NUTRITIONAL PROPERTIES OF DECAFFEINATED TEA WASTE

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I declare that this thesis is my original work except for quotations, excerpts, and summary that has been duly acknowledged.

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ABSTRAK

Tujuan kajian ini adalah untuk mengkaji beberapa sifat mineral yang terdapat dalam sisa-sisa teh. Ini adalah kerana sisa-sisa teh ini mempunyai potensi untuk dijadikan sebagai makanan haiwan. Kandungan mineral yang terlibat dalam kajian ini adalah natrium (Na), kuprum (Cu), kasium (Ca), besi (Fe), zink (Zn), Magnesium (Mg), kalium (K) dan fosforus. Nutrisi yang telah dikaji ialah lemak, protein dan khasiat. Bagi lemak, ia adalah diuji dengan menggunakan kaedah extrak soxhlet sistem. Bagi sisa-sisa teh ini, hanya 0.25% lemak terdapat dalam sisa-sisa teh yang diuji Oleh itu, jika sisa-sisa teh ini dijadikan sebagai makanan haiwan, ia perlu dicampurkan dengan tumbuh-tumbuhan lain seperti kacang soya. Protein diuji dengan menggunakan kaedah kjedahl. Peratus yang paling sesuai untuk makanan haiwan ialah 10.0% tetapi dalam sisa-sisa teh ini hanya mempunyai 5.85%. Tiga mineral yang terdapat dalam sisa-sisa teh ialah kuprum (0.1644 mg/g), besi (0.0860 mg/g) dan natrium (0.0467 mg/g). Di samping itu, amaun bagi mineral yang lain yang muncul dalam sisa-sisa teh ini ialah fosforus (0.0031 mg/g), kalsium (0.0124 mg/g), magnesium (0.0168 mg/g), zink (0.0318 mg/g) dan kalium (0.0350 mg/g). Semua nutrisi ini memainkan peranan dalam perkembangan haiwan.

ABSTRACT

The aim of this study was to determine some of the nutritional properties from the decaffeinated tea waste. The tea extract waste had the potential to be used as animal feed. Minerals content analysed in this study were sodium (Na), copper (Cu), calcium (Ca), Iron (Fe), Zinc (Zn), magnesium (Mg), potassium (K) and phosphorus. The nutritional properties that had been determined were lipid, protein and minerals. For the lipid, it was determined by undergo the process in soxhlet extraction system. There was only 0.25 w/w % of lipid found in the decaffeinated tea extract waste. The quality of decaffeinated tea waste can be improved by mixing with other plant like soy bean to become animal feed. Protein was determined according to the kjedahl digestion method. The optimal percentage of protein that need in poultry feed is 10.0 % but there was only 5.85 % of protein was present in tea extract waste. Three minerals found in decaffeinated tea extract waste were copper (Cu), iron (Fe), and sodium (Na) which were 0.1644 mg/g, 0.0860 mg/g and 0.0467 mg/g respectively. Besides that, amount of total other minerals that presented were phosphorus, 0.0031 mg/g, calcium, 0.0124 mg/g, magnesium, 0.0168 mg/g, zinc, 0.0318 mg/g and potassium, 0.0350 mg/g. These minerals play important roles in poultry growth.

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°C	Degree Celcius
%	Percentage
Abs	Absorbance
g	Gram
M	Molar
N	Normality
mg	Milligram
mg/L	Milligram per liter
mg/mL	Milligram per milliliter
mol/L	Mole per liter
µL	Microliter
µg	Microgram
mL	Milliliter
UV-Vis	Ultraviolet Visible
v/v	Volume over volume
w/v	Weight over volume

CHAPTER 1

INTRODUCTION

1.1 Introduction of Animal Feed

Livestock feed has been a major constraint to small scale farming. The most major constraint to increase in production is the inadequacy of feed and fodder resources both in quality and quantity. Besides that, for fish feed, there is shortage and increasing prices. Due to the natural, carnivorous feeding habit of most intensively farmed fish species, fish meal is the most important fish feed component (Kaushik., 2006). The increasing demand for fish meal for aquaculture and other animal feed production and declining fish meal production resulted in shortage and increasing prices.

To resolve the shortage and increasing prices of fish meal, a lot of researches are to be done to replace fish meal with others. Today, full fat soya meals, residues from oil seed processing, corn and wheat gluten as well as several other materials are usually used as feed ingredients. By replacing this fish oil with plant oil, it will reduce the overall need for marine resources. Another potential source of feeds for livestock and poultry is tea waste. According to FAO statistics, the world production of coffee green beans and tea was estimated to be of 742,000,000 and 352,000,000 tons, respectively (Morikawa., 2005). After extraction of the tea from the processed tea leaves, the remainders of the leaves are discarded as waste, and most of the waste is burned or dumped into landfills. Burning and damping of these waste management practices has a serious negative impact on the economy and the environment (Kondo *et al.*, 2004). The large amount of tea usage will produce a lot of waste. Thus using tea waste as feed and fodder for livestock and poultry is desirable.

The remaining nutrient, protein, fats and other minerals in the tea extracts wastes are concerned. This is important to define tea extracts wastes are suitable to be used as feed and fodder for the livestock, fish and poultry. Tea leaves contain variety of amino acids, proteins, tannins, polyphenols such as catechin, epicatechin gallate, epigallocatechin gallate, and vitamins (Yamamoto *et al.*, 1997) suggesting that tea waste may have potential as protein supplement in animal feed. Tea waste may be considered a valuable protein source consisting of 22 ~ 35% of crude protein (CP) (Kondo *et al.*, 2004). While tea waste contains high CP, it is known to contain a high proportion of tannins (Kondo et al. 2004). Animals fed tannin-rich diets show decrease in their feed intake (Silanikove *et al.*, 1994), increased fecal N excretion (Nunez-Hernandez *et al.*, 1991), reduced digestibility and less ruminal degradability (Woodward and Reed., 1997 ; Tolera *et al.*, 1997). Thus, due attention must be given to tannin of tea waste when it is used instead of commercial feed in livestock production. However, when Decaffeinated Tea Waste (DCTW) washed with boiled water during the process of extraction, DCTW will be free of tannic acid without much effects on their CP contents (Bizendra et al.,). This will be useful for feeding livestock without further processing which is very important for formulating economic rations.

Nutrients in feed are necessary for maintenance, growth, production and health of animals. More than 40% specific chemical compounds or elements are nutrients that necessary in diet to support life, growth and reproduction (Damron *et al.*, 2003). The specific chemical compounds are divided into six classifications according to their function and chemical nature. The six groups are water, proteins, carbohydrates, fats, vitamins and minerals. For good health and performance of the poultry and livestock, their diet must contain all this known nutrients in the proper amounts. Proteins are complex compounds made up of more than 23 organic compounds containing carbon, hydrogen, oxygen, nitrogen and sulfur. This are called amino acid. Amino acids are the essential nutrients, rather than the protein molecules itself. Feed proteins are broken down into amino acids by digestion. They are then absorbed and transported by the blood to the cells that assemble these amino acids into body protein (Buchanan *et al.*, 2002). Excess protein which is above what is required for growth or production is used as energy and the nitrogen excreted in the urine. Growing chickens should have 16-24% protein; growing turkeys, 24-28% protein; for egg production 15-17% protein. For maintenance that is no growing and production poultries are 10-12% protein (Canadian Poultry Consultants Ltd., 2008). Birds can make some amino acids from other protein but many amino acids are essential, that is they cannot be synthesized in adequate to meet grow and requirements so they must be present in the diet. This shows that the protein is required in the feed given to the poultry. Protein also is the main component in the fish feed to maintain the performance in the fish itself. Protein contain about 16% of nitrogen so the amount of protein (crude protein, not the digestible or usable protein) in a feed can be estimated by measuring the nitrogen content (Canadian Poultry Consultants Ltd., 2008).

Beside, carbohydrates also make up the largest portion of a poultry and livestock diet (Damron *et al.*, 2003). Carbohydrates common in poultry diets are starches, sugars, cellulose, and other non-starch compounds (Scott Beyer *et al.*, 2000). Cellulose and the non-starch compounds are typically classified as crude fiber. Most starch and sugars are used well by poultry but the fiber portion is not. This is because poultry lack certain digestive enzymes required to digest the various fiber components (Canadian Poultry Consultants Ltd., 2008).

Supplemental fats may increase energy used in poultry in association with a decreased rate of food passage through the digestive tract (Buchanan *et al.*, 2002). Fat has 2.25 times more energy than carbohydrates on an equal-weight basis. Like carbohydrates, they are organic compounds made up from carbon, hydrogen and oxygen which from fatty acids. Because they are higher in hydrogen and lower in oxygen than carbohydrates, fats have a higher energy value (ME) than carbohydrates (Canadian Poultry Consultants Ltd., 2008). Another function for the fats is for the absorption of vitamins A, D3, E and K even as a source of essential fatty acids. These fatty acids are responsible for membrane integrity, hormone synthesis, fertility, and hatchability.

The minerals needed in poultry and livestock are divided into two classes. These are macro minerals, which are needed in large quantities and the micro or trace minerals. Although micro minerals are required in a small quantity, the lack of an adequate dietary supply can be equally as detrimental to poultry as a lack of one of the macro minerals. This mineral is needed for the formation of straight, strong and rigid bones. Minerals are also needed for the formation of blood cells, blood clotting, enzyme activation, energy metabolism, and for the proper muscle function for the livestock and poultry (Damron *et al.*, 2003). Calcium, phosphorus and salt are needed in the feed. Dicalcium or defluorinated phosphates are the customary carriers of phosphorus and calcium for poultry diets. Micro minerals such as iron, copper, zinc, manganese and iodine are also needed in the feed.

The 13 vitamins required by poultry are classified as fat-soluble or water soluble. The fat-soluble group includes vitamins A, D3, E and K. The water-soluble vitamins are thiamin, riboflavin, nicotinic acid, folic acid, biotin, pantothenic acid, pyridoxine, vitamin B12 and chlorine (Damron *et al.*, 2003). Vitamin A is required for normal growth and development of epithelial tissue and reproduction in poultry. Vitamin D3 is required for normal growth and development of bones and for eggshell formation. Vitamin K is an essential part of blood-clot formation. Vitamin E is a powerful antioxidant (Scott Beyer *et al.*, 2000). Antioxidant which are found in tea waste can be included into feed to prevent rancidity of the fat in the diet (Buchanan *et al.*, 2002).

1.2 Objective

The objectives of this study are:

1) To determine the nutritional properties (lipid, protein and minerals) of decaffeinated tea extract waste.
2) To determine the feasibility of decaffeinated tea extract waste as animal feed.

1.3 Scope of Study

The tea samples are collected from Sabah Tea Plantation, Ranau, Sabah. After the fresh tea leaves were processed, the decaffeinated tea extract wastes were analyzed to determine the nutritional properties remaining. The nutritional properties obtained in tea waste will be compared with animal feed.

Lipids (Soxhlet extraction system) and protein (Kjeldahl method) content of the tea extracts waste were according to AOAC method. Determination of minerals (sodium, copper, calcium, iron, and zinc) was done by using Atomic Absorption Spectrophotometer (AAS) according to Gallaher *et al.* (2006) while phosphorus was according to (AOAC method 986.24).

The amount of lipids, protein and minerals that gained in the decaffeinated tea extract waste were compared with the other sources of animal feed that had be commercialised. The ratio among the minerals in animal feed was known according to the journal of the commercialised animal feed.

CHAPTER 2

LITERATURE REVIEW

2.1 Review on the Component of Tea

Tea is one of the most popular soft drinks in the world with an estimated total production of 3.2 million ton (year 2004)(Shen, L. Q *et al.*, 2008). The beverage is an infusion of dried leaves of camellia sinensis, and can be classified into the three types green, black, and oolong tea depending on the process of treatment of the leaves after harvesting (Schmidt, M *et al.*, 2005). Of the total amount of tea produced and consumed in the world, 78% is black, 20% is green, and less than 2% is oolong tea (Mukhtar & Ahmad., 2000; Luczaj & Skrzydlewska., 2005).

Tea leaves contain a variety of amino acids, proteins, tannins, polyphenols such as catechin, epicatechin gallate, epigallocatechin gallate, and vitamins (Yamamoto *et al.*, 1997). Tea leaves also contain fluoride which helps strengthen bones and teeth and fights cavities (Wissostsky., 2007). Green tea is considered to exert a number of beneficial health effects, and therefore, green tea products are widely used as dietary supplements (Schmidt, M *et al.*, 2005). Based on the nutritional properties in tea leaves, this suggests that tea waste may have the potential to be used as animal feed. Beside that, tea waste may be considered a valuable protein source consisting of 22 ~ 35% of crude protein (CP) (Kondo *et al.*, 2004).

2.2 Classification of Tea

Tea can be classified principally into three types which differ in how they are produced and in their chemical composition: green (unfermented), oolong (semi-fermented), and black (fully fermented) (Wang *et al.*, 2000).

Green tea is prepared by deactivating the enzymes of the fresh leaves, either by firing or by steaming, to prevent the enzymatic oxidation of catechins (Wang *et al.*, 2000). Therefore green tea contains high concentrations of monomeric polyphenols from the catechins group (Dufresne & Farnworth., 2001).

Oolong tea is prepared by firing the leaves shortly after rolling, and then drying the leaves (Wang *et al.*, 2000). Oolong tea is a partially oxidized product (Luczaj & Skrzydlewska., 2005). The characteristics of oolong tea are between black and green tea (Wang *et al.*, 2000).

Black tea undergoes a full fermentation stage before drying and steaming (Zuo *et al.*, 2002). This fermentation process results in the oxidation of simple polyphenols (catechins) to more complex condensed molecules which give black tea its typical colour and strong, astringent flavor (Wang *et al.*, 2000). Black tea is rich in tannins which gives it an astringent quality useful in treating diarrhoea and can help relieve certain types of headaches (Ferrara *et al.*, 2001). Damp black tea bags can also be placed over tired, red eyes or on insect bites to relieve itching and redness (Ferrara *et al.*, 2001).

2.3 Tea and Caffein

Caffeine is in numerous plants. It consumption helps with metabolism, weight loss and vitality. Caffeine ($C_8H_{10}N_4O_2$; 1,3,7- trimethylxanthine) is one of three methylxanthines (caffeine, theophylline and theobromine) in tea (Aynur Senol., 2006). It is a white compound, moderately soluble in water and organic solvents like ethanol, ethyl acetate, methanol, benzene, etc (Babu *et al.*, 2007). The average amount of caffeine from brewed tea leaves depend on the type of tea (black tea contains more caffeine than green tea), the brewing duration, and the water temperature (the higher the temperature, the greater the amount of caffeine drained from the leaves). About 120 000 tones of caffeine are consumed worldwide every year (Wang *et al.*, 2007).

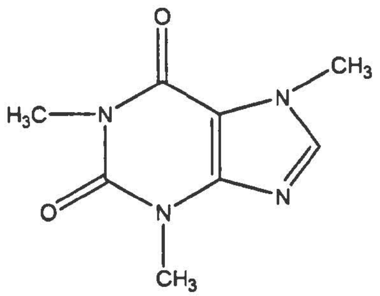

Figure 2.1 Molecular structure of caffeine (Spiller, 1998; Zuo *et al.*, 2002).

Figure 2.1 shows the molecular structure of caffeine. Caffeine is the major alkaloid present (about 2 – 5%) in dry leaves of *C. sinensis*, which also contains small amounts of theobromine (0.2 – 0.4%) and theophylline (about 0.02%) (Yang *et al.*, 2007b).

The table 2.1 presents the amount of caffein in various drinks, at 200 mg of beverage. In generally, the caffein that presented in coffee is much more higher than tea. Different amount of caffein present in various tea leaves. Black tea usually have higher caffein compared with green tea.

Table 2.1 Amount of caffein (mg) in various drink.

Espresso	50 - 150
Filter coffee	60 - 80
Instant coffee	40 - 70
Black tea	40 - 60
Oolong tea	30 - 50
Green tea	15 - 20
Red tea	0
Herb and fruit infusions	0

(Wissotsky, 2007)

2.4 Decaffeination of Tea

Decaffeination of tea leaves is required. It is because tannin in the leaves will affect the healthy of the animals. Animals fed tannin-rich diets showed decrease in their feed intake (Silanikove *et al.*, 1994). Tannins are known to reduce feed intake by decreasing palatability and/or ruminal turnover as well as the rate of digestion (Reed, 1995). Previous studies on crude tannins (CT) (Terrill *et al.*, 1992), (Wang *et al.*, 1996a) and (Wang *et al.*, 1996b) points out the importance of considering their dosage-dependent effect. While moderate concentrations of CT (20-40 g/kg DM) can have exert beneficial effects on the protein metabolism in ruminants, they had no effect on voluntary feed intake (Aerts *et al.*, 1999).

To decaffeinate tea leaves, hot water treatment is recommended. Hot water treatment is the most inexpensive method to decaffeinate tea leaves. Extraction time and ration of leaf to water has a statistically significant effect on decaffeination. When fresh tea leaves are decaffeinated with a ratio of tea leaf to water of 1:20 (w/v) at 100°C for 3 min, caffeine concentration was decreased from 23.7 to 4.0 mgg^{-1}, while total tea catechins decreased from 134.5 to 127.6 mgg^{-1}; 83% of caffeine was removed and 95% of total catechins was retained in the caffeinated leaf (Liang *et al.*, 2007). Chloroform or methylene chloride is an effective solvent for isolating caffeine from tea leaves. However, it is not widely accepted by consumers because of its toxicity (Sakanaka., 2003). Other than that, decaffeination using supercritical carbon dioxide is effective and leaves no solvent residue (Chang, Chiu, Chen, & Chang., 2000, 2001), but it needs expensive equipment. Sawdust lignocelluloses column can be used to separate caffeine from tea extracts too (Sakanaka., 2003), but they are difficult to use for decaffeination of tea leaves.

2.5 Composition of Tea

Fresh tea leaves contain upon an average (related to dry substance mass): 36% polyphenolic compounds, 25% carbohydrates, 15% proteins, 6.5% lignin, 5% ash, 4% amino acids, 2% lipids, 1.5% organic acids, 0.5% chlorophyll as well as carotenoids and volatile substances constituting less than 0.1% (Graham., 1992; Luczaj, & Skrzydlewska., 2005) . It must be noted that the chemical composition of tea leaves varies with climatic conditions, season, position on the flushing shoot, age of leaf, and cultural practices (Spiller., 1998). Composition of the tea leaves are compared to the poultry nutritional needs to ensure the tea waste can be used as the poultry feed.

Table 2.2 is the composition of green and black tea. The data is based on the extractable solids present for black and green tea. There are many components that included in green and black tea which are needed for animal or human health and development.

Table 2.2 Composition of green and black tea.

	Green Tea (%)	Black Tea (%)
Amino acids	3	3
Aroma	Trace	Trace
Caffeine	3	3
Catechins	30	9
Flavonols	2	1
Lipids	3	3
Organic acids	2	2
Other carbohydrates	4	4
Other methylxanthines	<1	<1
Other minerals/ ash	5	5
Other polyphenols	6	23
Peptides/ proteins	6	6
Potassium	5	5
Simple polyphenols	2	3
Sugars	7	7

Theaflavins	-	4
Theanine	3	3

(Source: Spiller, 1998)

2.5.1 Minerals

Minerals are inorganic elements found in nature that function in the body as structural components and regulators of body processes. Minerals serve two of the three basic functions of nutrient in food: (a) as the building blocks for body tissue; (b) components for enzymes involved in the regulation of metabolism; and (c) do not provide a source of caloric energy (Williams., 2005).

Human and animal studies shows that optimal intakes of elements such as sodium, potassium, magnesium, calcium, manganese, copper, zinc and iodine could reduce individual risk factors, including those related to cardiovascular disease (Sanchez-Castillo *et al.*, 1998; Ozcan., 2004).

The average tea infusion, is prepared by steeping the dried leaves in near-boiling water, and contains little protein, vitamins, fibre or carbohydrate, but may be a source of some essential dietary metals and metal binding polyphenols. For instance, tea could be an important source of manganese and the large amount of potassium in comparison with sodium that could be beneficial for hypertensive patients (Fernandez *et al.*, 2002).

2.5.2 Major Minerals

Major minerals are those required in amounts greater than 100 mg per day and they represent 1% or less of bodyweight. These include calcium, phosphorus, magnesium, sulfur, potassium, chloride, and sodium (Ozcan., 2004; Williams., 2005). Table 2.3 shows the major functions, deficiency and excess symptons of major minerals.

Table 2.3 Major functions, deficiency, and symptons of overdoze of major minerals.

Minerals	**Major Functions**	**Deficiency Symptons**	**Symptons of overdoze**
Calcium, Ca	Component of bones and teeth; enzyme activation; muscle contraction; cell membrane potential; blood pressure; nerve impulse transmission; blood clotting.	Osteoporosis; muscle cramps; rickets; rickets; impaired muscle contraction;	Constipation; heart arrhythmias; calcification of soft tissues; kidney stones; inhibition of trace mineral absorption
Chloride, Cl_2	Negative ion in extracellular fluid; hydrochloride acid formation in stomach; nerve impulse conduction	Growth failure in children; muscle cramps; mental apathy; appetite loss;	Hypertension; vomiting
Magnesium, Mg	Bone mineralization; maintainces of teeth; metalloenzyme; nerve impulse transmission; protein synthesis; smooth muscle contraction; glucose metabolism	Muscle weakness; apathy; muscle twitching; muscle cramps; appetie loss; cardiac arrhythmias; confusion; depressed pancreatic hormone secretion;	Nausea; electrolyte imbalances; vomiting; diarrhea
Potassium, K	Positive ion in intracellular fluid; same functions as sodium, but intracellular; glucose transport into cell	Dehydration; muscle weakness and cramps; paralysis; confusion; hypokalemia; appetite loss; apathy; irregular	Muscle weakness; triggers vomiting; hyperkalemia; inhibited heart

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