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The Effects Of Polyphenol Rich Peanut Skin Diets On Weight Gain, Lipid Profile, And Blood Chemistry In Rats

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THE EFFECTS OF POLYPHENOL RICH PEANUT SKIN DIETS ON
WEIGHT GAIN, LIPID PROFILE, AND BLOOD CHEMISTRY IN RATS

by

Erika Robinson Hayes

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Department: Animal Sciences
Major: Animal Health Science
Major Professor: Dr. Tracy Hanner

North Carolina A&T State University
Greensboro, North Carolina
2011

School of Graduate Studies
North Carolina Agricultural and Technical State University

This is to certify that the Master's Thesis of

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Greensboro, North Carolina
2011

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DEDICATION

I would like to dedicate this body of work to all of the people that have supported me throughout my academic career. Thank you for your support and guidance. I would like to acknowledge the one person that has believed in my abilities and my dreams to succeed, my husband, Antonio. He is truly my inspiration to continue in my quest for success.

BIOGRAPHICAL SKETCH

Erika Robinson Hayes was born on May 9, 1979, in Greensboro, North Carolina. She received her Bachelor of Science degree in Laboratory Animal Science from North Carolina Agricultural and Technical State University in 2005. She is a candidate for Master of Science in Animal Health Science in 2011.

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LIST OF ABBREVIATIONS

°C	Celsius
°F	Fahrenheit
ALT	Alanine Transaminase
AMY	Amylase
BUN	Blood Urea Nitrogen
Ca	Calcium
CRE	Creatinine
dl	Deciliter
g	Gram
GAE	Gallic Acid Equivalent
GLU	Glucose
H ₂ SO ₄	Sulfuric Acid
HDL	High Density Lipoprotein
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
LDL	Low Density Lipoprotein
mg	Milligram
ml	Milliliter
N	Nitrogen
Na ₂ CO ₃	Sodium Carbonate
NaOH	Sodium Hydroxide

nm	Nanometer
TC	Total Cholesterol
TC/HDL	The ratio of Total Cholesterol to High Density Lipoprotein
TG	Triglycerides
TP	Total Protein
μl	Microliter
μm	Micrometer
VLDL	Very Low Density Lipoprotein
Vol	Volume

ABSTRACT

Hayes, Erika Robinson. THE EFFECTS OF POLYPHENOL RICH PEANUT SKIN DIETS ON WEIGHT GAIN, LIPID PROFILE, AND BLOOD CHEMISTRY IN RATS. (Advisor: Tracy Hanner), North Carolina Agricultural and Technical State University.

Peanut skin is a by-product of the peanut processing industry that is typically disposed. However, peanut skin contains significant amounts of protein, lipids, dietary fiber and is rich in antioxidants such as phenolics. The objectives of this study were to (1) evaluate the overall impact of peanut skin consumption on the growth of young lab rats; (2) investigate the effects of peanut skin consumption on blood plasma cholesterol profile in laboratory rats; and (3) evaluate the effect of peanut skin consumption on the functions of major animal organs by measuring the changes of related biomarkers and gross pathology. Twenty five 7-week old female Sprague Dawley rats were randomly assigned to one of five groups. Each group was fed one of five different diets for 8 weeks. The diets were: G1- standard Purina 5001 rodent diet; G2- Purina-5001 + 1% cholesterol; G3- Purina-5001 + 1% cholesterol and 2.5% peanut skin, G4- Purina-5001 + 1% cholesterol and 5.0% peanut skin; and G5- Purina-5001 + 1% cholesterol and 10.0% peanut skin. In each group, the rat's average food consumption and total body weight were measured weekly. The hematological parameters and lipid profile of blood were evaluated biweekly using pooled blood samples. At the time of euthanasia, the gross pathology of rats was performed and the brain, liver, heart, lung, kidney, and spleen were weighed and visually checked for abnormalities. Results showed that rats in G2-G5 consumed more feed and gained more weight than rats in G1. Rats in G4 and G5 had lower triglycerides and total cholesterol than rats in G2. During the first few weeks, rats fed with the peanut skin diets

had higher LDL and lower HDL than rats in G2. However, this trend reversed after 5 weeks. Compared to G2, rats in G4 and G5 showed lower ALT, BUN and glucose levels, particularly at later feeding stages. Organs of rats in G2 were larger than the organs in other groups. This study suggests that long term consumption of diets containing $\geq 5\%$ peanut skin may significantly lower the health risks associated with consuming a high cholesterol diet.

CHAPTER 1

INTRODUCTION

Peanut is an important crop and oil seed. The world's peanut production was 34.4 million metric tons in 2008-2009 and the United States is currently the third largest peanut producer (USDA, 2010). Peanut is a source of plant protein, dietary fiber, unsaturated fatty acids, B-vitamins, vitamin E, magnesium, copper and phosphorous. Numerous bioactive substances, including polyphenols are present in peanuts (Griel, Eissenstat, Juturu, Hsieh, & Kris-Etherton, 2004). Peanuts are widely used to produce peanut oil, peanut butter, peanut flour, peanut protein concentrate/isolate, and roasted peanut snacks. Peanuts are also used to develop infant formula and peanut milk. The food industry's use of peanuts generates a significant amount of peanut skin, a by-product with little value, usually 2-3% of the total peanuts produced. It is estimated that world production of peanut skin is on the magnitude of 750,000 tons annually, with the only current market being low value animal feed applications (Sobolev & Cole, 2004). Peanut skin contains 16.6% oil, 12.32% protein and 2.83% ash (Nepote, Grosso, & Guzman, 2002) and is typically used as animal feed or decomposed. Peanut skin is also rich in polyphenols such as catechins, A-type and B-type procyanidins (also called condensed tannins), some phenolic acids and reveratrols (Nepote, Grosso, & Guzman, 2002; Lou, Yamazaki, Sasaki, Uchida, Tanaka, & Oka, 1999; Yu, Ahmedna, Goktepe, & Dai, 2006).

Polyphenols are secondary metabolites of plants and the most abundant antioxidants in the diet. Main dietary sources of polyphenols are fruits and plant derived beverages such as teas, fruit juices and wines. Vegetables, cereals, chocolate and dry

legumes also contribute to intake of polyphenol (Scalbert, Johnson, & Saltmarsh, 2005). Numerous in-vitro and in-vivo studies show that dietary polyphenols have antioxidant, anticarcinogenic, cardiopreventive, and anti-inflammatory effects. Some polyphenols were reported to improve bone health, cognitive function, and to reduce postmenopausal symptoms. Epidemiological studies have shown that a diet rich in polyphenols is inversely associated with cardiovascular disease (Arts & Hollman, 2005). Fruits and vegetables rich in polyphenols may help to reverse hyperlipidemia, alter the atherogenicity of the LDL particle (Lampe, 1999) and protect the cholesterol in LDL from oxidation (Brouillard, George, & Fourgerousse, 1997).

Human studies have shown that diets rich in procyanidins decrease/inhibit lipid peroxidation of LDL cholesterol and increase free radical scavenging capacity (Natella, Belelli, Gentili, Ursini, & Scaccini, 2002; Fuhrman, Lavy, & Aviram, 1995). Studies in different cell lines, animal models and human epidemiological trials suggest a protective role of dietary polyphenols against different types of cancers. The in vitro antioxidant activity of peanut skin polyphenols, and their protective effect against lipid oxidation and bacterial growth in ground beef have been reported (Yu, Ahmedna, Goktepe, & Dai 2006; Yu, Ahmedna, & Goktepe, 2010). However, the health benefits of peanut skin polyphenol in animal models have not been reported.

The objectives of this study were to (1) evaluate the overall impact of peanut skin consumption on the growth of young lab rats; (2) investigate the effects of peanut skin consumption on blood plasma cholesterol profile in laboratory rats; and (3) evaluate the

effect of peanut skin consumption on the functions of major animal organs by measuring the changes of related biomarkers and gross pathology.

CHAPTER 2

LITERATURE REVIEW

2.1 Diseases Associated with Hypercholesterolemia in Humans

Increased levels of dietary cholesterol can aid in the development of cardiovascular diseases. Cardiovascular diseases which include hypertension, atherosclerosis, myocarditis, and coronary heart disease are leading causes of death in the United States and the world. Elevated levels of the lipoprotein fractions, LDL, IDL and VLDL are regarded as *atherogenic* (prone to cause atherosclerosis). It was hypothesized that oxidation of LDL modifies the LDL particles. These modified particles are then taken by macrophages inside the arterial wall and form atherosclerotic plaque (Zock & Katan, 1998). Atherosclerosis may occur when plaque made up of cholesterol, calcium, and other fats build up on the inner surface of blood vessels. Plaque grows thick leading to a narrowing of blood vessels and restriction of blood flow. High cholesterol levels have been associated with coronary artery plaque formation in coronary heart disease. A heart attack may occur if a coronary artery plaque ruptures (Kovalala, 2005). A 10 year follow up study conducted in Japan found that low serum total cholesterol (<160 mg/dl) levels are associated with high mortality from intraparenchymal hemorrhage while high levels (>260 mg/dl) are associated with high mortality from coronary heart disease (Cui, Iso, Toyoshima, Date, Yamamoto, Kikuchi et al., 2007).

The level of cholesterol in blood can be increased by consuming a diet high in cholesterol and fat. Dietary cholesterol originates from meat, poultry, fish, seafood and dairy products. There are three different kinds of dietary fats: saturated, polyunsaturated,

and monounsaturated (Perkins & Visek, 1983). Saturated fat is the main dietary cause of elevated blood cholesterol. Saturated fats are found predominately in foods from animal sources and in a few plant derived products, principally coconut and palm oils (Perkins & Visek, 1983). Polyunsaturated and unsaturated fats are found in fish, nuts, seeds and vegetable oils. Both polyunsaturated and monounsaturated fats may help to lower the blood cholesterol level when used to replace the saturated fats in the human diet. Along with cholesterol, saturated fats may cause atherosclerosis (Perkins & Visek, 1983). There are currently several methods to treat coronary heart disease, including medications (such as beta-blockers and Ca channel blockers), non-surgical methods (such as angioplasty, atherectomy), and surgical methods (such as bypass and off-pump bypass).

Obesity has also been linked to elevated cholesterol levels, placing individuals at risk for a range of health issues including high blood pressure, diabetes, heart disease, and stroke. Physicians test cholesterol levels to determine how much cholesterol is carried by low density lipoproteins (LDL) and how much is carried by high density lipoproteins (HDL). Higher levels of HDL, the most preferred form of lipoproteins, do not occur with obesity, aid in lowering heart disease and stroke risks. (Smolak & Thompson, 2009). Obesity has become a very serious problem in America. One in three children or adults are considered to be obese. Children are less engaged in physical activity although many schools now have a mandatory implementation of physical education (Smolak & Thompson, 2009). In addition to genetic factors, unhealthy eating behavior and lack of physical activity are two major contributing factors of obesity. Gastric by-pass surgery is a last resort method to extreme obesity. However, gastric

by-pass surgery is not indicated for the obese individual who is unwilling to make long term dietary behavior changes (Blass, 2008). Therefore, preventing obesity is the best solution by maintaining healthy weight through diet and exercise.

2.2 Cholesterol

Cholesterol is a soft, fat-like, waxy substance found in blood serum and in somatic cells. Normal levels of cholesterol in blood serum are important because it is important in maintaining a healthy body. It is used for producing cell membranes and some hormones, and assists in other bodily functions such as, digestion and building muscle (Kovala, 2005). About 20–25% of total daily cholesterol production occurs in the liver; other sites of high synthesis rates include the intestines, adrenal glands, and reproductive organs. Major dietary sources of cholesterol include cheese, egg yolks, beef, pork, poultry, and shrimp (USDA, 2008). The amount of cholesterol present in plant-based food sources is generally much lower than animal based sources (Behrman & Gopalan, 2005). Plant products such as flax seeds and peanuts contain cholesterol-like compounds called phytosterols, which help lower serum cholesterol levels by inhibiting absorption (Ostlund, Racette, & Stenson, 2003).

Cholesterol is synthesized in four different stages. Initially, 2 acetyl-CoA molecules condense to form acetoacetyl-CoA. A third acetyl-CoA is added and condenses to form 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) (The University of Bristol, 2007). HMG-CoA is then reduced by HMG-CoA reductase to form mevalonate. Mevalonate is decarboxylated to form isopentenyl pyrophosphate. Isopentenyl pyrophosphate is then condensed to form farnesyl pyrophosphate. Two

molecules of farnesyl pyrophosphate are then condensed to form squalene, which in turn, forms lanosterol, which is converted to cholesterol (Rhodes, Stryer, & Tasker, 1995).

Bate, Rumbold, & Williams (2007) designed a basic flow chart of the synthesis of cholesterol (see Figure 2.1).

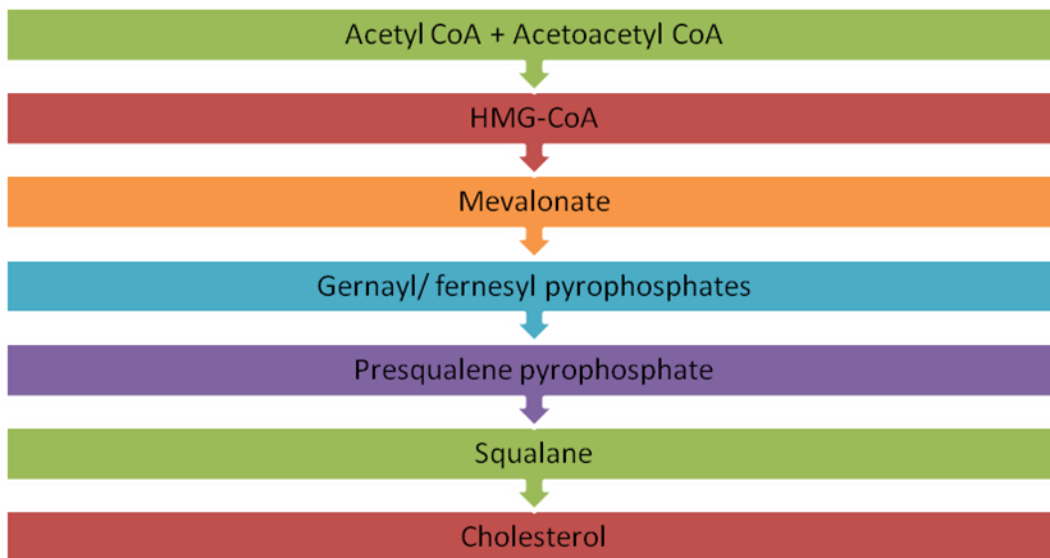


Figure 2.1. Flow Chart of Cholesterol Synthesis

Vehicles which transport cholesterol throughout the blood stream are known as lipoproteins. There are five main classes of lipoproteins: 1) very low density lipoproteins (VLDL), 2) intermediate density lipoproteins (IDL), 3) low density lipoproteins (LDL), 4) high density lipoproteins (HDL), and 5) chylomicrons. In general, lipoprotein particles range in size from 10 to 100 nm. Lipoproteins are composed of a hydrophobic core containing cholesterol esters, triglycerides, fatty acids and fat-soluble vitamins. The surrounding hydrophilic layer is composed of various apolipoproteins, phospholipids, and cholesterol (Sigma-Aldrich, 2007). Chylomicrons are the largest but least dense of the

lipoproteins. They contain only 1-2% protein, 85-88% triglycerides, ~8% phospholipids, ~3% cholesteryl esters and ~1% cholesterol. Chylomicrons are produced for the purpose of transporting dietary triglycerides and cholesterol absorbed by intestinal epithelia.

Chylomicron assembly originates in the intestinal mucosa. VLDL's are slightly smaller than chylomicrons in terms of size, density and lipid content. VLDL's are approximately 25-90 nm in size, with a density of ~0.98 and contain 5-12% protein, 50-55% triglycerides, 18-20% phospholipids, 12-15% cholesteryl esters and 8-10% cholesterol (Sigma-Aldrich, 2007).

LDL contains 20-22% protein, 10-15% triglycerides, 20-28% phospholipids, 37-48% cholesteryl esters and 8-10% cholesterol. HDL contains approximately 55% protein, 3-15% triglycerides, 26-46% phospholipids, 15-30% cholesteryl esters and 2-10% cholesterol. LDL and HDL transport both dietary and endogenous cholesterol in the plasma. LDL is the main transporter of cholesterol and cholesteryl esters and makes up more than half of the total lipoprotein in plasma. LDL is absorbed by the liver and other tissues via receptor mediated endocytosis. The cytoplasmic domain of the LDL receptor facilitates the formation of coated pits; receptor-rich regions of the membrane (Sigma-Aldrich, 2007). The recommended values for total cholesterol, LDL, and HDL are <200mg/dl, <100mg/dl, and >40mg/dl, respectively. The National Institutes of Health (NIH) has developed guidelines for healthy cholesterol levels (see Table 2.1). Following these guidelines, can help assist in a healthy lifestyle. These recommendations are of great importance because cholesterol levels have been used a biomarkers to determines the risk of cardiovascular diseases.

Table 2.1

National Recommendations for Cholesterol

Total Cholesterol	
<200	Desirable
200-239	Borderline High
>240	High
LDL Cholesterol	
<100	Optimal
100-129	Near Optimal/Above Optimal
130-159	Borderline High
160-189	High
>190	Very High
HDL Cholesterol	
<40	Low
>60	High

2.3 Hematology Values

Blood tests are one of the most important tools that can be used in evaluating health status. There are serum chemistry tests available to evaluate major organ function along with normal blood levels for cholesterol. Liver function can be evaluated by testing for alanine transaminase (ALT). ALT is found in blood serum and in several body tissues, but is most commonly associated with the liver. It catalyzes two parts of the alanine cycle. ALT is currently one of the most reliable markers of hepatocellular injury, necrosis, and congestive heart failure (Giboney, 2005). In rats the normal ALT values are dependent on the species of rat being studied. Kidney function may be evaluated by testing for levels of serum creatinine (Cr), blood urea nitrogen (BUN) and glucose (GLU). Creatinine is created from the chemical waste that is generated from the

metabolism of muscle in the body. High levels of creatinine can be an indicator of kidney dysfunction (Beard, Taylor, and Miller, 1957). In humans, reference ranges of creatinine are 0.7 to 1.3 mg/dL in men and 0.6 to 1.1 mg/dL in women (Abcar, Chan, and Yeoh, 2004). In rats, ALT values are species dependent.

Pancreatic function may be evaluated by testing for normal glucose metabolism and amylase activity. Amylase is involved in the breakdown of dietary starch into glucose. Glucose is the body's main source of energy. Blood glucose levels are regulated by insulin and glucagon, hormones produced by the pancreas. Insulin is released into the blood when the amount of glucose in the blood rises to dangerous levels and causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle (Gibney, 2005). When blood glucose levels are too low, glucagon is released to initiate the breakdown of glycogen. If glucose levels remain too high over time, diabetes can occur and may cause damage to the cardiovascular and renal systems (Gibney, 2005). Normal values of glucose for humans generally range from 70 to 120 grams per deciliter (g/dL) and depend on factors such as weight, age, and environment (American Diabetes Association, 2006). Amylase which is produced by pancreatic cells may be affected by abnormal pancreatic function. Injury to the pancreas results in an increase in serum amylase levels.

Ammonia metabolism occurs in the liver where it is converted to BUN and then excreted by the kidneys. BUN measures the amount of nitrogen in the blood and is a way to measure renal function. Elevated BUN may be an indicator of moderate to severe renal failure. Low BUN levels are not as significant but can indicate a problem with the liver

(Gennari, 2001). As with the previous biochemical values, rat values are species dependent. In humans, the normal range is 10-15mg/dL (Gennari, 2001).

2.4 Polyphenols

Fruit, vegetable, and nut consumption have been associated with reduced risks of some forms of cancer, heart disease, stroke, obesity, and other chemical diseases. This is partially contributed to their antioxidant capability. Polyphenols are secondary metabolites of plants. Polyphenols contribute to the color and taste of many fruits and vegetables. Polyphenols can also exist in certain amounts in grains and legumes. Polyphenols are classified into different groups as a function of the number of phenol rings that they contain and the structural elements that bind these rings to one another. Distinctions can be made between the phenolic acids, flavanoids, stilbenes, and lignans. The flavanoids share a common structure consisting of two aromatic rings (A and B) that are bound together by three carbon atoms that form an oxygenated heterocycle (ring C). Flavanoids may be divided into six subclasses as a function of the heterocycle that is involved: flavonols, flavones, isoflavones, flavanones, anthocyanidians, and flavanols (catechins and proanthocyanidins). The most abundant flavanoids found in foods are flavonols, and the main representatives are quercetin and kaempferol. Red wine and tea contain up to 45mg/L of flavonols. For years, wine has been praised for its rich antioxidant properties. Flavonol compounds are present in the glycosylated form. Fruit often contains between 5 to 10 different flavonol glycosides (Clifford, 1999).

Flavonols accumulate in the outer and aerial tissues of plants where their biosynthesis is stimulated by light. Concentration differences can exist between different

pieces of fruit on the same tree and even between different sides on one piece of fruit, depending on the level of exposure to sunlight (Price, Breen, Valladao, & Watson, 1995). Flavones are less common than flavonols in fruit and vegetables. Celery and parsley are the only important edible sources of flavones (Manach, Morand, Jimenez, Rémésy, & Scalbert, 2004). Isoflavones are flavonoids with a very similar structure to estrogens. Although isoflavones are not steroids, they have hydroxyl groups in positions 7' and 4' in a configuration comparable to the hydroxyls in the estradiol molecule. Isoflavones have pseudohormonal properties on them, which includes the ability to bind to estrogen receptors. Isoflavones are also known as phytoestrogens. Isoflavones are found almost exclusively in legumes, particularly in soy beans (Manach et al, 2004).

Flavanols exist in both monomer form, which are catechins, and the polymer form, which are proanthocyanidins. Catechins are found in different types of fruit, such as apricots which are the richest source. Catechins are also present in red wine, but green tea and chocolate are by far the richest sources (Lakenbrink, Lapczynski, Maiwald, & Engelhardt, 2000). Proanthocyanidins, which are also known as condensed tannins, are dimmers, oligomers, and polymers of catechins that are bound together by links between C4 and C8 (C6) (Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998). Anthocyanins are the pigments that are dissolved in the vacuolar sap in the epidermal tissues of flowers and fruit (Mazza & Maniati, 1993). Anthocyanins are stabilized by the formation of complexes with other flavonoids. Anthocyanins are found in red wine, certain cereals, and certain leafy and root vegetables, but they are very abundant in fruit. Cyanidin is the

most common anthocyanidin in foods, especially in the skin of fruit (Manach et al., 2004).

Procyanidins are polyphenolic compounds that are usually found in pine bark (Rohdewald, 1998) grape (seeds and skin) (Escribano-Bailon, Gutierrez-Fernandez, Rivas-Gonzalo, & Santos-Buelga, 1998) cocoa, cranberries and apples (Hammerstone, Lazarus, & Schmitz, 2000). Monomeric procyanidins are polyhydroxyflavan-3-ol monomers, and are often called catechins; which includes catechins, epicatechin, galocatechin, epigallocatechin, and their gallic acid esters (catechins gallate and epicatechin gallate) (Yu et al., 2006). Lignans are formed of two phenylpropane units. The richest dietary source of lignans is linseed. Other cereals, grains, fruit and certain vegetables also contain traces of these same lignans, but concentrations in linseed are ≈ 1000 times as high as the concentrations in other food sources (Adlercreutz, 1997). Stilbenes are found in low quantities in the human diet. One of these, resveratrol, for which anticarcinogenic effects have been extensively studied, is also found in low quantities in wine, grape skin, and peanut skin also contain a certain level of resveratrol (Bertilli et al., 1998; Bhat & Pezzuto, 2002; Vitrac, Vercauteren, Deffieux, & Mérillon, 2002). The polyphenol fraction of wine includes phenolics acids, trihydroxy stilbenes, oligomer proanthocyanidins, and the flavonoids (Soleas & Goldberg 199; Salah, Miller, Paganga, Tijburg, Bolwell, & Rice-Evans, 1995). The antioxidant effectiveness of some of these polyphenolic compounds have been found to be more potent than vitamin E in their free radical scavenging properties in addition to their ability to prevent LDL oxidization (Nardini, Aquino, Tomassi, Gentili, Felice, & Saccini, 1995). Grape seed

proanthocyanidins were found to possess cardioprotective abilities by functioning as an in-vivo antioxidant and their ability to directly scavenge reactive oxygen species including hydroxyl and peroxy radicals (Sato, Maulik, Ray, Bagchi, & Das, 1999).

Additionally, there have been many studies that involve the health benefits of plant extracts. In one particular study, green tea was added to the diet of rats which included 1% cholesterol and 10% coconut oil to investigate the serum antioxidative activity of the polyphenols in green tea. Researchers found an increase in HDLs and decrease in total cholesterol due to polyphenol addition to diet of rats (Yokozawa, 2002). A study published in 2008, tested the polyphenols in peanut skins as a way to decrease cholesterol. The preliminary results showed a reduction in serum cholesterol levels in rats as a result of the addition of peanut skins to the diet (Shimizu-Ibuka, Udagawa, Kobayashi-Hattori, Mura, Tokue, Takita et al., 2009). These studies offer insight to the potential health benefits of polyphenols.

2.5 Justification/Importance of the Study

There are numerous studies that confirm the health effects of polyphenols in the diet. For instance in one Japanese study, the results suggested that polyphenolic substances derived from cocoa powder may contribute to a reduction in LDL cholesterol, an elevation in HDL cholesterol, and the suppression of LDL oxidation (Seigo, Midori, Akiko, Yuko, Takaaki, Naomi et al 2007). The first human study to find that a tea product lowers LDL cholesterol was completed by a researcher in Vanderbilt University Medical Center (Pasley, 2003). In this particular study, a 375 mg capsule made up of 75 mg of theaflavins (flavanoids from black tea) 150 mg of catechins (flavanoids from green

tea) and 150 mg of other tea polyphenols was used instead of liquid tea. The ingredients of the capsule were equivalent to seven cups of high-quality black tea or 35 cups of green tea. The results of this study resulted in an overall reduction of LDL cholesterol by 16% (Pasley, 2003).

A study of blueberry consumption showed the cholesterol-lowering potential of blueberries was similar to that of effective drugs. The blueberries contain a compound pterostilbene, which is an antioxidant that is similar to resveratrol, another antioxidant identified in grapes and red wine that is also believed to lower cholesterol. Many epidemiological studies have suggested that there is an association of red wine and the prevention of cardiovascular disease. The lower coronary heart disease mortality in France has been related to the high intake of red wine (Renaud & de Lorgeril, 1992.) Studies have indicated that the high content of polyphenolic components in red wine could be a key factor in the prevention of life-style related diseases (Belleville, 2002; Sun Simonyi, & Sun, 2002).

A large body of evidence consistently shows that consumption of tree nuts and peanuts is associated with a decreased risk of coronary heart disease. The Nurses' Health Study showed an inverse association between nut consumption and the risk of coronary heart disease (Griel et al., 2004). It was reported that the substitution of fat from one ounce of nuts for the equivalent energy from carbohydrate and saturated fat reduced coronary heart disease risk by 30% and 45%, respectively (Hu & Stampher, 1999). The health benefits associated with nuts are thought to reflect their nutritional profile including their nutrient density, fatty acid profile, and presence of bioactive compounds.

A couple of the bioactive substances that are present in nuts are flavanoids and resveratrol which are known for their association with decreased cholesterol and coronary heart disease risks (Griel et al., 2004). The health benefits of red wine and grape seeds have been extensively studied. However, peanut skin is also rich in polyphenols. According to Yu et al. (2006), the red skin of peanuts contained 12-13% phenolics, which consisted of mainly A-type and B-type procyanidins. In addition, peanut skin also contains certain amounts of protein, fat/lipid and non-digestible carbohydrates. In vitro studies have shown that peanut skin polyphenols have 2-3 times the antioxidant activity of vitamin C (Yu et al., 2006). It is important to investigate the health benefits of peanut skin and peanut skin polyphenols in vivo using animal models.

CHAPTER 3

MATERIALS AND METHODS

3.1 Peanuts and Peanut Skin Removal

Raw peanuts were obtained from Peanut Processor's in Dublin, North Carolina. Peanut kernels were frozen overnight. Then the skins were removed manually by the direct peeling method. The peanut skins were dried in a Fischer Scientific Isotemp vacuum oven and ground into a powder using a Retsch PM100 Ball Mill (Haan, Germany).

3.2. Proximate Composition of Peanut Skins

Crude Fiber Analysis: The crude fiber in peanut skin was determined by AOCS method Ba 6-84, AOAC 962.09 (Filter Bag Technique) using an Ankom 200 Fiber Analyzer (Macedon, New York) using the procedures in the Ankom manual. The procedures include fat extraction steps (acetone,), acid hydrolysis, alkaline hydrolysis, drying and ashing. This method determines the organic residue after digesting with 0.255N H₂SO₄ and 0.313N NaOH. The analysis was conducted in triplicate. Detailed procedures are described below.

Filter bags were weighed (W1) before adding samples, then 0.95-1.00 g peanut skin (W2) powder was directly weighed into each filter bag. A blank bag was weighed and included in run to determine blank bag correction (C1). The bags were then sealed using a heat sealer, placed into a 250 ml container, and then acetone was added to the container to extract fat from peanut skin samples. Acetone was added in the container just

enough to cover the bags. The container was then secured with a lid and soaked for 10 minutes. This process was repeated one additional time with fresh acetone.

After the acetone was poured out, the bags were placed on a wire screen to dry. The dry bags were placed on a bag suspender tray and the tray was then submerged in a vessel containing 1950ml of 0.225N H_2SO_4 solution. The vessel was tightly sealed with a lid. Samples in the bags were extracted for 45 minutes under heat and agitation. At the end of extraction the agitator and heat were turned off. The exhaust valve was opened and the hot solution was released before opening the lid. After the solution had been exhausted, the exhaust valve was closed and then the lid was opened. Following the rinse, 1950ml of 95°C water was added to rinse the bags for 3 minutes with agitation. The lid was closed but not completely tightened. The rinse was repeated two additional times. 1950ml of 0.313M of NaOH was added to the vessel with agitation and heated for 45 minutes. After going through the same process as that with 1950 ml of 0.225 N_2SO_4 , the bag was rinsed three times as described above. This process was repeated two additional times.

The filter bags were removed from the suspender trays and the water was gently pressed out to remove excess water. The bags were soaked in acetone for two minutes, in a 250 ml beaker, then removed and pressed gently to remove any excess acetone. The bags were spread out to dry by air. The dry bags were then placed in an oven at 105°C for two and a half hours. The bags were removed from the oven and placed in desiccant pouches until cooled to ambient temperature and weighed (W3). The ash was placed in a pre-weighed crucible and placed in a furnace for two hours at 550°C. The ash was then

cooled in desiccators and weighed for the organic matter calculation. Percentage of crude fiber in each sample is calculated as follows:

$$\% \text{ Crude Fiber} = 100 \times (W3 - (W1 \times C1)) / W2$$

Where, W1= bag weight, W2= sample weight, W3 = weight of organic matter, C1= ash corrected blank bag factor

Crude Protein Analysis: The crude protein content in peanut skins was determined by the AOAC method 990.03 (AOAC, 1990) utilizing a Leco Truspec CN Analyzer (Leco Corporation, St. Joseph, MI). This is a combustion based elemental analyzer. For protein determination, it measures percent of total nitrogen after sample is completely combusted. The conversion factor of 6.25 was used to convert percent N to percent protein. The machine was calibrated using EDTA powder which contains 9.2% nitrogen. Powdered peanut skin sample was weighed in a special aluminum foil (0.1500g). The sample was wrapped and placed in an auto-sampler tray. After entering sample information (name, weight, and conversion factor) in the computer, the analyzing cycle started by clicking Analyzer button from computer. The computer calculated the percent protein automatically according to the nitrogen detected and sample information entered. Protein determination of peanut skins was conducted in triplicate.

Crude Fat Analysis: This analysis was done utilizing the Foss Soxtec 2050 Extractor (Foss, Eden Prairie, Minnesota). The analysis was conducted in four replications. Four samples of powdered peanut skin each 2.00g were weighed (W1) into 4 thimbles. After the extractor was turned on along with the heater, the thimbles were placed in thimble holders and loaded into the extractor. Then the extraction cups (W2)

each containing 60 ml of petroleum ether were loaded in the machine. When the extraction cycle started, thimbles containing samples were submerged in heated petroleum ether. When the cycle ended, the thimbles and extraction cups were removed. Extraction cups contained fat extracted. Cups were weighed (W3), and the weight differences before and after extraction were the fat weight. Percent fat in peanut skin was calculated by following equation:

$$\% \text{ Fat} = (W3 - W2) \times 100 / W1$$

Where, W1 = peanut skin sample weight, W2 = weight of empty cup, W3 = weight of cup containing extracted fat.

3.3 Extraction and Determination of Phenolics in Peanut Skin

Extraction of phenolics: Eighty percent ethanol was used to extract phenolics from the peanut skin. Thirty millimeters of solvent was added in a 50ml centrifuge tube containing 1.000g of peanut skin. The mixture was homogenized by using a PT2100 Polytron Homogenizer (Brinkman, Switzerland) for 2 minutes, and then centrifuged at 3000g for 15 minutes at room temperature. The volume (v) of combined supernatant was collected in an amber bottle. The precipitate was extracted twice by the same procedure described above. The volume of combined supernatant was measured and filtered through a Whatman Anotop syringe filter (0.2µm) and stored in a freezer until use. The extraction of the peanut skins was performed in triplicate.

Phenolics Determination: The determination of total phenolics was completed by using the Folin-Ciocalteu method (Singleton et al., 1999). Briefly, 20µl of sample solution or standard solution was added to a 4 ml glass vial, followed by adding 1.28ml

of distilled water and 100µl of Folin-Ciocalteu reagent (Sigma-Aldrich, St. Louis, MO). The mixture in the vial was mixed using a Vortex and left at room temperature for 8 minutes. Then, 0.6ml of the Na₂CO₃ solution was added. The vial was capped and left at room temperature in the dark for 2 hours. The absorbance of the sample was measured at 765nm using a Genesis 10 Spectrometer (Fisher Scientific). Four replications were conducted. The determination was conducted in four replications.

Gallic acid (Sigma-Aldrich, St. Louis, MO) was used as the standard to develop standard curve according to the procedure described above. Total phenolics (mg/ml) in the extract was calculated using a calibration curve developed using a set of gallic acid solutions with known concentrations (0-100 mg/ml), and the result was expressed as a gallic acid equivalent (GAE) (mg gallic acid/ml extract). The phenolic concentration of the peanut skin powder was calculated as follows:

$$\text{Phenolic Content (mg/g)} = \text{total phenolics (mg/ml)} \times (v) \text{ (ml)} / \text{sample weight (g)}$$

3.4 Animal Experiment

Experimental Design: Twenty-five seven week old Sprague Dawley rats were obtained from Harley Sprague-Dawley, Inc (Indianapolis, Indiana). On arrival to North Carolina Agricultural and Technical State University, all animals were subjected to a physical examination by the technical staff under direction of the clinical veterinarian to ensure satisfactory health status. Rats were randomly divided into five groups. Rats received one of five experimental diets, G1- standard Purina 5001 rodent diet; G2- Purina-5001 + 1% cholesterol; G3- Purina-5001 + 1% cholesterol and 2.5% peanut skin, G4- Purina-5001 + 1% cholesterol and 5.0% peanut skin; and G5- Purina-5001 + 1%

cholesterol and 10.0% peanut skin. Table 3.1 shows the calculations for the inclusion of peanut skin powder for each of the five diet groups.

Table 3.1

Calculation of Feed with the Inclusion of Peanut Skin Powder

Groups	Peanut Skin Powder (g)	Rodent Chow (g)	Total Feed (g)
G1	0	1000	1000
G2	0	1000	1000
G3	25	975	1000
G4	50	950	1000
G5	100	900	1000

Note. G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4-control + 1% cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin

Housing condition: Rats were housed in individual stainless steel suspended wire mesh cages in a room maintained at $21 \pm 3^\circ\text{C}$, $50 \pm 10\%$ humidity, with 12 hour light-dark cycles. After a seven-day adaptation period all rats were fed one of the experimental diets for eight weeks. All animals were maintained in accordance with university animal care policy regulations.

Measurement of feed consumption and weight gain of rats during feeding period: The animals were offered 100g of feed initially. The animals feed was weighed every two days. Their feed consumption was calculated by subtracting the amount left in the feeding cups by the amount offered. The animals were weighed weekly using an Arbor 1605 balance. Their weight gain was calculated by subtracting the current weight from the previous weekly weight.

Blood Collection: The animals were placed in an inhalation chamber and anaesthetized by inhalation of 1% isoflurane. Blood was collected retro-orbitally from the

animals for weekly analysis. The amount of blood collected from each animal varied based on the animals health and availability of blood. Blood was collected in heparinized micro-hematocrit capillary tubes placed on a hematology mixer. Once the samples were analyzed, the remaining blood samples were stored in a -20°C freezer.

Evaluation of major organ functions by hematological analysis: Hematological analysis was performed utilizing the Abaxis VetScan HM II Hematology analyzer from TW Medical Veterinary Supply (Lago Vista, Texas). The rotors used were the Comprehensive Diagnostic Profile from Abaxis. The blood used for the analysis was pooled from each animal of that corresponding treatment group. The amount of blood needed was 100µl. The blood chemistry profile included Alanine Transferase, (µ/l), Amylase (µ/l), Blood Urea Nitrogen (mg/dl), Creatinine (mg/dl), Glucose (g/dl), Total Protein (g/dl).

Blood lipid profile: The cholesterol profile of rat blood was performed utilizing the Abaxis VetScan II Blood Chemistry Analyzer from Abaxis (Union City, California). The rotors used were Lipid Panel Reagent Disc from TW Medical Veterinary Supply (Lago Vista, Texas). Blood samples of five rats in the same treatment group were pooled and used for analysis. The amount of blood needed for each group was 100µl. The items analyzed were total cholesterol (TC) (mg/ml), high density lipoprotein (HDL) (mg/ml), low density lipoprotein (LDL) (mg/ml), very low density lipoprotein (mg/ml) (VLDL), and triglycerides (TG) (mg/ml).

Necropsy: Animal necropsy was performed for gross examination for pathological change and organ weight analysis. The heart liver, spleen, adrenal glands,

ovaries, brain, thymus, and lungs were all weighed. All organs were stored in 10% buffered formalin.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Proximate Composition of Peanut Skin and Feed

The composition of the peanut skins was very important to this study. This analysis was necessary to determine if the inclusion of peanut skin in the rat feed could cause significant change in the diet composition of the rat diet. Table 4.1 shows proximate compositions of peanut skin and original rat feed. The peanut skin contained 19.41% fiber, 16.60% fat, 12.32% protein, 2.83% ash.

Table 4.1

Proximate Composition and Total Phenolics of Peanut Skin Used

Component	% Fiber	% Fat	% Protein	% Ash	Total Phenolics (mg/g skin)
Content	19.41	16.60	12.32	2.83	7.29 ± 0.26

The inclusion of peanut skin in the rat feed at 2.5-10% did not cause significant changes in feed protein content, but resulted in increased total fat and fiber, and decreased ash content in the feed. The inclusion of peanut skin at the level of 2.5, 5.0 and 10.0% also contributed 0.18, 0.36 and 0.73% of total phenolics to the rat feed, respectively as shown in Table 4.2.

Table 4.2

Compositions of Feed Containing 0, 2.5, 5.0 and 10.0% Peanut Skin

Groups	% Replacement	% Cholesterol	% Fiber	% Fat	% Protein	% Ash	% Polyphenol
G1	0	0	5.10	5.70	23.90	7.00	0
G2	0	1	6.00	4.50	23.00	8.00	0
G3	2.5	1	6.33	4.80	22.73	7.87	0.18
G4	5.0	1	6.67	5.11	22.47	7.48	0.36
G5	10	1	7.34	5.72	21.93	7.48	0.73

Note. G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4-control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin

4.2 Feed Consumption and Weight Gain/Change as Affected by Peanut Skin

Figure 4.1 shows the feed consumption among different feed groups throughout the eight week testing period. Figure 4.1 also shows that G5 had the highest food consumption among the different groups during the feeding period. In contrast, G1 had the lowest average feed consumption during the feeding period. This was to be expected because G1 did not have the addition of peanut skin powder to its diet. This could be attributed to the palatability of the peanut skin powder that was added to the feed. G3 and G4 also had relatively low average food consumptions. From the results of the chart, there were no major differences in the consumption patterns of feed between the groups.

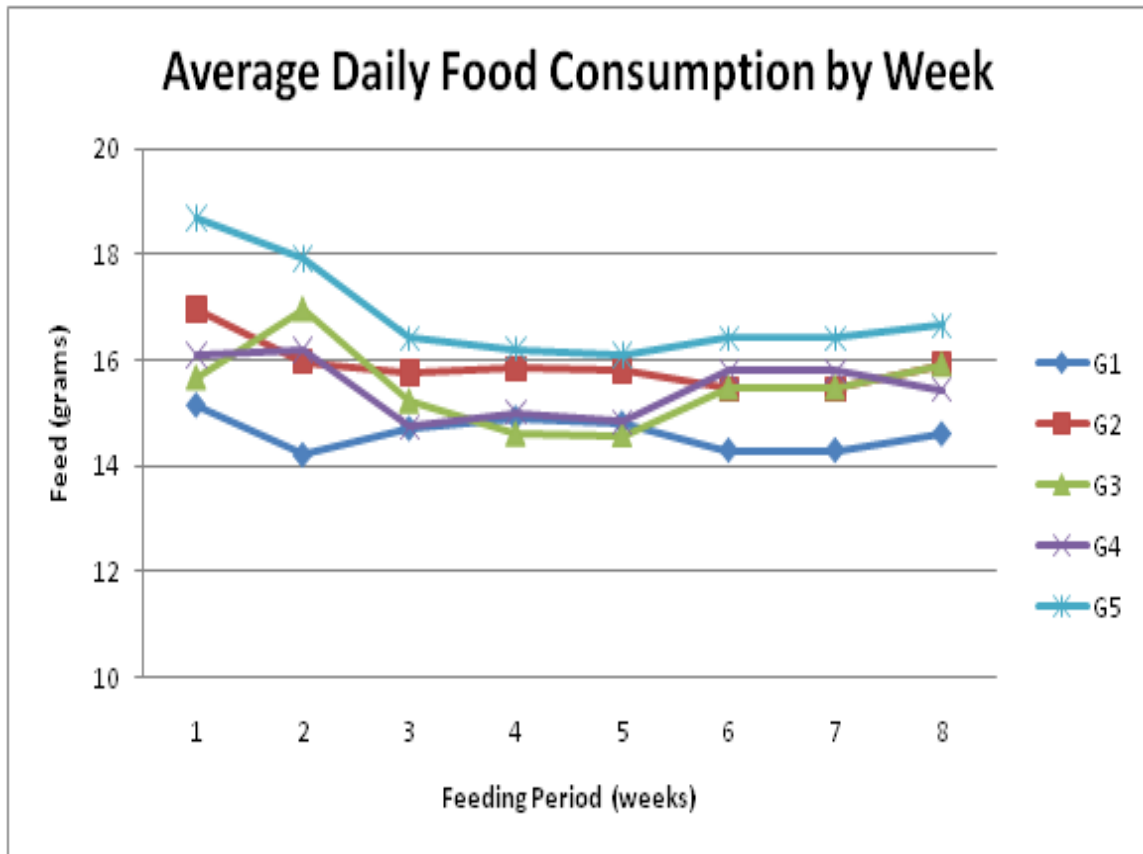


Figure 4.1. The Effects of peanut skin polyphenol on feed consumption

When viewing Figure 4.1 note that G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5-control + 1% cholesterol + 10.0% peanut skin.

4.2.1 Weight Changes of Rats During Feeding Period. As can be seen in Figure 4.2, G1 has the lowest average weight. It does appear that G2 and G5 have the highest body weights. Referencing back to Figure 4.1, G1 has the lowest average food consumption; G2 and G5 have the highest average food consumption rates. The food consumption rates can be a contributing factor in the noticeable weight gain of the rats in G2 and G5. When viewing Figure 4.2 note that G1-Control; G2-Cholesterol;

G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5 control + 1% cholesterol + 10.0% peanut skin.

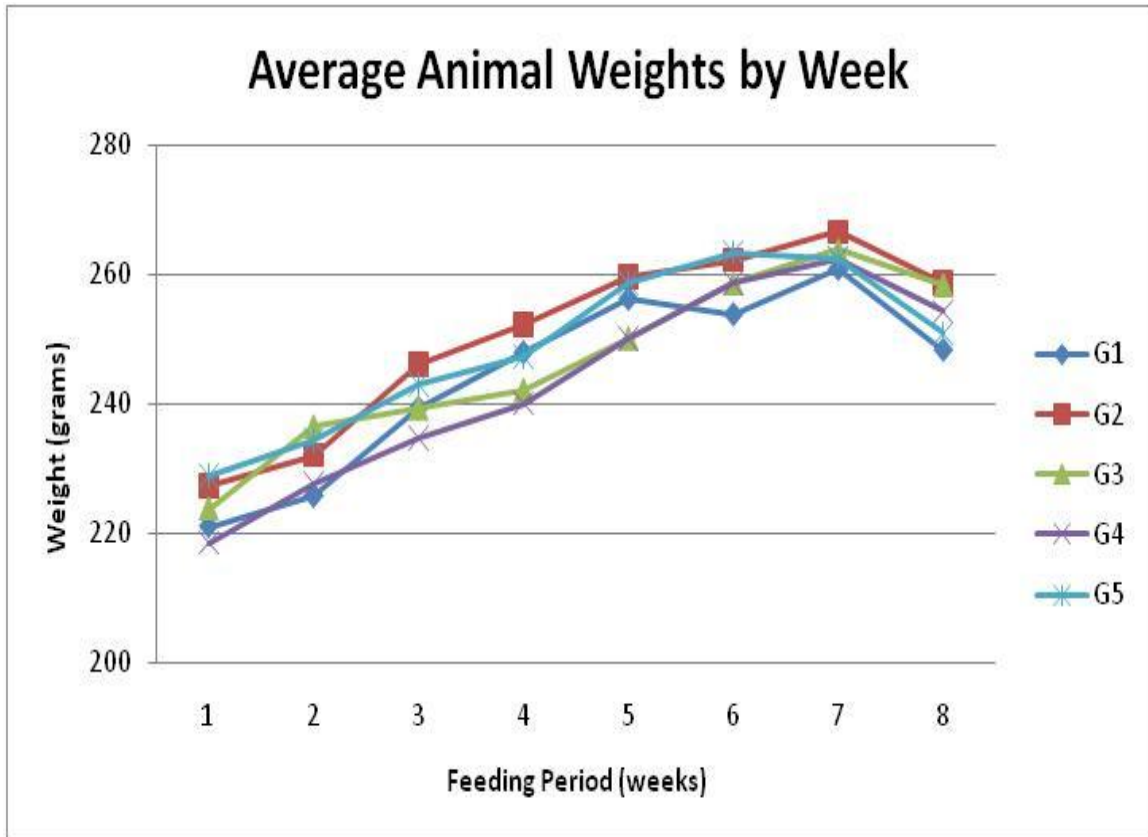


Figure 4.2. Effects of different diets on the average weights of rats during the 8 week feeding period

The presence of percent cholesterol in the diet could be an attributable factor for the large body weight of rats in the treatment Groups (G2 to G5) and the addition of peanut skin slightly leveled off the weight gain of rats (G3 to G4). The largest average weight gain was observed in group 5, which was corresponded to the highest feed consumption (see Table 4.3). Therefore, it is possible that the addition of peanut skin in the diet may be able to control weight gain caused by the addition of cholesterol to the

diet. Under the experiment condition, adding up to 10% of peanut skin to the diet did assist in suppressing the weight gain of the rats.

Table 4.3

Weight Gain from Week 0 through Week 8

Groups	W1- W0	W2- W0	W3- W0	W4- W0	W5- W0	W6- W0	W7- W0	W8- W0	Average Weight (g)
G1	5.24	9.94	23.56	32.16	40.48	38.08	45.14	32.58	28.40
G2	11.26	22.3	36.22	42.38	49.88	52.44	56.76	48.92	40.02
G3	12.82	25.52	28.38	31.2	39.2	47.76	52.8	47.48	35.65
G4	12.32	21.46	28.46	33.82	43.84	52.5	56.22	48	37.08
G5	22.82	28.46	36.96	41.36	52.64	57.38	56.48	44.82	42.62

Note. G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4-control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin

4.2.2 Blood Chemistry Profile. The blood chemistry tests performed did not show any abnormal results for the parameters analyzed. All of the test results were within normal ranges for Alanine Transaminase (ALT), Blood Urea Nitrogen (BUN), Creatinine (CRE), Amylase (AMY), total protein (TP) and Glucose (GLU), as seen in Figures 4.3, 4.4, 4.5, 4.6, 4.7, and 4.8 respectively. ALT is found in blood serum and in several body tissues including liver, lung, pancreas, kidney, heart, and muscles; but, is most commonly found with the liver. Please note the following while reviewing Figure 4.3, G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5 control + 1% cholesterol + 10.0% peanut skin.

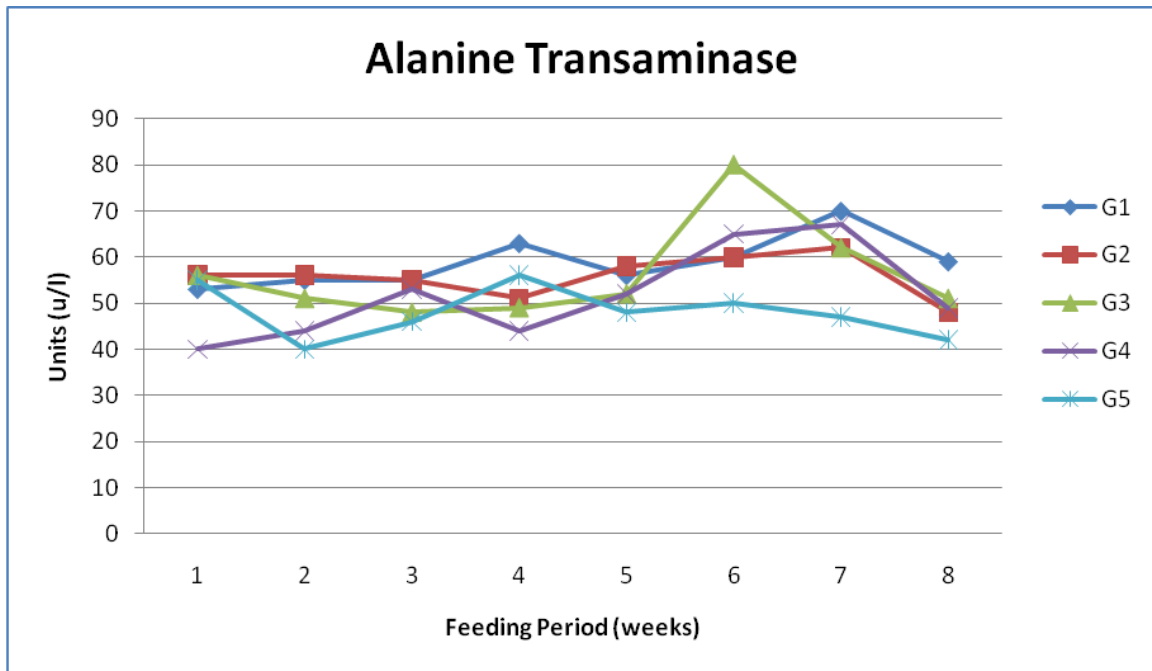


Figure 4.3. Effect of peanut skin contents in the diet on Alanine Transaminase

ALT is currently one of the most reliable markers of hepatocellular injury, disease or necrosis. Additionally, ALT is used as a bio-marker for many different disorders such as fatty liver (Bose, Alvarenga, Tejero, Voruganti, Proffitt, Freeland-Graves, et al 2009). The increase of ALT may be an indication of liver damage or disease. Figure 4.3 shows that peanut skin containing diets did not change the ALT over the study period compared to the control group G1. We can conclude that during the period of this study (eight weeks), ALT values were not high enough to indicate liver damage or necrosis.

BUN measures the amount of nitrogen in the blood and is an efficient way to measure renal function. Elevated BUN may be due to kidney disease as well as congenital heart failure. Low BUN may be due to liver failure or malnutrition/over hydration (Gennari, 2001). According to the BUN value of rats in control group 1 (G1),

the normal BUN range for rats should be 14.3-19.9 mg/dl, with the exception of a few data points (see Figure 4.4).

Figure 4.4 indicated that the peanut skin powder could have an effect on the BUN levels. Over time, the groups with the addition of peanut skin had lower levels of BUN. Again, the levels of BUN could be attributed to the high food consumption during the feeding period as seen in Table 4.4. There was not an increase in protein in the diet or decrease in fluid intake to explain the inconsistencies in BUN measures. Perhaps the pooled blood might explain these changes in BUN. Note that G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

Creatinine is created from the chemical waste generated from the metabolism of muscle in the body. Creatinine is also measured with BUN to identify kidney dysfunction. High levels of creatinine can be an indicator of kidney dysfunction (Beard et al., 1957). Creatinine levels rise when the kidneys are not working properly. The normal range of Creatinine is 0.3-0.5 (mg/dl). Figure 4.5 shows the creatinine levels of groups over the study period. The finding from this analysis indicates that the creatinine levels could have been measured inaccurately, which could explain the unpredictable trend in the data points. The incorrect measurement could be either computer or human error. Note that G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0%) peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

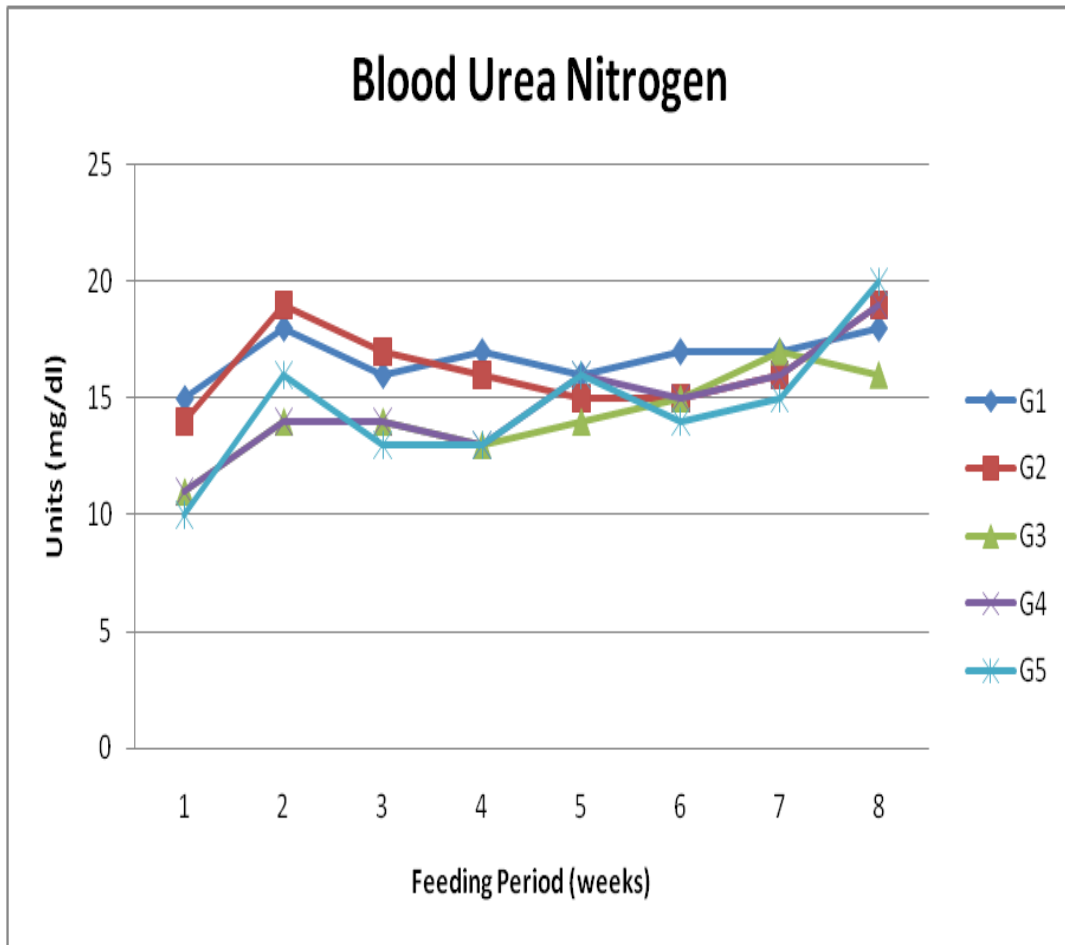


Figure 4.4. Effect of peanut skin contents in the diet on Blood Urea Nitrogen

Glucose is derived from the carbohydrates in foods and is the main source of energy used by the body. The normal blood glucose levels in rat is 76- 147 (mg/dl). Figure 4.6 shows the blood glucose levels of rats from the different treatment groups taken during the study period. Blood glucose levels of rats in the treatment groups G3, G4 and G5 were not different from that in control groups G1 and G2. Therefore, inclusion of peanut skin in the rat diet up to 10% does not appear to cause a negative effect on rat blood glucose levels and presumably does not impact kidney function. Note

that G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0%) peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

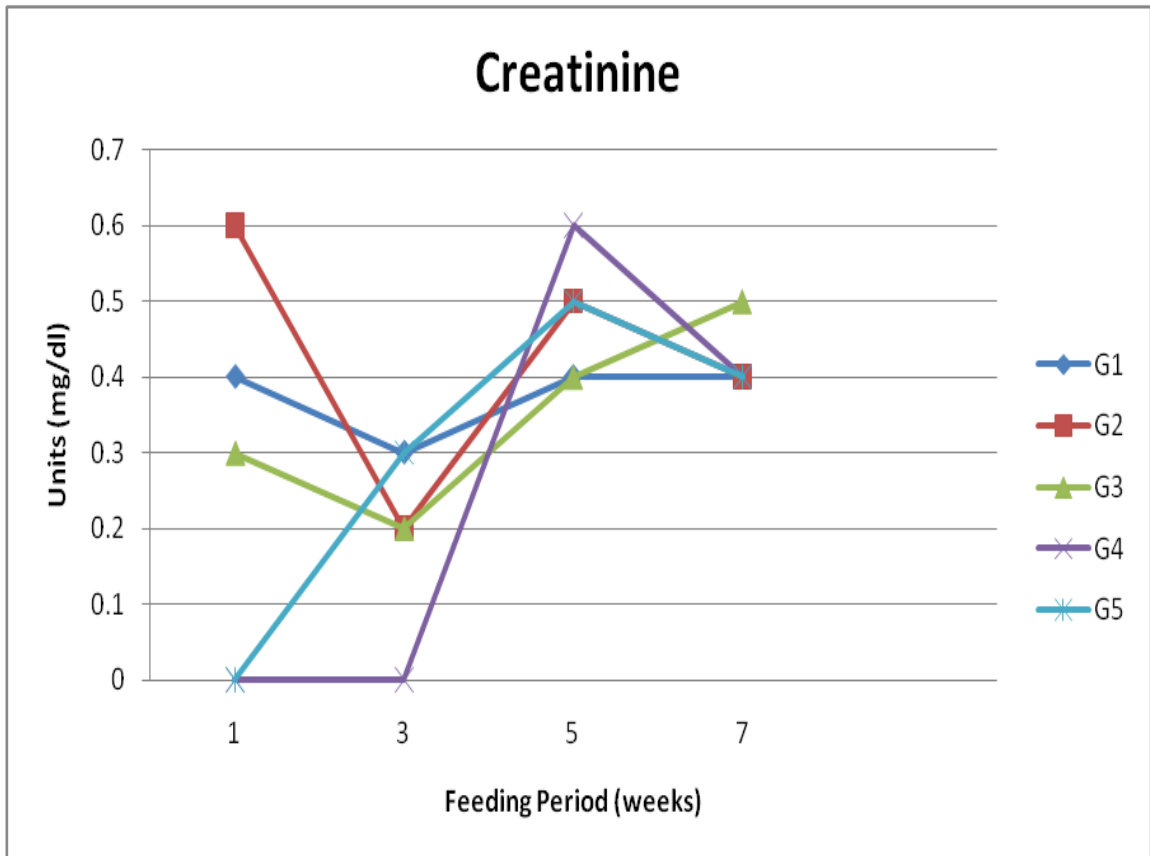


Figure 4.5. Effect of peanut skin contents in the diet on Creatinine

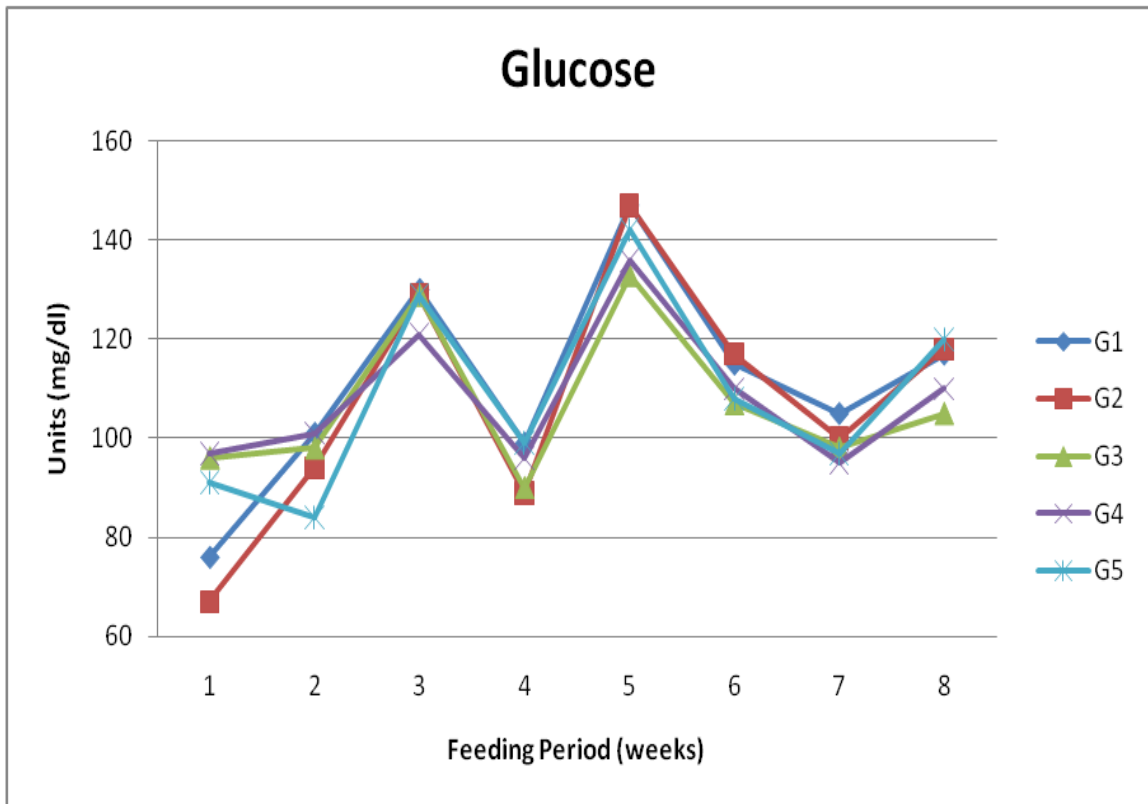


Figure 4.6. Effect of peanut skin contents in the diet on the blood glucose of rats

Amylase is an enzyme that aids in the digestion of carbohydrates (Owyang, 2007). It is produced in the pancreas and the salivary glands. Amylase production can be a biomarker for diseases of the pancreas. The normal range for rats is 720-817 (u/l).

Figure 4.7 shows the amylase levels of rats from the different treatment groups over the study period. High cholesterol diet resulted in higher blood amylase (G2) and the inclusion of peanut skin in the high cholesterol diet did not show consistent effect on the blood amylase level. For example, amylase in the G1 and G3 were consistently lower than G2. However, G4 and G5 were not. Please note that G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

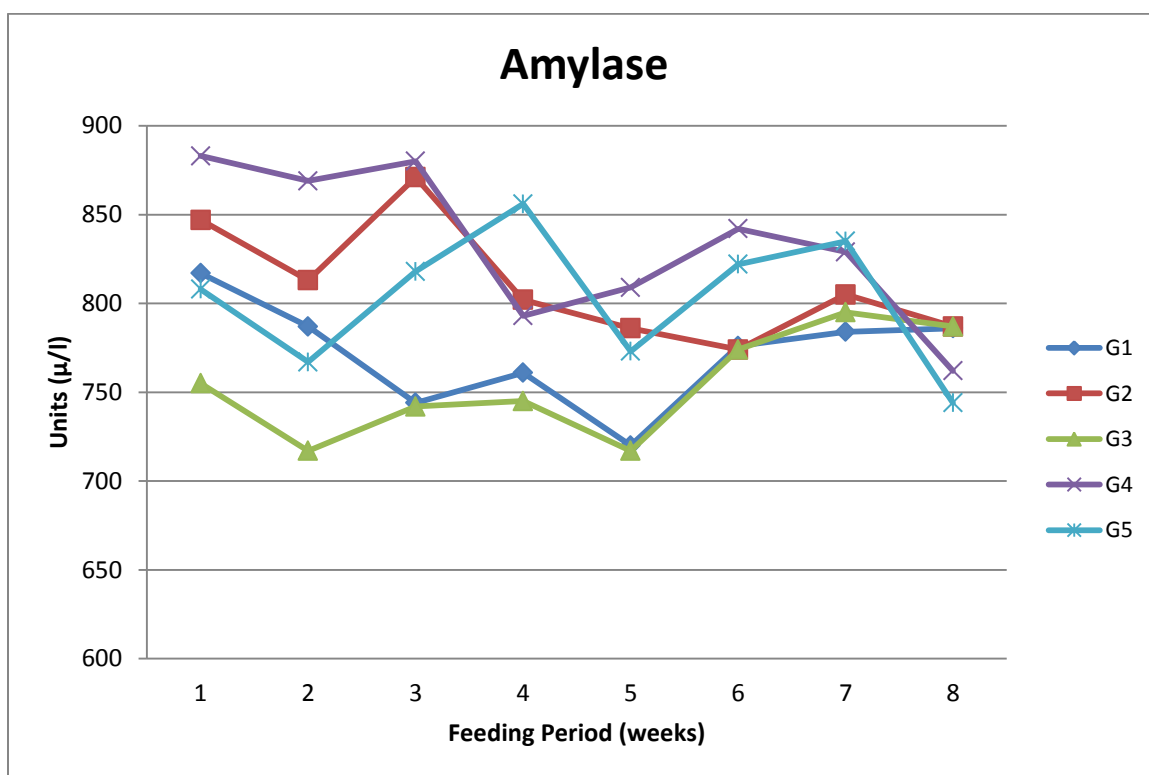


Figure 4.7. Effect of peanut skin contents in the diet on amylase of rats

Total blood protein is used as a biomarker for liver dysfunction and nutritional problems (Bazari, 2007). Figure 4.8 shows that the normal range for rats is 7-7.7 (g/dl). Consumption of high cholesterol diet (G2) appeared to elevate total protein, but inclusion of peanut skin in the high cholesterol diet showed a lower total blood protein with exception of G4. The reader should note that G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

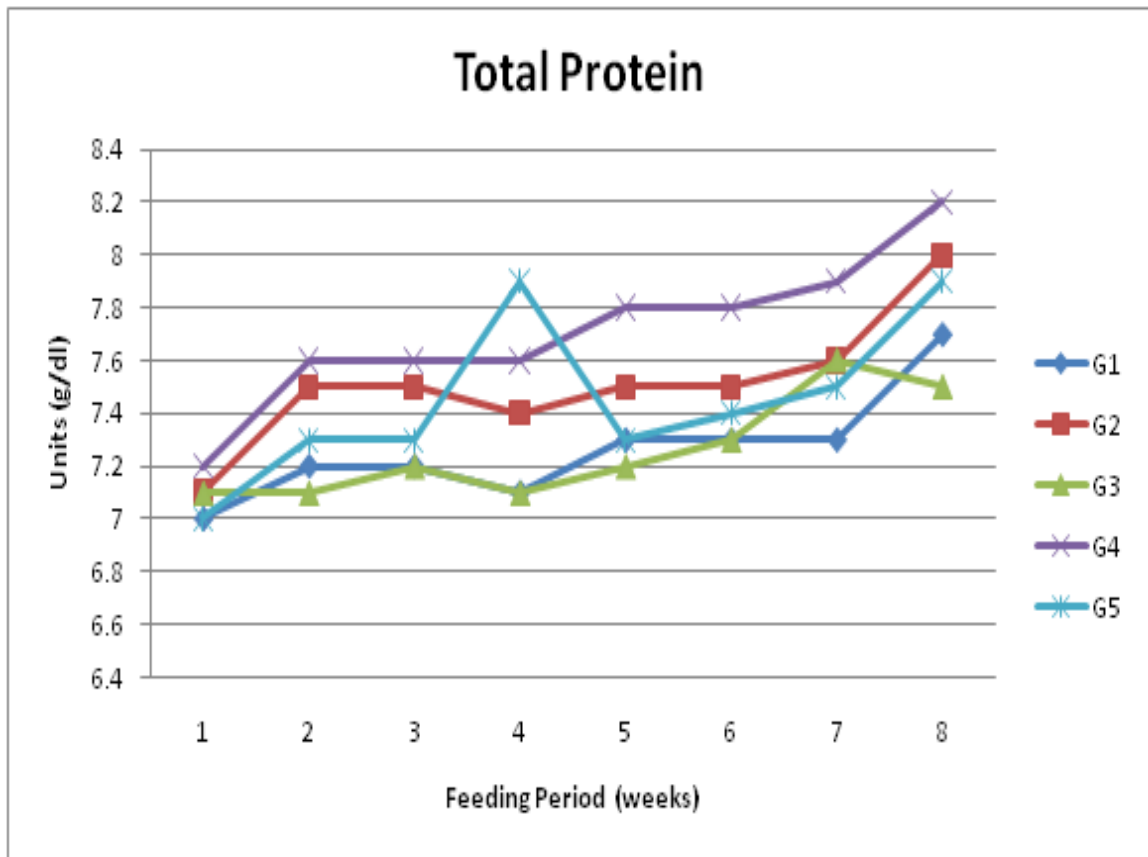


Figure 4.8. Effect of peanut skin contents in the diet on total protein of rats

The reason that rats in group four had higher total protein in the blood is unknown. Because total protein (TP) analysis was done using pooled blood samples, if one rat had very high blood TP, the results of the whole group would be affected. Therefore, a better design study is needed to confirm the result presented here.

4.2.3 Blood Lipid Profile. The items analyzed were total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (mg/ml), the ratio of (TC/HDL) and very low density lipoprotein (VLDL). The cholesterol profiles are very important because they are risk indicators of certain cardiovascular diseases such as stroke and heart disease. Total cholesterol is tested as a preventative measure against

cardiovascular disease. The more elevated the TC levels are the higher the risk for plaque to build up on the artery walls and to cause health problems with the cardiovascular system such as stroke and heart disease (Welson, 2006). The normal range of TC in rat blood is 81.7-123.9. The results from the study are within normal limits.

Figure 4.9 shows the TC levels of the different groups of rats over the study period. From the results of Figure 4.9, it appears that there were no differences among the groups. Not surprisingly, G1 which did not include extra cholesterol in the diet had the lowest levels of serum cholesterol. Compared with rats from G2, rats in the peanut skin containing diets had lower total cholesterol levels, particularly at later feeding stages, except those in G4. It would have been expected that the rats would have a decrease in their serum cholesterol based on the increasing amount of peanut skin added. Note that (G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4-control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

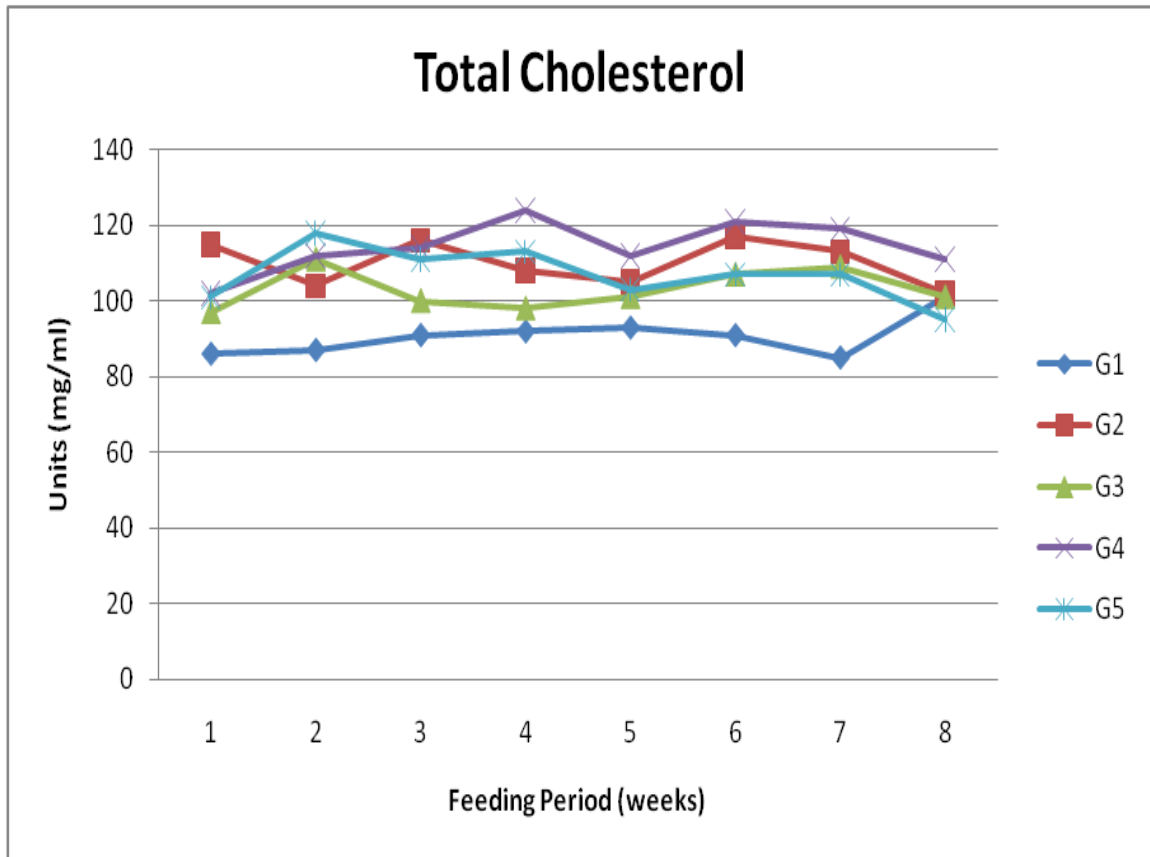


Figure 4.9. Effect of diet with peanut skin polyphenol on Total Cholesterol level of rat sera

The groups with the highest amount of peanut skin added did not consistently have the lower cholesterol levels. Additionally, the blood cholesterol levels could be attributed to the amount of food consumed as well; Figure 4.1 displays that G5 had the highest food consumption among the groups with the added cholesterol and peanut skin powder during the trial period. The total blood cholesterol of rats in G5 was high at an early stage of feeding but decrease significantly at later stage of feeding. These results indicate that long term feeding of peanut skin could offset the cholesterol level caused by high cholesterol diets.

The main function of HDL is to assist with the removal of excess cholesterol from within walls of the blood vessels and then carry it to the liver, where it is then broken down and removed from the body in the bile. Low levels of HDL can be an indicator of an increased risk for cardiovascular disease (Catapano, Bernini, and Corsini, 1993). According to the HDL of G1 (see Figure 4.10) the normal range of HDL for rats is 59.7-70.7(mg/ml). Figure 4.10 shows that feeding high cholesterol diet resulted in significant decrease of HDL in experimental rats (G2). HDL level increased gradually in the treatment groups fed with 5 and 10% peanut skin. Therefore, we can assume if the rat with low blood HDL stop consuming high cholesterol diet but continued peanut skin diet, the HDL level will increase. When viewing Figure 4.10 note that G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

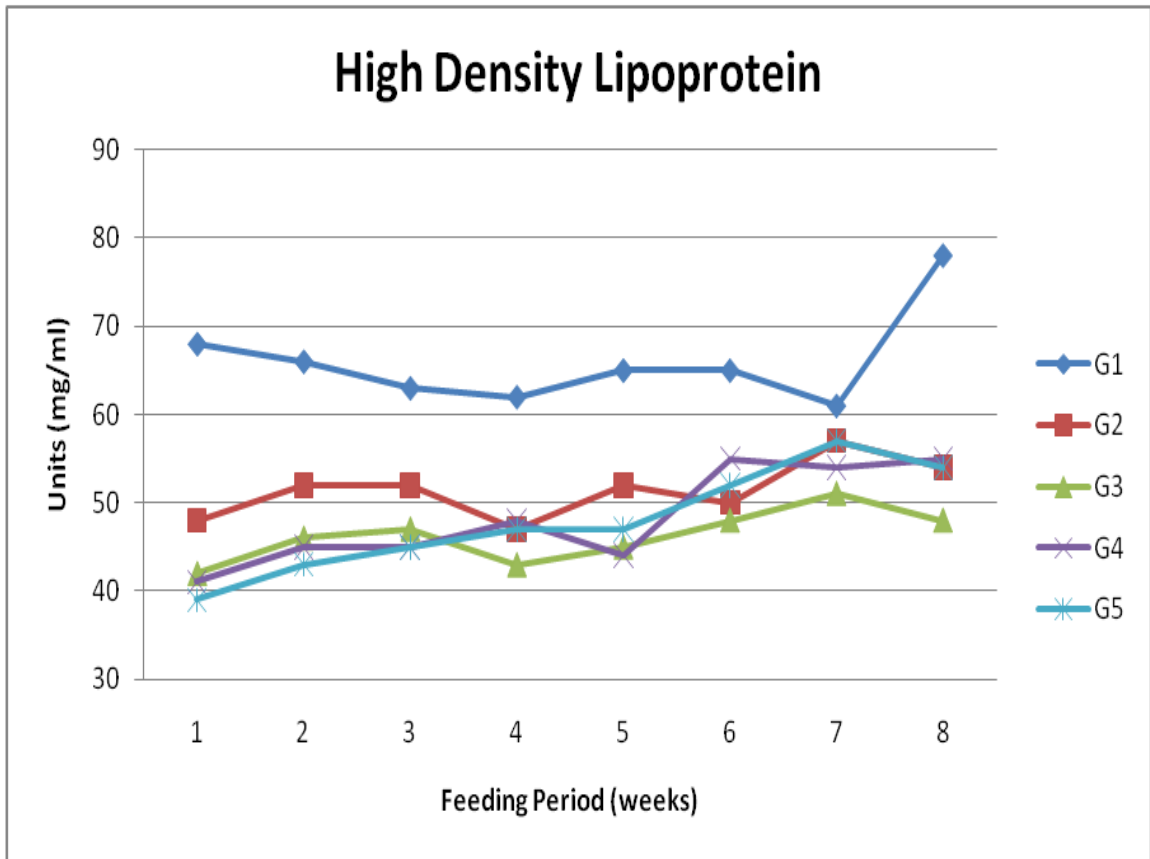


Figure 4.10. High Density Lipoprotein of rats in different treatment groups

Triglycerides are the chemical form of fat exists in food as well as in the body. Triglycerides are also present in the blood plasma and are also associated with blood cholesterol in the form of plasma lipids. Calories that are ingested during a meal and not immediately used by the body tissues are converted to triglycerides and then transported to fat cells to be stored. Triglycerides in blood are often tested as a preventative measure against cardiovascular disease. Having high triglyceride levels can cause an increased risk for cardiovascular disease however there are no apparent consequences for low triglyceride levels (Welson, 2006). Figure 4.11 shows the Triglyceride results from the different groups over the study period. Based on the controls the normal range of

triglycerides in rat blood is 52.4- 92.7(mg/dL). The results show that adding 1% cholesterol to the diet significantly increased the blood triglyceride levels of rats in G2. The reader should note that G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

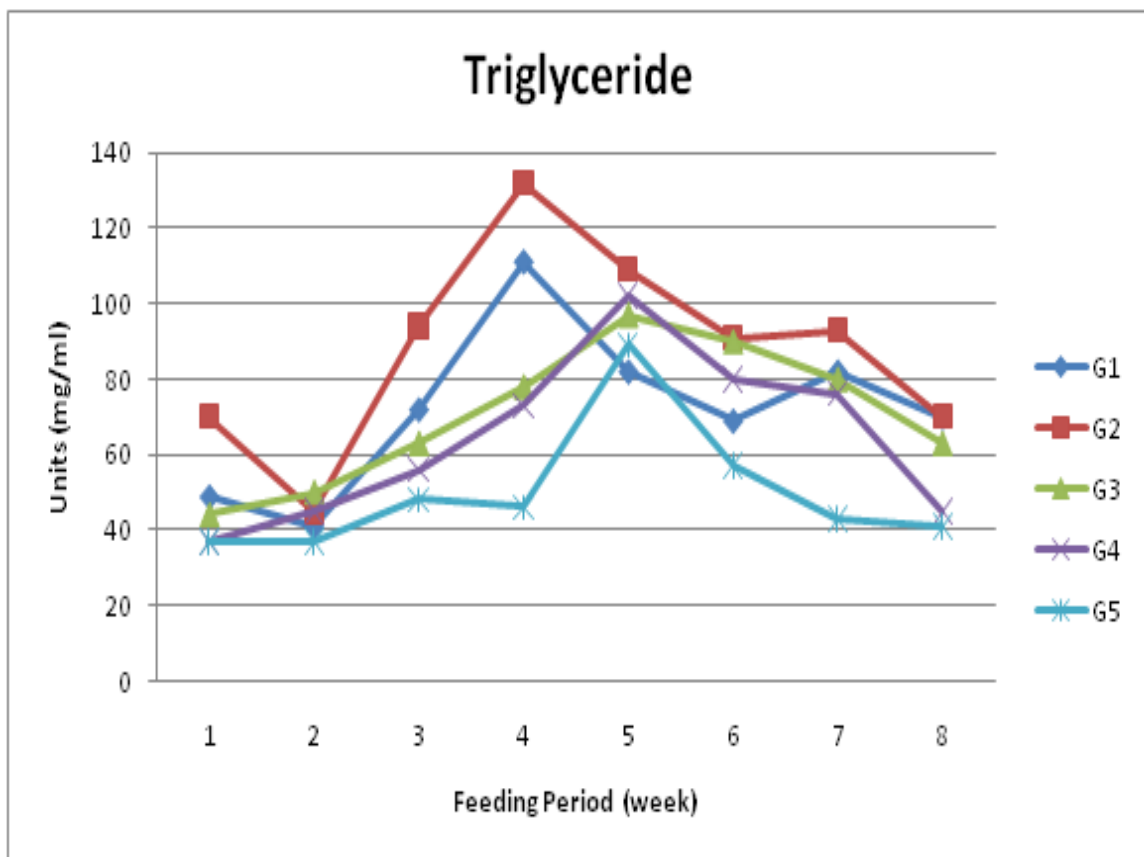


Figure 4.11. Triglyceride levels of rats in different treatment groups

However, the addition of peanut skin did have a positive effect on the triglyceride levels. The triglyceride levels of rats in all treatment groups and control group reached their highest level between feeding week four and week five, and then exhibited a

declining trend. All rats fed peanut skin containing diets had lower blood triglyceride levels. Rats in G5 had the lowest levels of blood triglycerides corresponding to the highest level of peanut skin powder added. G2 that had the additional cholesterol and no peanut skin had the highest levels of triglycerides, with the control close behind. Therefore, peanut skin effectively reduced the serum triglyceride level of rats fed a cholesterol containing diet. Adding peanut skin in rat diet may significantly reduce blood triglyceride in human which may reduce the risk for cardiovascular disease.

Figure 4.12 shows the ratio of Total TC/HDL of rats in different groups during the study. No significant difference in TC/H was observed among the treated groups, with the exception of G1. The results among the groups with the peanut skin did not show that the addition of peanut skin made a difference in TC/HDL ratio. Although the addition of peanut skin in the diet resulted in relatively low HDL levels as shown in Figure 4.10, the lower total cholesterol levels in rats fed with diet containing larger amounts of peanut skin polyphenol (see Figure 4.9) offset the TC/H ratios of rats in peanut skin fed groups. Adding 1% cholesterol to the diet did increase the TC/HDL of G2 due to the increase of TC and decrease of HDL. The addition of peanut skin in the diets did not affect the TC/HDL until week four. After week four, TC/HDL began to decrease corresponding due to the decrease of TC (see Figure 4.7) and the increase of HDL (see Figure 4.8). The reader should note the following for Figure 4.12 G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1% cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

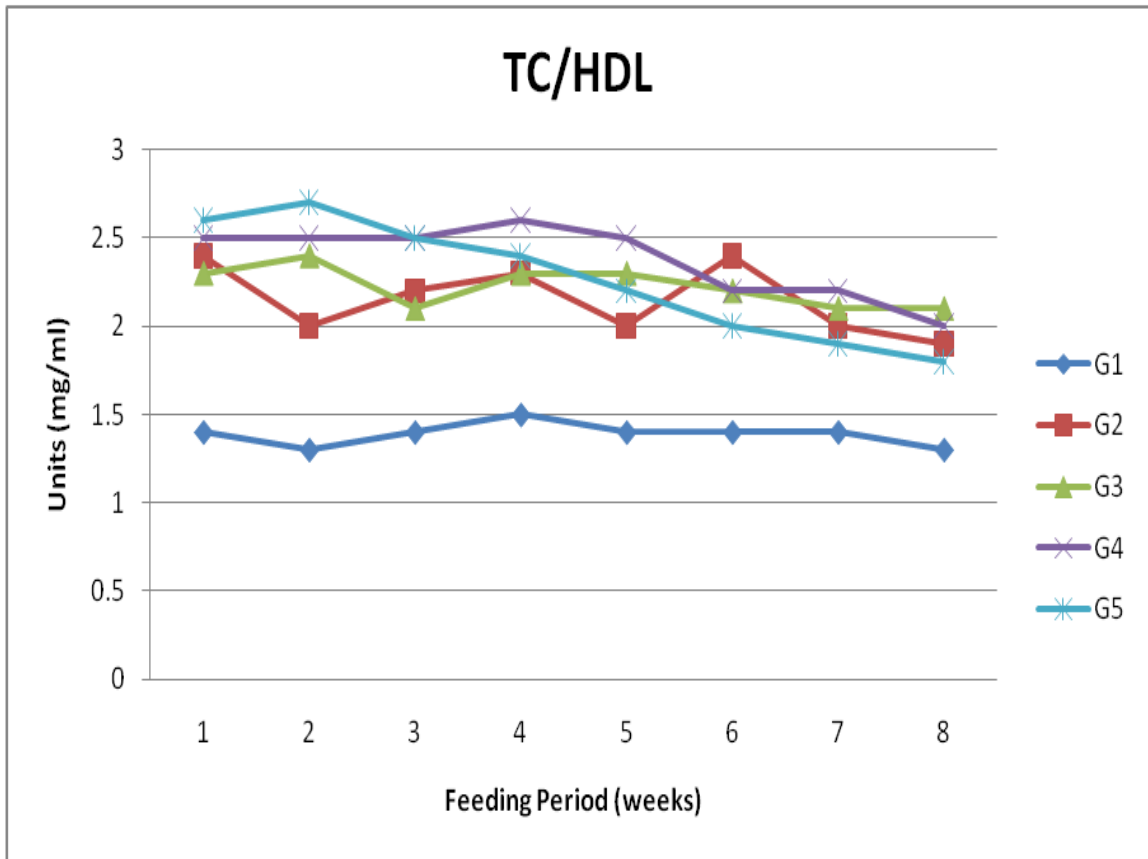


Figure 4.12. TC/HDL of rats in different treatment groups

LDL is measured to aid in the determination of the risk for cardiovascular disease. When LDL levels are considered to be too elevated, the risk for cardiovascular disease is increased; when the LDL levels are lowered the risks for cardiovascular diseases are decreased (Myant, 1990). The normal range for LDL in healthy rats is 8.8-14.9(mg/dL) based on the data from the control group. The blood LDL levels of treatment groups were out of the normal range. Figure 4.13 shows changes of serum LDL in the different groups during the eight week experimental period. Note that G1-Control; G2-Cholesterol; G3-

control +1% cholesterol + 2.5% peanut skin; G4- control + 1% cholesterol + 5.0% peanut skin; G5- control cholesterol + 10.0% peanut skin.

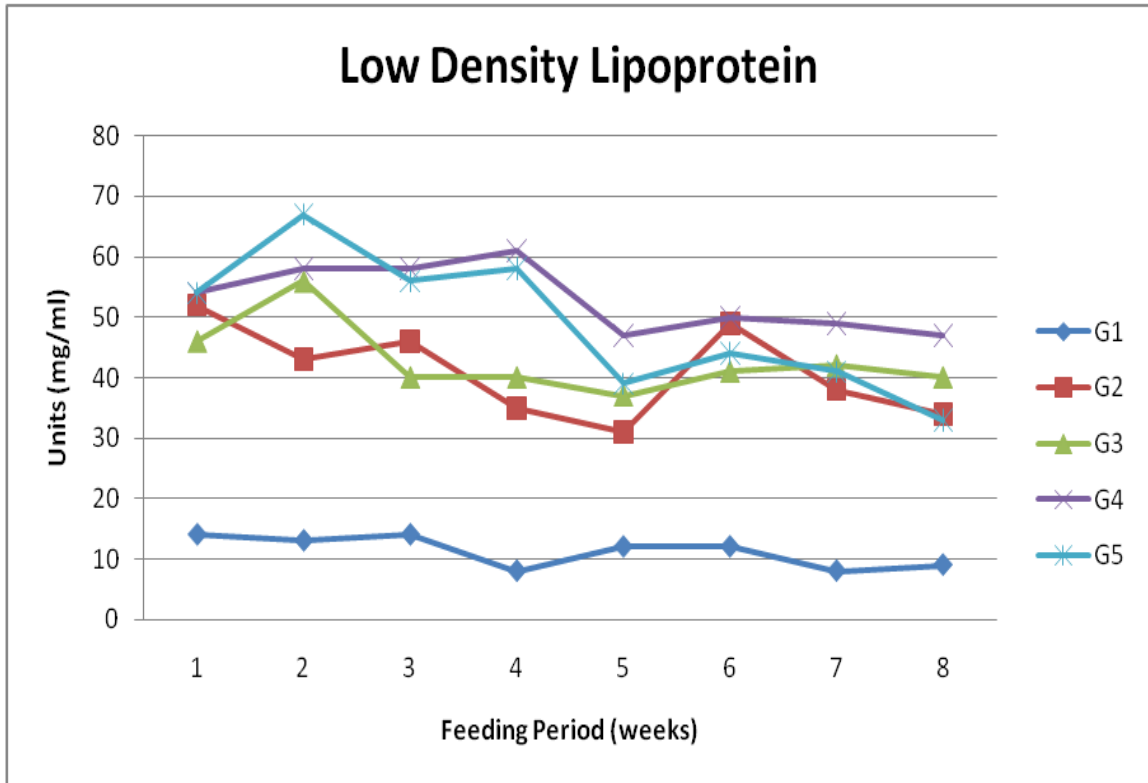


Figure 4.13. Low Density Lipoprotein of rats in different treatment groups

Data show that rats fed with cholesterol containing diet had significantly higher LDL levels in their blood sera than rats in the control group. Higher peanut skin inclusion appeared to increase blood LDL of rats during the first four weeks of feeding; this might be due to the higher consumption of feed and cholesterol shown in Table 4.1. When the content of peanut skin in the diet was low (2.5% G3), no difference in LDL between rats in G2 and G3 was observed. However, from week five, LDL of rats in treatment Groups G3, G4 and G5 began to decrease. Therefore, for peanut skin to exhibit its health benefits

due to higher fiber and polyphenol content, it takes at least four to five weeks of continuous feeding.

Figure 4.14 shows the blood VLDL levels of rats from the different groups over the experimental period. Data show some distinct differences amongst the groups. It appears that the addition of peanut skin may have a positive effect on the VLDL levels. G5 with the highest level of added peanut skin powder had the lowest levels of VLDL. G2 that had the additional cholesterol but no peanut skin had the highest levels of VLDL, with the control closely behind. Recent studies (Sarwar, Aspelund, Eiriksdottir, Gobin, Seshasai, Forouhi et al 2010; Mora, Otvos, Rifai, Rosenson, Buring, and Ridker, 2009) have found that VLDL played an important role in the development of cardiovascular diseases. Therefore, the VLDL cholesterol lowering effect of peanut skin is important in the reduction the risk of cardiovascular diseases risks. Note the following while reviewing Figure 4.14, G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

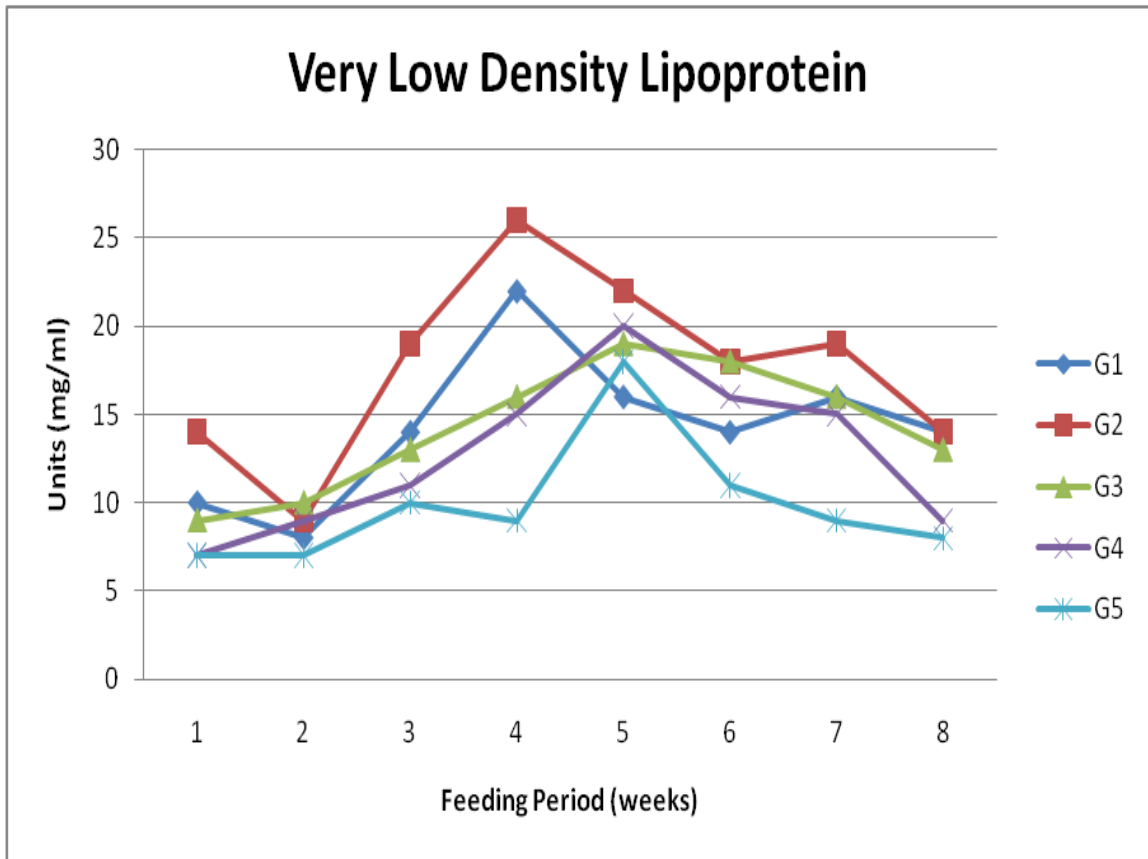


Figure 4.14. Very Low Density Lipoprotein of rats in different treatment groups

4.2.4 Animal Organ Weights. The organs that were examined at necropsy were the spleen, heart, kidneys, brain, adrenal glands, lungs, and liver. Figure 4.15 shows that the average organ weight of rats in group 2 (G2) was higher than that of rats in group G1. Adding 5-10% of peanut skin in diets (G4 and G5) suppressed organ enlargement caused by the presence of cholesterol. For example, the average liver weights of rats in G4 and G5 were 5.3% and 10.6% lighter in weight than that of rats in G2. The kidney weights of G4 and G5 was 7.9 and 3.4% lighter in weight than that of G2, respectively. The spleen weights of G4 and G5 were 12.8 and 10.5% lighter than that of G2. Therefore, long term consumption of high peanut skin diet may have beneficial effects on the prevention of

fatty organs or organ enlargement. The percent weight change of each type of organ was calculated as follows:

$$\text{Percent change} = (\text{organ weight of G3 or G4 or G5} - \text{organ weight of G2}) * 100 / \text{organ weight of G2}$$

The reader should note the following while viewing Figure 4.15 G1-Control; G2-Cholesterol; G3- control +1% cholesterol + 2.5% peanut skin; G4- control + 1%cholesterol + 5.0% peanut skin; G5- control + 1% cholesterol + 10.0% peanut skin.

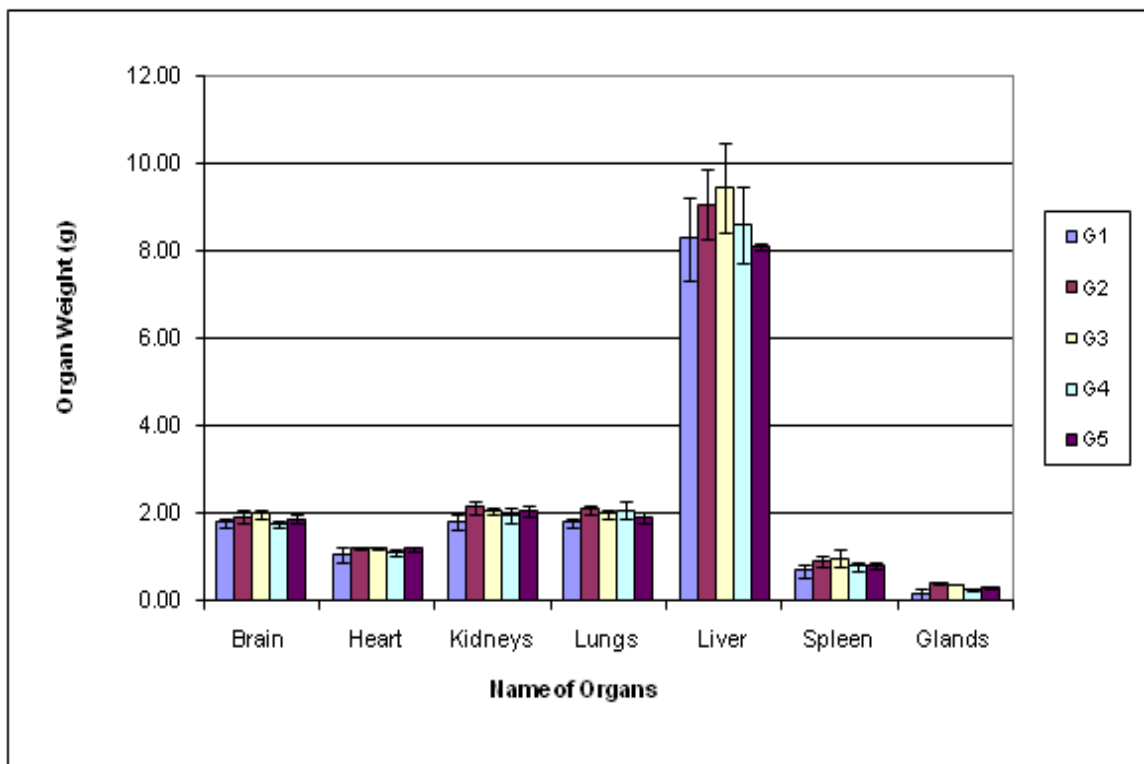


Figure 4.15. The effect of peanut skin diet on average organ weights at necropsy

CHAPTER 5

CONCLUSION

This study found that feeding female rats with peanut skin containing diet: (1) did not inhibit the growth of the rats; (2) reduced serum triglyceride; (3) reduced total cholesterol; (4) reduced VLDL cholesterol; and (5) suppressed organ enlargement caused by the high cholesterol diet. Adding peanut skin in the diet also appeared to improve the functions of liver, kidney, and pancreas as reflected by the better blood ALT, BUN, amylase and protein levels of rats in peanut skin diets. However, the study also found that inclusion of peanut skin in the rat diet resulted in higher LDL and lower HDL levels in the blood samples taken in the first four to five weeks of feeding period. This is controversial with the majority of published studies since consumption of polyphenol rich foods tends to reduce blood LDL and increase HDL. The age of rats used in this study might be a contributing factor of such controversy. In addition, because all blood chemistry and lipid profile analysis was done using pooled blood samples of the rats in the same treatment group due to the lack of experimental resources, it hard to tell if some of the data were outliers. Therefore, more studies are needed to confirm these important findings. Further, studies are also needed to evaluate the lipid lowering effect of peanut skins as affected by gender and age of rats.

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