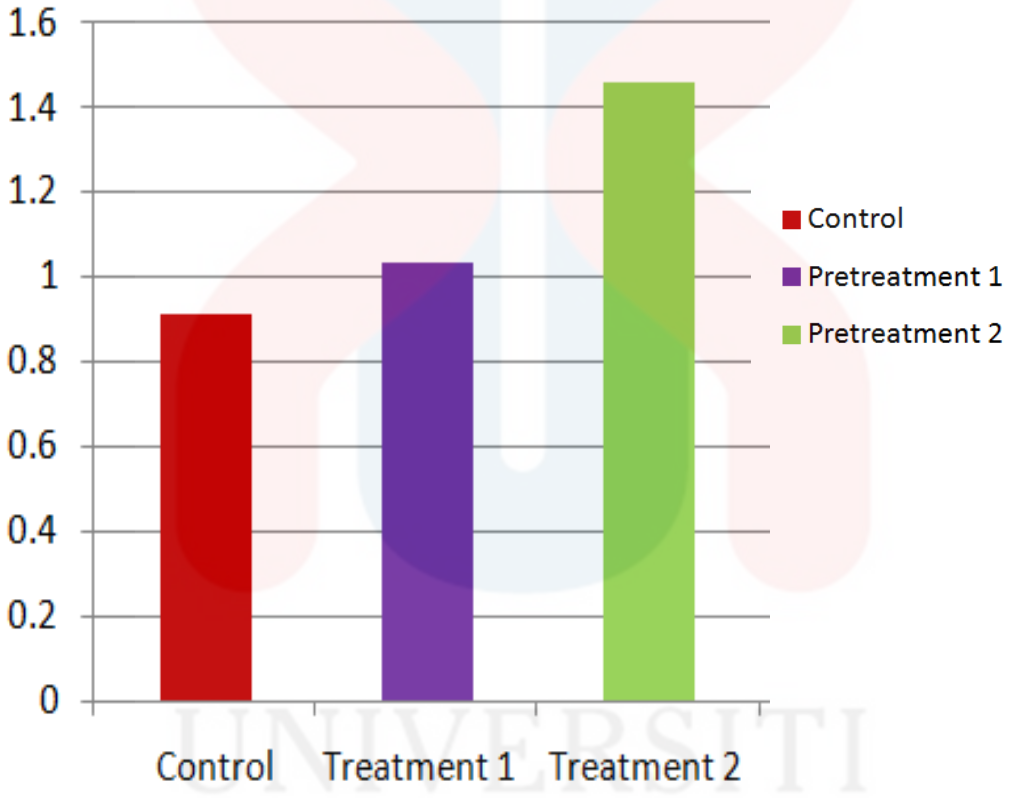

Figure 4.4. Comparison Cu in different treatments.

4.3 Fe

The mineral value of Fe is higher in treatment 1 with (3.73±0.71) while the lowest value is treatment 2 (

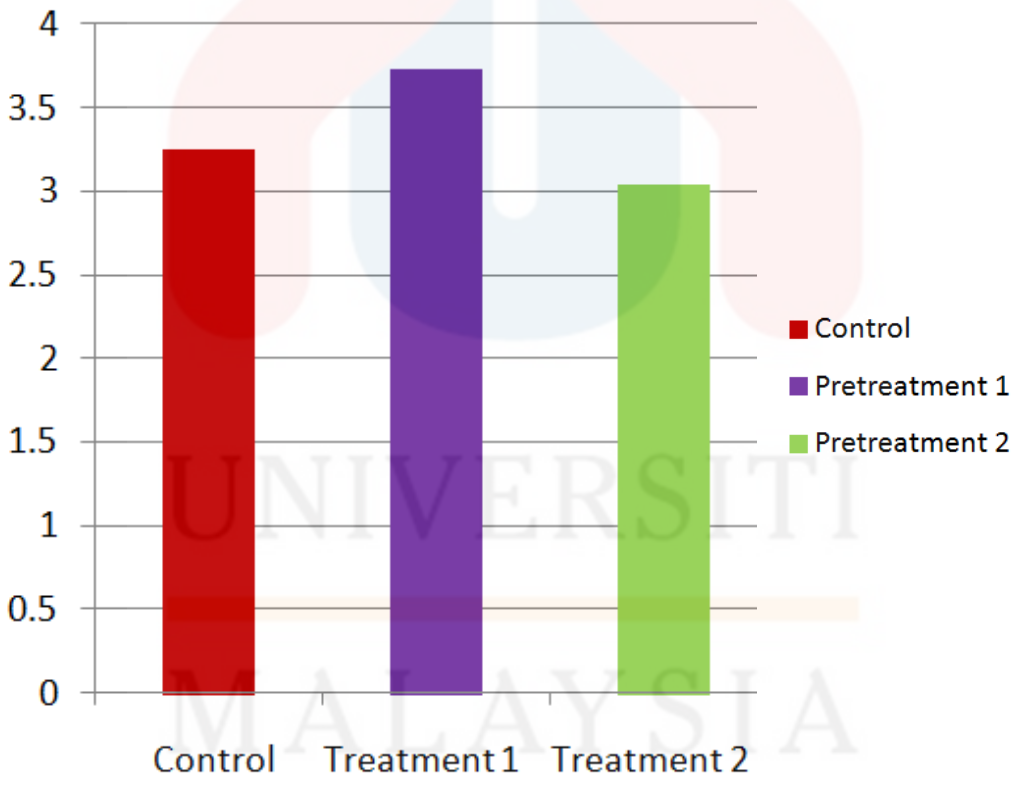

Figure 4.5. Comparison Fe in different treatments.

4.4 Mg

The highest value of Mg was control treatment .

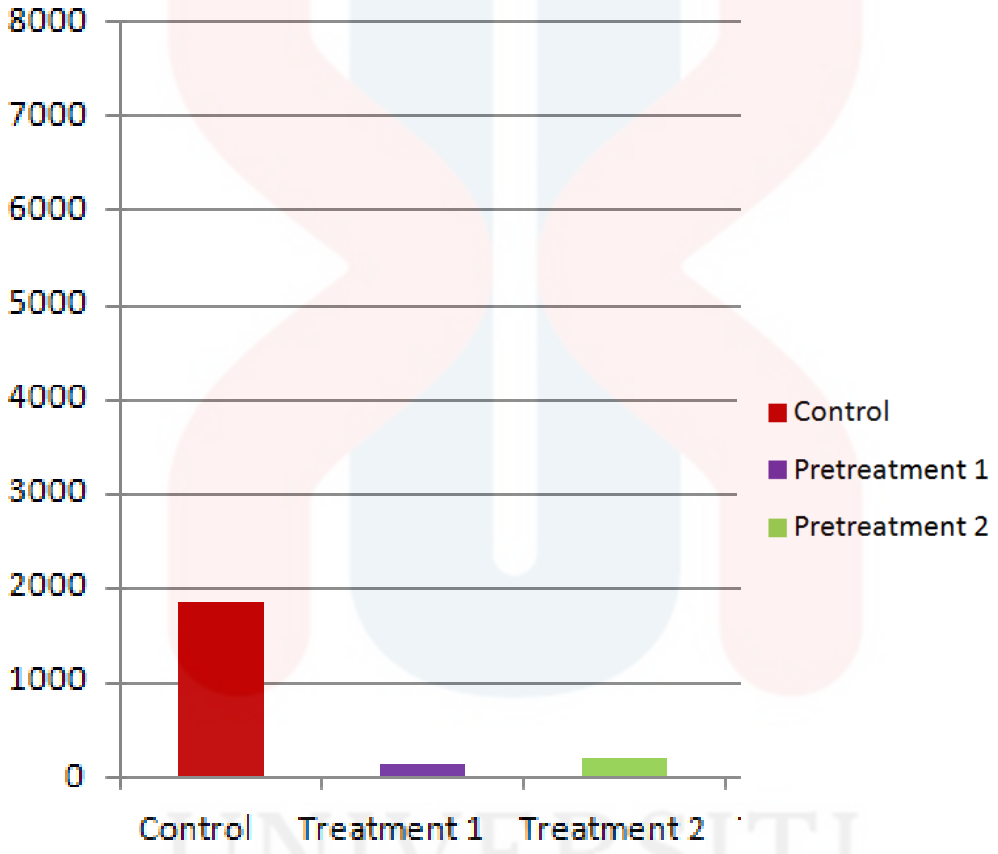

Figure 4.6. Comparison Mg in different treatments.

4.5 Zn

The highest value of Zn is a control group with (1.54±.019) and lowest value of Zn is treatment 2 (1.30±0.21)

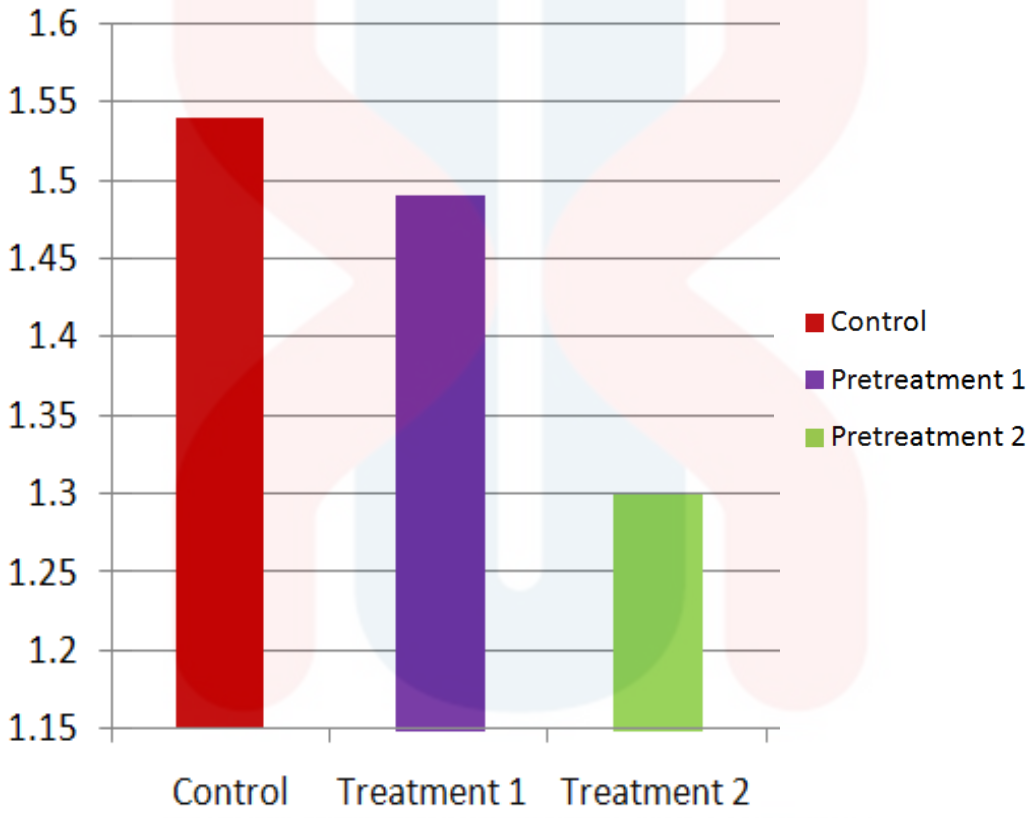

Figure 4.7. Comparison Zn in different treatments.

CHAPTER 5

DISCUSSION

All animals require mineral growth, especially productivity and production milk for dairy livestock. All type of mineral are need on sufficient level, if the mineral are inadequate or exceed the range value it (Schweinzer et al., 2017) can cause a several symptom that reduce the growth, productivity and may lead to a critical level which can cause mortality.

The normal mineral level in goat value reported by Schweinzer et al., (2017) showed the range of five different types of mineral which demonstrated 26.7- 36.5 mg/l Mg, 88.2 – 120.2 mg/L Ca, 0.8 – 1.11 mg/L Cu, 0.65 – 1.24 mg/L Zn and 1.28 – 1.41mg/l Fe.

The range value of Ca was reported in journal Schweinzer et al.,(2017) is between 88.2 mg/l to 120.2 mg/l that in line to research finding by Baumgartner (2009) which was 88.2 mg/l to 112.2 mg/l. In addition, both the data value above is supported to the value finding written by Pugh and Baird (2012) (89 mg/L - 117 mg/l). Thus, the data value gain from this study found that Ca value in all pretreatment have no significance value ($p > 0.05$) compare to all data mention above (control 4205.57 mg/l; treatment 1 354.20 mg/l; treatment 2 334.40 mg/l. In general, the exceed of Ca usually happen in pregnant goat where the Ca value is elevated in their bone which cause milk fever.

The value data gained from this research for Cu value in control treatment (0.91 mg/l) and treatment 1 (1.03 mg/l) are significant different in between the range ($p < 0.05$) stated by (Schweinzer et al., 2017) which are 0.8 to 1.11 mg/l. The previous study conducted by Schweinzer et al., (2017) reported that the mean value of adequate amount of Cu are 8 to 10 mg/kg DM. Adequate amount of Cu are crucial in animal body as it involved the formation of RBC (haemoglobin) and it is required for enzymatic activity.

One previous study investigated different element of mineral in goat's blood sample which was obtained from the mean value of serum reported by Baumgartner (2009). Based on Schweinzer et al., (2017) again, the amount of Fe are between 1.28 mg/l to 1.41 mg/L. However, the value obtained from this study are differ from journal above which is value of Fe in control treatment are 3.25 mg/l. Deficiency in Fe will lead to anaemia where the red blood cell are fewer than the normal rate in ruminant's body. Meanwhile, highest amount of Fe in goat serum will cause iron toxicity and may also depress copper absorption and thereby impair copper status in ruminants (Schonewille et al., 2017). This may due to numerous factors such as not enough feed supplied or poor quality dietary plane. However, based on data tabulated in table 4.2, the amount of Fe treatment 1 (3.73 mg/l) is higher than treatment 2 (3.04 mg/l) This is because the animals are given inconsistent supply of feed (OPF and Napier grass). The ruminants may give different part of OPF (whole OPF, petiole and leaflet) during the feeding trial.

The range value of Mg approximately 26.7 mg/L to 36.5 mg/L according to Schweinzer et al., (2017) in comparison with mean value from the same journal state that Mg value is 39.3 mg/L. Furthermore, these data value above are differing from both journal written by Baumgarter (2009) and Pugh and Baird (2012) with value range of Mg

are between 19.4 mg/l to 26.7 mg/l and 28mg/l to 36 mg/l. The data obtained from study have no significant ($p<0.05$) differ toward all data value above (control 1845.90 mg/L; treatment 1 354.20 mg/L; treatment 2 334.40 mg/L)

The mean value of Zn showed in control data was 1886.5 mg/L (Baumgartner 2009). In contrary, the value data of control treatment that obtained from this study is 1.54 mg/L which differs from range stated in journal (Schweinzer et al., 2017) which are between 0.65 mg/L to 1.24 mg/L and mean value stated above which obtained by Baumgartner (2009). In this study, all the treatments showed no significant value ($p>0.05$) on value of Zn in goat serum (treatment 1 1.49 mg/L; treatment 2 1.30 mg/L). The recommended trace element allowance for goats is 50 mg/kg DM. For this reason, the deficiency of Zn may cause of insufficient amount of feed given and thus lead to lower Zn intake.

There are various factors that cause the data value was interrupted. The total laboratory testing process can be divided into three phase – preanalytic, analytic and post analytic. Error that may affect the accuracy and the reliability of results can occur in any of the phases. Typically it is the analytic type of errors that most frequently come to mind as the reason for an incorrect result. It also can be error on preanalytic phase which including mishandling using pressing the OPF using sugar cane pressing machine. Inconsistent pressing of OPF and this will lead to higher amount of water contained in the OPF fibres.

Besides that, inadequate nutrients of feed supplied to meet the animal nutrient requirements. The feed given to the animals may not meet the nutrients requirements as the supplied Napier grass and OPF during the feeding trial are using different cultivars. Last but not least, different OPF cultivars supplied during the feeding. Different cultivars have different amount of nutrients and minerals. Maturity and age of the cultivars will affect the nutrients and mineral content in the plant.

The supplied OPF may give the whole OPF, leaflets and also only petiole. Different fraction of OPF may contain slightly different amounts of nutrients. A few studies has been carried out showing that all types of minerals were highest in the leaflet compared to other fraction of OPF with 0.529 mg/L of Ca; 0.168 mg/L of Mg; 3.04 mg/L of Cu; 13.20 mg/L of Zn; 121.67 mg/L of Fe (ISLAM et al., 2000).

Other than that, the improper amount of feed given. Basically, goat required amount of feed from 3% of their body weight. The amount of feed supplied should be differs from time to time. This is because, the animals are in the growing phase and required a sufficient amount of feed this can be supported by Hart & Schauer (2015) where yearling and 2 year old bucks have greater nutrient requirement since they are still growing.

According to E. J. McCaughey et al. (2016) studies on key factors influencing the incidence of haemolysis. They reported that haemolysis is the pre-analytical error in the laboratory. Determinant factor and proper step need to be practiced to reduce the haemolysis to be happening. This report gives evidence that haemolysis was given significant effect of incorrect measurement of analysts like mineral which lead to erroneous laboratory results Melissa K.(2009) states that temperature during collection and long transportation, storage were may give effect to the quality of sample this is because blood needs the ambient temperature to clot and form serum

Contamination during storage as it was stored in the freezer for 2 weeks before analysis by AAS machine may contribute to other factor. The contamination also can be happen during dilution where the measuring volume of lanthanum is not accurate as the pipette was not really functioning.

Lastly, the distilled water may be interference the data value obtained from this experiment result. Basically, deionised water was used in dilution by referring to (Elmer, 1996) as an ion in the water were already removes the ion. This claim can also be supported by research done by Tavares et al. (2011) that deionized water was effective purification water system for its use for all analytical step in Ca, Cu, Fe, Mg and Zn evaluation in animal samples. The ions in water will be interference and dissolving the sample in distilled water will make the whole test give the wrong answer. This is because the water with ions in it is also quite a lot more electrically conductive than water without ions in it. This can be support by Marvrodineanu (1977) that the trace element content of distilled water stored in polyethlene increased for some element after 30 days storage. As general rule, distilled water should be used as soon as possible after preparation.

CHAPTER 6

CONCLUSION

6.1 Conclusion

In conclusion, the mineral contains are vary between treatment where we can conclude that all mineral showed no significant value which mean the treatment does not give effect to mineral in goats serum. However, only Cu has significant value in control treatment and fresh OPF (treatment 1 and 3). Ca, Fe, Mg and Zn found to be highest in fresh treatment and Cu was higher in pressed OPF (treatment 2). To obtain reliable trace element data, it is necessary to appreciate the problems which may arise and the precautions which need to be taken all the way through sample collection, sample storage, laboratory analysis and data interpretation.

6.2 Recommendation

In addition, as recommendation deionised water is needed and practical step in handling this research need to be followed to prevent error and interference in the result. Furthermore, the practical handling must be followed to reduce contamination of sample. The supplied of OPF and napier need to be in consistent fraction which due to inconsistency of quality feed supplied may cause the content of mineral in goat serum was interference. Other than that, the pen house for this research need to be improve as it is not suitable for this research where it required individual feeding but then the feed trough inside this pen house cause the goat waste their feed so effect the feed intake which further related to mineral analysis. Besides that, the goat easily can go into other pen to eat the feed that belong to other goat.

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APPENDICES

Ca

Duncan[a]

Pretreatment_	N	Subset for alpha = 0.05
		1
Pretreatment 3 (Napier + Pellete + OPF fresh)	3	334.4000
Pretreatment 1 (Napier + pellete + OPF fresh)	3	354.2000
Pretreatment 4 (Napier + Pellete + OPF fresh)	3	2708.4333
Control (Napier +Pellete)	3	4205.5667
Pretreatment 2 (Napier + Pellete + OPF pressed)	3	79586.8667
Sig.		.176

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Cu

Duncan[a]

Pretreatment_	N	Subset for alpha = 0.05
		1
Control (Napier +Pellete)	3	.9137
Pretreatment 1 (Napier + pellete + OPF fresh)	3	1.0260
Pretreatment 4 (Napier + Pellete + OPF fresh)	3	1.1177
Pretreatment 2 (Napier + Pellete + OPF pressed)	3	1.3923
Pretreatment 3 (Napier + Pellete + OPF fresh)	3	1.4583
Sig.		.155

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Fe

Duncan[a]

Pretreatment_	N	Subset for alpha = 0.05
		1
Pretreatment 4 (Napier + Pellete + OPF fresh)	3	2.7847
Pretreatment 3 (Napier + Pellete + OPF fresh)	3	3.0380
Control (Napier +Pellete)	3	3.2483
Pretreatment 2 (Napier + Pellete + OPF pressed)	3	3.4500
Pretreatment 1 (Napier + pellete + OPF fresh)	3	3.7253
Sig.		.131

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Mg

Duncan[a]

Pretreatment_	N	Subset for alpha = 0.05
		1
Pretreatment 1 (Napier + pellete + OPF fresh)	3	123.8300
Pretreatment 3 (Napier + Pellete + OPF fresh)	3	182.4333
Pretreatment 4 (Napier + Pellete + OPF fresh)	3	696.9000
Control (Napier +Pellete)	3	1845.9033
Pretreatment 2 (Napier + Pellete + OPF pressed)	3	7298.3000
Sig.		.184

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Zn

Duncan[a]

Pretreatment_	N	Subset for alpha = 0.05
		1
Pretreatment 3 (Napier + Pellete + OPF fresh)	3	1.2953
Pretreatment 2 (Napier + Pellete + OPF pressed)	3	1.4207
Pretreatment 4 (Napier + Pellete + OPF fresh)	3	1.4677
Pretreatment 1 (Napier + pellete + OPF fresh)	3	1.4907
Control (Napier +Pellete)	3	1.5407
Sig.		.328

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Ca	Between Groups	1451678522 8.209	4	3629196307. 052	.963	.469
	Within Groups	3770052800 7.240	10	3770052800. 724		
	Total	5221731323 5.449	14			
Cu	Between Groups	.663	4	.166	1.041	.433
	Within Groups	1.592	10	.159		
	Total	2.256	14			
Fe	Between Groups	1.582	4	.396	.951	.474
	Within Groups	4.158	10	.416		
	Total	5.740	14			
Mg	Between Groups	109839052.0 57	4	27459763.01 4	.855	.523
	Within Groups	321341841.8 60	10	32134184.18 6		

	Total	431180893.918	14			
Zn	Between Groups	.104	4	.026	.358	.833
	Within Groups	.727	10	.073		
	Total	.831	14			