

1.0 INTRODUCTION

Dietary manipulation through the consumption of specific plant materials containing phytochemicals such as phytosterols and tannins been proven to be effective treatment methods for the regulation of glucose and lipids in the blood (Tang et al., 1998). Studies have shown that plants containing these sterols such as the aloe-vera can be used to reduce visceral fat accumulation, and improve hyperlipidemia and hyperglycemia in rat (Eriko et al., 2008). *In-vitro* studies have also shown that phytosterols (PS), dissolved in diacylglycerol (DAG) oil (PS/DAG) was very effective at a relatively low dose to lower the blood cholesterol and lipoprotein concentrations for hypercholesterolemia patients with a low response to pravastatin (Masao et al., 2008).

Phytosterols, distinguishable by corresponding variations of the side chains or additional methyl groups (Ramadan and Morsel, 2003), are found in food of plant origin especially seed and oils in concentration up to 5%. Phytosterols are well known for their cholesterol lowering and anti carcinogenic effect as evident in animal and human studies (Engel and Schubert, 2005). They lower not only the total serum cholesterol concentration but also the ratio of concentration of low density lipoprotein to high density lipoprotein bound cholesterol in the serum (Pouteau et al., 2003).

Phytosterols compete with the cholesterol for absorption into mixed bile salt and acids micelles, the vehicle for absorption of lipophilic compounds in the intestine (Piironen et al., 2000). In addition, the higher hydrophobicity of phytosterols is more readily absorbed into the micelles than cholesterol. The greater parts of dietary cholesterol unavailable for re-absorption are thus excreted with the faeces.

The anti-hyperglycaemic properties of the phytosterols lower the blood glucose purportedly by causing a strong inhibitory action against intestinal absorption of glucose in the body (Beppu et al., 2006), but the mode of their action is not fully understood.

Another plant substance that could be of importance in the dietary manipulation is the tannins. They do not reduce high lipid or glucose in the body but they tend to improve on feeding and growth performance in animals. Tannins at low concentration are known to improve feed and protein efficiency in animals (Garry, 2008). This occurred by the reduction of internal gastrointestinal parasitism and protein degradability. In the ruminants tannins also bind to the feed protein which effectively reduce the excessive break down of protein in the stomach (Getachew et al., 2000a) and increase the availability of high quality proteins for absorption in the lower guts (Gatechew et al., 2007). Tannins also alter fermentation of carbohydrates and protein (Barry and Ducan, 1984), microbial protein synthesis (Makkar et al., 1995) and improves protein digestibility at low concentration (Bressani et al., 1983).

Tamarind and avocado seed contain phytosterols such as the beta-sitosterol, campesterol and stigmaterol (Engel and Schubert, 2005; Karen, 2001), but phytosterols in tamarind seed (e.g. 590mg/kg; Gebhardt and Thomas, 2002) is lower than that of avocado seed (e.g. 826mg/kg; Leroux et al., 2002). Avocado seed also contains fatty acids with olefinic, acetylenic bonds, furanoic acids, dimers of flavanol (Maseko et al., 2006) and oligomeric proanthocyanidins (Elkin and Lorenz, 2009) which could account for other nutritional importance. Tamarind seed contain higher tannins (e.g. 101.89g/kg; Gu et al., 2003) than the avocado seed (e.g. 2.45g/kg; Agostini, 1995).

In the present study the effects of avocado or tamarind seed were evaluated on growth performance, blood glucose and cholesterol levels, and glycogen in liver of rats in three different experiments. The objectives of this study was to determine if these seeds can modulate these parameters that are related to hypertension and diabetics and at the same time improve feeding and growth performance in rats. To achieve this, the seeds were introduced in three rat's models; normal rats, hypertensive rats and rats exposed to a hyperglycaemic condition (rats were fed on high sucrose diet (HSD)).

2.0 LITERATURE REVIEW

2.1. TAMARIND

Tamarind (*Tamarindus indica* L.) is a plant in the family of Leguminosae under the genus *Tamarindus*. Common names of tamarind are tamarind, tamarindo, tamarin, and sampalok. Tamarind is native to tropical Africa but is extensively cultivated in India, North America and many tropical areas of the world (Khandare, 2000).

2.1.1 Cultivation

Adaptation: Tamarind is well adapted to semi-arid tropical conditions, although it does well in many humid tropical areas of the world with seasonal high rainfall. Tamarinds can grow 80 feet high with a spread of 20 to 35 ft (Morton, 1987). The leaves are normally evergreen but may be shed briefly in very dry areas during the hot season (Doughari, 2006). The five-petal tamarind flowers are borne in small racemes and are yellow with orange or red streaks. The flower buds are pink due to the outer colour of the 4 sepals which are shed when the flower opens (Tamale et al., 1995). Tamarind fruits are 3 - 8 inch long, brown, irregularly curved as pods and are borne in abundance along the new branches. As the pods mature, they fill out and the juicy acidulous pulp turns brown or reddish-brown. When fully ripened, the shells are brittle and easily broken.

The pulp dehydrates to a sticky paste enclosed by a few coarse stands of fibre. The pods may contain from 1 to 12 large, flat, glossy brown, obviate seeds embedded in the brown, edible pulp. The pulp has a pleasing sweet/sour flavour and is high in both acid and sugar. It is also rich in vitamin B and high in calcium (Morton, 1987).

Tamarinds grows best in well drained soils which are slightly acid but do not tolerate cold wet soils (Doughari, 2006). The young tamarind trees require adequate soil moisture until they become established, but the mature ones do quite well without supplemental irrigation (Tamale et al., 1995). The tamarind is not very demanding in its nutritional requirements. Young trees are fertilized every 2 - 3 months with a 6-6-3 NPK or similar analysis fertilizer. Microelements, particularly iron may be required for trees in alkaline soils (Morton, 1987).

Tamarind fruits mature in late spring to early summer. They may be left on the tree for as long as 6 months after maturity so that the moisture content will be reduced to 20% or lower. Fruits for immediate processing are often harvested by pulling the pod away from the stalk. Mature trees are capable of producing 350 lb. of fruit a year. Ripe fruit in humid climates is readily attacked by beetles and fungi, so mature fruit should be harvested and stored under refrigeration (Tamale et al., 1995). Tamarinds may be eaten fresh, but they are most commonly used with sugar and water in the American tropics to prepare a cooling drink. The pulp is used to flavour preserves and chutney to make meat sauce. Candy can be made by mixing the pulp with dry sugar and moulding it into desired shapes (Adiza, 2010).

2.1.2. Phytochemistry

The tamarind seed contains crude proteins, crude fibre, crude fat and tannins in various weights 131.3, 67.1, 48.2 and 56.2g/kg-1 respectively with trypsin inhibitor activity of 0.8 where most tannin being located in the testa (Bhatta et al., 1999). It contains fifteen fatty acid mainly palmitic (14–20%), stearic (6–7%), oleic (15–27%), linoleic (36–49%), arachidic (2–4%), behenic (3–5%) and lignoceric (3–8%) acids and phytosterols such as β -sitosterol (66–72%), campesterol (16–19%) and stigmasterol

(11–14%) (Andriamanantena et al., 1983). The seed contains flavonoids such as the anthocyanidins and oligomeric proanthocyanidins (Pumthong, 1999). The seeds also contains phenolic antioxidants such as 2-hydroxyl-30, 40-dihydroxyacetophenone, methyl 3,4-dihydroxy-benzoate, 3-4dihydroxyphenyl acetate and epicatechin (Aengwanich et al.,2009). The organic content is 975.4 g/kg DM, neutral detergent fibre (NDF) 755.2g/kg DM, acid detergent fibre (ADF) 725.4g/kg DM, acid detergent lignin 421.9g/kg DM, ash in ADF 2.1g/kg DM, total phenol content 155g/kg DM (Bhatta et al., 2001).

2.1.3. Medicinal uses

Tamarind fruits are used traditionally as cathartic, astringent, febrifuge, antiseptic and refrigerant purposes (Khandare, 2000) while tamarind seed husk act as a source of tannin to manipulate fermentation or nutrient digestion to the advantage of lactating cows instead of the cost additive efforts in detannifying it (Bhatta et al., 1999). The xyloglucan polysaccharide derived from tamarind seeds are used as a potential gels (formed by *in situ* gelation of the xyloglucan gel) for precutaneous administration of non steroid anti inflammatory drugs (Takahashi et al., 2002) otherwise a vehicle for oral drug delivery (Kawasaki et al., 1999). Recent studies has also revealed that tamarind fruit is a good source of compounds active on complement system (Anapaula et al., 2007) and was also showed that the xyloglucan gel formed from tamarind seed can be used as a sustained vehicles for intraperitoneal administration of Mytomycin C (Suisha et al., 1998). Tamarind intake appears to have additional beneficial effects on the mobilization of deposited fluoride from bone, by enhancing urinary excretion of fluoride (Arjun et al., 2004).

Tamarind is also used as a raw material for the microbial production of citric acid (Hemant et al., 2002). The Malabar tamarind may be effective in the treatment of obesity (Sergio, 2004), but the mechanism is not fully understood. Tamarind has also been used to reduce the calculogenic properties in urine (Anasuya and Sasikala, 2006). The seed extract has been used as a replacement for phosphoric acid citric acid and other acids that are added to soft drink as a result of its high pH and flavor profile equivalent to or better than beverages sweetened with aspartame (Zablocki and Pecore, 1996). The seed extract also exhibits antioxidant potentials by reducing lipid peroxidation invitro and anti-microbial activity (Aengwanich et al., 2009).

2.2. AVOCADO

Avocado belongs to the flowering plant family of *Lauraceae*, genus *Persea* and specie *Persea americana*. It is commonly called avocado, alligator pear (English); aguacate, palta (Spanish). Avocado probably originated in southern Mexico but was cultivated from the Rio Grande to central Peru before the arrival of Europeans (Zamet, 1996).

2.2.1. Cultivation

Avocados do well in the mild-winter areas. Some hardier varieties can be grown in the cooler parts and along the Gulf Coast. Avocados do best some distance from ocean influence but are not adapted to the desert interior (Standley, 2004). Avocado is a dense, evergreen tree, shedding many leaves in early spring. It is fast growing and can with age reach 80 feet, although usually less, and generally branches to form a broad tree. (Crane et al., 2007). Avocado leaves are alternate, glossy, elliptic and dark green with paler veins. They normally remain on the tree for 2 to 3 years (Zamet, 1996).

Avocado flowers appear in January - March before the first seasonal growth, in terminal panicles of 200 - 300 small yellow-green blooms. Each panicle will produce only one to three fruits.

The flowers are perfect, but are either receptive to pollen in the morning or shed pollen the following afternoon (type A), or are receptive to pollen in the afternoon, and shed pollen the following morning (type B). About 5% of flowers are defective in form and sterile. Production is best with cross-pollination between types A and B. The flowers attract bees and hoverflies and pollination usually good except during cool weather. Off-season blooms may appear during the year and often set fruit. Some cultivars bloom and set fruit in alternate years (Lahav and Whiley, 2002). The flesh of avocados is deep green near the skin, becoming yellowish nearer the single large, inedible ovoid seed.

Avocado flesh is hard when harvested but softens to a buttery texture. Wind-caused abrasion can scar the skin, forming cracks which extend into the flesh. Seeds may sprout within an avocado when it is over-matured, causing internal moulds and breakdown. High in monounsaturated fat, the oil content of avocados is second only to olives among fruits, and sometimes greater (Zamet, 1996). Avocado trees flourish in decomposed granite or sandy loam soil. They do not survive in locations with poor drainage. The trees grow well on hillsides and should never be planted in stream beds (Lahav and Whiley, 2002). Over irrigation can induce root which is the most common cause of avocado failure. Avocados tolerate some salts, though they will show leaf tip burn and stunting of leaves. Deep irrigation will leach salt accumulation (Zamet, 1996). Avocado seeds may be needed to be fertilized using a balanced fertilizer, four times yearly. Older trees need nitrogenous fertilizer applied to them. Yellowed leaves (chlorosis) indicate iron deficiency.

This can usually be corrected by a chelated foliar spray of trace elements containing iron. Mature trees sometimes suffer from zinc deficiency (Chen et al., 2009). The time of harvest depends upon the variety. Some are ripe in 6 - 8 months from bloom while others can take up to 12 - 18 months. Fruits continue to enlarge on the tree even after maturity. Avocados are allowed to colour fully before harvest. Avocado fruits can be stored at (40 - 50°) F for up to six weeks but some avocado discolour quickly and require immediate consumption (Chen et al., 2009).

2.2.2. Phytochemistry

The avocado fruit has a lot of nutrients. This includes its high content of essential minerals, potassium, vitamin b6, vitamin E and B complex. The avocado seed also contains various classes of natural products such as phytosterols and triterpenes (Werman et al., 1990), fatty acids with olefinic, acetylenic bonds, furanoic acid, dimmers of flavonols and oligomeric proanthocyanidins, β -D- glucoside of 8-hydroxyabscisic acid and epi-dihydrophaseic acid β -d-glucoside (Maria et al., 2004). The proximate analyses of the fruit on dry basis shows 4.2%, ash 8.1% protein 70%, oil 7.2% fiber and 10.5% carbohydrate. It also contains tannins 2.45g/kg wt of the seed (Agostini, 1995). Oil content was found to change in terms of maturity from 49.5% after harvest to 70% (dry basis) after 13 days of storage. Thus ripe avocados afford the maximum amount of oil from the raw material (Buenrostro et al., 1986).

2.2.3. Medicinal uses:

Leaf and seed extracts have been used for a variety of medical application, including treatment of diarrhoea and dysentery and as an antibiotic. (Lahav and Whiley, 2002). It contains nutrients which are beneficial in the synthesis of skin protein called

collagen (Ding et al., 2007). The fruit is considered one of the most potent anti-oxidant fruit in the world because of its high content of mono unsaturated fats (Soong et al., 2004), thus people consuming a special avocado based diets showed lower cholesterol levels. It is noteworthy that ethnopharmacology of Aztec and Maya cultures used decocts of avocado seeds as a potent agent to treat mycotic and parasitic infections (Oberlies et al., 1998).

Avocado seeds preparations are traditionally used as anti inflammatory (Villamar et al., 1994). The avocado leaves aqueous extract can be very toxic to horses but not for cows, while the lipophilic extract are toxic (Oelrichs et al., 1995). Some lipids isolated from the avocado fruits have shown a selective activity against human prostate Aden carcinoma (Oberlies et al., 1998). Avocado fruit has a skin healing effects which may be due to the positive influence on fatty acid (Carranza et al., 1995). The non toxic ACC-inhibiting substance could be a beneficial tool to suppress fat accumulation and hence to avoid obesity (Hashimura et al., 2001).

It is reported that the aqueous leaves extract of the avocado has some vaso relaxant effect on isolated rats and this effect is dependent on the synthesis or release of endothelium –derived relaxing factors as well as the release of the prostanoid, thus inhibiting Ca^{2+} influx through calcium channels (Yasir et al., 2010). The presence of two glycoylated abscisic acid derivate in avocado seed (Maria et al., 2004) could ameliorate the symptoms of type 2 diabetes, targeting perioxisome proliferator-activated receptor gamma in a similar manner as the thiazolinediones class anti- diabetic drugs (Bassaganya-Riera et al., 2010).

2.3. CHOLESTEROL

Cholesterol is a waxy steroid metabolite found in cell membrane. It serves as a medium for permeability and fluidity and it is transported in the blood plasma of all animals as an essential structural component of mammalian cell membranes. Cholesterol from the diet is required but in small quantity. This is because high blood cholesterol is an indicator for diseases like the heart disease (Emma, 2009).

2.3.1. Cholesterol metabolism

Physiology: Cholesterol is synthesized from simpler substances (acetyl CoA) within the body. A high level of cholesterol in the body is usually associated with atherosclerosis though it is depended on how it is transported within lipoproteins (Brunzell et al., 2008). The average daily cholesterol synthesized in an adult of about 70kg is 1g and the total body content is about 35g. Additional daily dietary intake of 200-300mg is required and the body compensate for cholesterol intake by reducing the amount synthesized (Segrest et al., 2008). Cholesterol is usually recycled in the body as about 50% of the excreted cholesterol via the bile into the digestive tract is reabsorbed by the small intestine back into the blood stream (Haines, 2001). Cholesterol is required for the building and maintenance of the body membranes, as it regulates the fluidity over the range of physiological temperatures (Warnick et al., 1990).

The hydroxyl group on cholesterol interacts with the polar head groups of the membrane phospholipids and sphingolipids, while the bulky steroid and hydrocarbon chain are embedded in the membrane, alongside the non polar fatty acid chain of the other lipids. In this structural role, cholesterol reduces the permeability of the plasma membrane to protons (positive hydrogen ions) and sodium ions (Haines, 2001).

Studies using methyl β -cyclodextrin (M β CD) to remove cholesterol from plasma membrane showed the importance of cholesterol in the structuring and functioning of invaginated caveolae and clathrin-coated pits, including caveola-dependent and clathrin-dependent endocytosis (Pawlina and Ross, 2006).

Cholesterol is also essential in cell signaling processes, assisting in the formation of lipid rafts in the plasma membrane (Otvos, 1999). Many neurons such as the myelin sheath which is rich in cholesterol provide insulation for more efficient conduction of impulse (Superko et al., 2002). Cholesterol is converted into bile salt in the liver and then stored in the gall bladder. These bile salts solubilize fats in the digestive tract and aid in the intestinal absorption of fat molecules as well as fat-soluble vitamins A, D, E, and K (Smith, 1991).

It is also an important precursor molecule for the synthesis of Vitamin D and the steroid hormones, including the adrenal gland hormones cortisol aldosterone as well as the sex hormones, progesterone, estrogens and testosterone and their derivatives (Shepherd et al., 1995). Some researchers have also indicated that cholesterol act as an antioxidant (Smith, 1991). Animal fats are good sources of cholesterol as they contain varying quantities of triglycerides, with lesser amounts of phospholipids and cholesterol (Christie, 2003). Major sources of cholesterol include cheese, egg yolks, beef, pork, poultry and shrimp (Vanessa et al., 2009). Cholesterol are not found in plant-based food but could be found in flax seeds and peanuts in the form of cholesterol-like compounds called phytosterols which are reported to lower serum cholesterol levels in animals (Ostlund et al.,2003).

Synthesis and Regulation of cholesterol

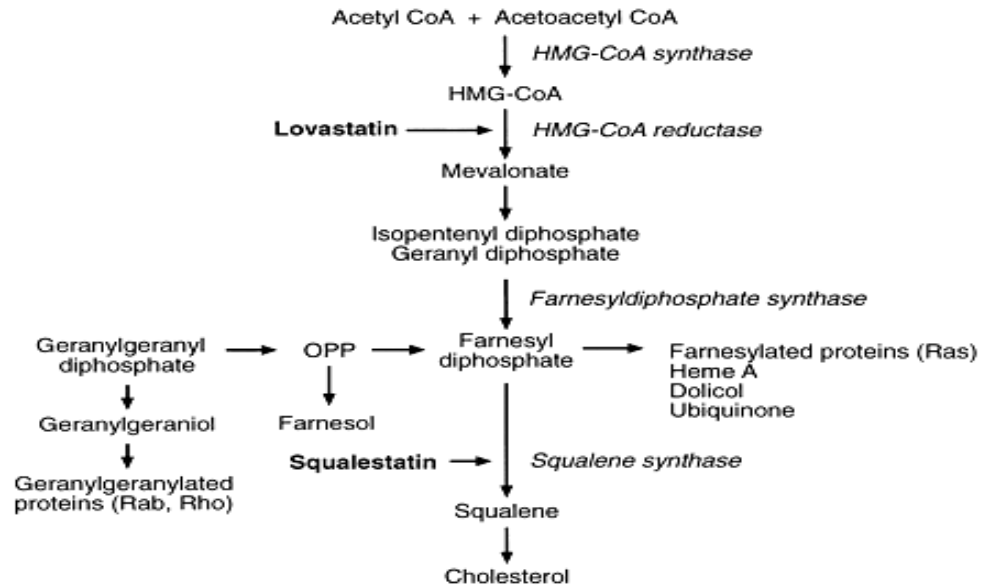


Figure 2.1: One molecule of acetyl CoA and acetoacetyl-CoA are dehydrated to form 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) which is enzymatically reduced to mevalonate by HMG-CoA reductase, a rate limiting step and irreversible step in cholesterol synthesis. The mevalonate is then converted to 3-isopentenyl pyrophosphate in three reactions that require ATP which is then decarboxylated to isopentenyl pyrophosphate, a key metabolite for various biological reactions. Three molecules of isopentenyl phosphate is condensed to form farnesyl pyrophosphate through the action of geranyl transferase. Two of farnesyl pyrophosphates condenses to form squalene by the action of squalene synthase in the endoplasmic reticulum. Oxidosqualene then cyclizes squalene to form lanosterol. Finally lanosterol is then converted to cholesterol (Rhodes et al., 1995)

Cholesterol synthesis occurs in the liver, intestines, adrenal glands and reproductive organs. Biosynthesis of cholesterol is directly regulated by cholesterol levels present, though the homeostatic mechanisms involved are only partly understood (Criqui, 1994). A high intake cholesterol from food leads to a net decrease in endogenous production, whereas a lower intake lead to an increased production of cholesterol and the synthesis can also be turned off if the blood cholesterol is high (Espenshade and Hughes, 2007).

2.3.2. Clinical significance.

Hypercholesterolemia: This is an abnormal condition characterized by high cholesterol concentration of low density lipoprotein (LDL) and a lower concentration of functional high density lipoprotein (HDL) which could lead to cardiovascular disease because of the development and increase of atheroma in arteries (atherosclerosis). These diseases could lead to myocardial infarction (heart attack), stroke and peripheral vascular disease (Brunzell et al., 2008). The balance in LDL and HDL are mostly genetically determined but can be affected by body build, medications, food choices and other factors (Durrington, 2003).

It is suggested that daily total blood cholesterol (HDL+ VLDL) of less than 200mg/dl is desirable for the body (Warren et al., 2007). Serum cholesterol levels of 200-239 can be regarded as borderline while values greater than 240mg/dl are considered high (January, 1988). Other causes of high cholesterol are diabetes mellitus and metabolic syndrome, kidney disease (nephritic syndrome), hypothyroidism, cushing's syndrome, anorexia nervosa, sleeping deprivation, Zieve's syndrome, antiretroviral drugs, diets, bodyweight and physical activities (Grundy et al., 2004).

Hypocholesterolemia: This is an abnormal low cholesterol level ($< 160\text{mg/dl}$ or 4.1 mmol/l) which occurred as a result of disease or illness such as depression, cancer, and cerebral hemorrhage that have lowered the production of cholesterol in the body (Warren et al., 2007). Other possible causes of low level cholesterol include statins, hyperthyroidism or over active thyroid gland, adrenal insufficiency, liver disease, malabsorption, malnutrition, celiac disease, abetalipoproteinemia, hypobetalipoproteinemia, manganese deficiency, Smith-Lemli-Opitz syndrome, Marfan syndrome and leukemias (Marini et al., 1989).

Increased use of medication to suppress cholesterol may result in lowering cholesterol excessively leading to the formation of metabolic disorders (Warren et al., 2007). Due to this, dietary manipulation or control has become a topic of research interest in an effort to stabilize the cholesterol concentration in the body (Maria et al., 2005).

2.4. CARBOHYDRATE

Carbohydrates are organic compounds with the general formula $C_m(H_2O)_n$. They are divided into four chemical groupings: monosaccharides, disaccharides, oligosaccharides and polysaccharides. The monosaccharide (example glucose, fructose and galactose) and disaccharides (example sucrose and lactose) are regarded as sugars and they have lower molecular weight (Flitsch and Ulijn, 2003). Oligosaccharides (fructooligosaccharides and galactooligosaccharide) and polysaccharide (starch and glycogen) serves as energy storage whereas cellulose in plants and chitin in arthropods serves as structural components.

Plant derived materials rich in starch or sugar such as cereals, bread, pasta, candy, jams, and desserts can be regarded as carbohydrate food (Maton et al., 1993).

2.4.1. Carbohydrate metabolism

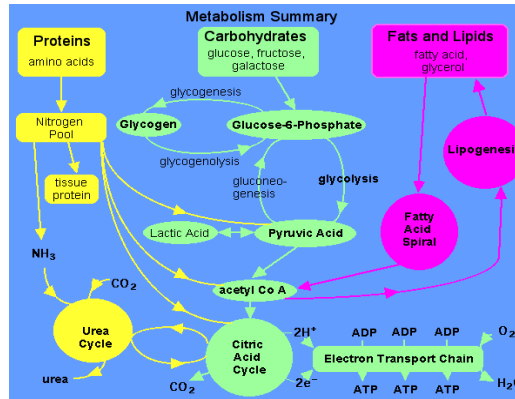


Figure 2.1: Glycolysis, glycogenolysis, and gluconeogenesis metabolic pathways

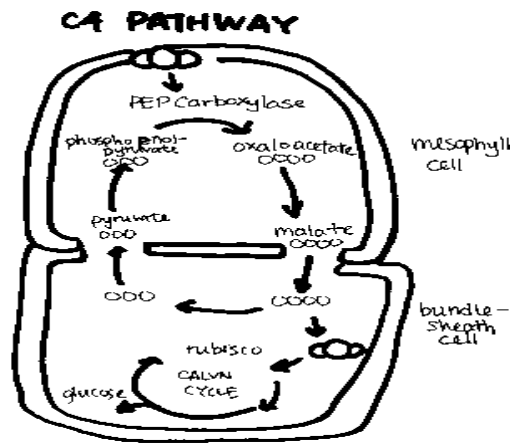
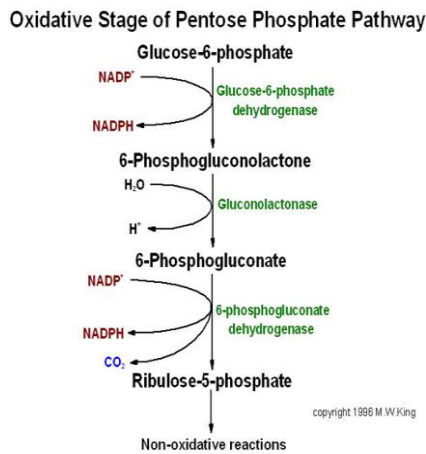


Figure 2.2: Pentose phosphate pathway

Figure 2.3: Carbon fixation

Figure 2a: Glycolysis; breakdown glucose molecule to produce ATP and pyruvate. The pyruvate formed may enter krebs cycle in aerobic microbes, glycogenesis; excess glucose are reconverted into glycogen and stored for use in future and to prevent osmotic pressure build up inside the cell, glycogenolysis; regeneration of glucose from glycogen to maintain glucose supply in the tissue, gluconeogenesis; synthesis of glucose molecules from simple organic molecules such as amino acid to generate glucose. Figure 2b: The pentose phosphate pathway; hexoses sugar are converted into pentose sugar along NADPH regeneration. Figure 2c: carbon fixation; carbon (iv) oxide is reduced to obtain carbohydrate (Andreas et al., 2009).

The body keeps a steady supply of glucose in the body through glucoregulation. The insulin which is made by the beta cells in the pancreas in the body plays important role in glucoregulation by stimulating the tissues or cells to take up glucose from circulation after a carbohydrate meal (George and Stephen, 1991). It also stimulates the storage of glucose in the form of glycogen (Claude et al., 2003). Low levels of blood glucose (<70mg/dl) releases glucagon from the alpha cells in the pancreas (Andreas et al., 2009). Glycogen reverses the process leading to glycogen breakdown and lipid release from storage cells (George and Stephen, 1991). The glucose released is only those in the liver but not those from the muscles cells as they lack the ability to release glucose to the blood (Andreas et al., 2009).

2.4.2. Clinical significance

Various diseases are associated with carbohydrate metabolism such as diabetes mellitus, lactose intolerance, fructose intolerance, galactosemia and glycogen storage disease (Benagiano et al., 1997). The onset of diabetes mellitus can lead to other chronic diseases such as hypertension, coronary heart disease and dyslipidemia (Sidika et al., 1996).

Diabetes mellitus: This is a condition in which the blood glucose is high, either because the body does not produce enough insulin or because cells do not respond to the insulin produced (Susumu et al., 2003). This condition could result polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). There are three main types of diabetes mellitus namely Type-1 diabetes, Type-2 diabetes and gestational diabetes. In the Type-1 diabetes, the body is unable to produce insulin due to loss of beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. This type of diabetes can be further classified as immune-mediated or idiopathic (Rother, 2007).

Type-2 diabetes is characterized by insulin resistance which may be combined with relatively high insulin secretion (Carlos et al., 2003). Hyperglycemia can be reversed by a variety of measures and medications that can improve insulin sensitivity or reduce glucose production by the liver (Susumu et al., 2003). However as this condition progresses, the impairment of insulin secretion occurs and therapeutic replacement of insulin and or a diet manipulation may be required (Rother, 2007).

The gestational diabetes mellitus involves relatively inadequate insulin secretion and responsiveness which could have resulted due to pregnancy and could improve or disappear after parturition (Heydari, 2010). Gestational diabetes is fully treatable but it requires careful medical supervision (Carlos, 2003). A person with diabetes mellitus is defined as having fasting plasma glucose $\geq 7.0\text{mmol/l}$ (126mg/dl), plasma glucose $\geq 11.1\text{mmol/l}$ (200mg/dl) two hours after a 75g oral glucose load (WHO, 1999). In the case of diabetes mellitus a management therapy involves measures that attempt to maintain blood sugar levels as close to normal (euglycemia) as possible without presenting undue danger (Benagiano et al., 1997). This can be best done using a dietary management, exercise and appropriate medication if necessary.

2.5. THE RELEVANCE OF USING RATS IN HUMAN STUDIES

Rats are used as laboratory animals because of their genetic consistency. The rats raised for animal testing are tested for any genetic defects that may affect the results of the experiments. Only rats with known genetic histories are candidates for testing. Carefully bred rats with documented genetic histories are used in animal testing for a number of reasons, including their frequent reproduction, genetic purity and similarities to human biology (Michael, 2010).

Since rats tend to breed frequently, their offspring can also be tested for any genetic abnormalities possibly caused by exposure to the test product. By so doing the researchers would know any genetic predispositions towards weight gain or cancer formation and solution can be achieved (Michael, 2010). The laboratory tests performed on rats are deemed necessary to check the safety of chemicals used in medicines, food products and cosmetic. Rats were used because they are mammals and their systems react to test chemicals in a similar way to those of a human.

For any chemical to be considered safe enough for human consumption or exposure, the chemical compound or drug must first be tested on experimental animals (rats or other mammals); (Trevor et al., 2001). With respect to food and safety rats are often fed extremely high amounts of a new food additive or injected with large doses of a new chemical compound. Theoretically, if the test product is completely safe for humans, it should not matter if the laboratory rats received even two hundred times the recommended levels (Raymond et al., 2010).

Rats also reproduce quickly and tend to have large litters, so researchers do not have to wait long to evaluate test results in generations. The grandsons and granddaughters of original test rats could appear within months, not years as it would take in human subjects. When looking for potential health hazards, examining various generations of a test subject all at once can provide more definitive proof of a product's safety or potential threat. Overseers such as the Federal Food and Drug Administration (FDA) require this level of proof before even considering human clinical trials. Although the issue of animal testing remains controversial, there can be no doubt that the use of rats in laboratory studies has provided a number of advances in the medical, food and cosmetics industries (Mark et al., 2009).

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1. Animal feed

Rat chow made from soybean meal was purchased from local feed manufacturer (Gold Coin Malaysia). Tamarind (*Tamarindus indica*) and avocado (*Persea americana*) were purchased from local markets. Soybean milk (Nutrisoy Inc.) powder supplements were purchased from local pharmaceutical stores.

3.1.2. Experimental animals

Male Sprague-Dawley (SD; n=56) and spontaneously hypertensive (out bred of Wistar-Kyoto rats; SHR (n=28), 6-8 weeks old were randomly selected from University of Malaya animal house. These animals were individually caged (600x380x200) mm³ at all time had access to rat chow and fresh water *ad libitum*.

3.2 METHODS

3.2.1 Preparation of ground tamarind and avocado seed

The pulp of fresh tamarind and avocado fruits were removed and the seeds were washed out of residual flesh. A total weight of about 8kg of seeds from each fruits was obtained and the seeds were dried in the oven (50⁰C) and ground separately into fine particles of about 50µm particle size.

3.2.2 Preparation of diets containing ground tamarind or avocado seed.

The basal diet (BD) was mixed with the ground tamarind or avocado seeds at the following inclusion: 2%, 4% and 8% wt/wt. High sucrose diet (HSD) was prepared by adding 30% wt/wt sucrose to the BD (see Tables 2-6).

Equivalent weight of soymilk was added into the diets to balance the difference in nitrogen and energy content as a result of the seeds inclusion. Diets and faecal nitrogen (N) were determined by Kjeldahl procedure and crude proteins were calculated as $N \times 6.25$ (Balogun and Fetuga 1986). Crude fiber, ash and ether content were determined according to AOAC method (Lynch et al., 1997).

3.3 EXPERIMENTAL PROCEDURES

Three different and independent experiments were conducted. In the first experiment (experiment 1) SD rats (n=28) were randomly assigned into seven groups of four each and served with test diets (BD) containing avocado or tamarind seed at the various inclusion 2, 4 and 8 %, respectively where 0% inclusion served as control in the group. In the second experiment (experiment 2), SD rats (n=28) were also randomly assigned into seven groups of four each and fed with HSD containing 2, 4, and 8 % avocado or tamarind seed and control 0% respectively. In the third experiment (experiment 3), SHR rats were assigned into seven groups accordingly and fed with BD. Initial body weight (IBW), body weight gain (BWG), feed intake (FI) and faecal output from each rat were measured at the beginning and every seven days thereafter for twenty-eight days. The amount of weekly diet ingested was calculated as the difference in the total weight of feed offered at the beginning and the balance at the end of the week.

At the end of the feeding, the animals were sacrificed. Blood samples were collected into heparinized tubes and kept cold at 4°C for further analysis. Liver samples of (approximately 5g) were collected and stored at – 20°C for liver glycogen estimation.

3.3.1 Feeding performance analysis

The weekly data collected were then used to calculate daily feed intake according to Monia et al. (2006) with the following formula:

$$\text{Feed intake (g/day)} = \frac{\text{Feed placed} - \text{Feed remaining}}{7 \text{ days}}$$

Fecal dry matter (DM) was determined after drying faeces collected in 24hrs at 105°C to constant weight (Monia et al., 2006).

Macro nutrients digestibility were assessed as the difference between daily DM intake and 24h DM excretion in faeces according to Monia et al. (2006).

$$\text{Digestibility} = \frac{\text{Dietary DM intake} - \text{Faecal DM excretion}}{\text{Dietary DM intake}} \times 100$$

The feed conversion efficiency (FCE) was determined by the following formula (Monia et al., 2006)

$$\text{FCE} = \frac{\text{Feed consumed (DM) in 28 days (g)}}{\text{Body weight gained in 28 days (g)}}$$

The protein efficiency ratio (PER) is the weight gain of the growing rat divided by intake total protein intake during the feeding period (Monia et al., 2006) according to the following formula:

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Body weight of rat in 28 days (g)}}{\text{Total Protein intake in 28 days (g)}}$$

3.4 BIOCHEMICAL ANALYSIS

Livers were cleaned of excess blood whereas coagulated blood samples were centrifuged (2500g, 10mins) and the serum harvested prior to storage at -20°C until required for analysis. Liver glycogen content was determined according to Vats et al. (2004). Serum glucose concentration was determined according to modified Trinder method (Monia et al., 2006) whereas cholesterol was determined using commercial kits from Chemo Lab (Malaysia) refs 87656 and 86516, respectively.

3.4.1. Estimation of blood glucose

Serum glucose content was determined by mixing 0.1ml of serum with 1.0ml of water, 1.0ml of 5.0% zinc sulphate and 1.0ml of 0.25N sodium hydroxide. The mixture was then centrifuged (2500g, 10mins) and the supernatant (1.0ml) was transferred into a test tube containing 1.0ml of alkaline copper reagent followed by boiling in water bath for ten minutes. The mixture was cooled by placing the tubes under running water for three minutes. Arseno-molybdate reagent (1.0ml) was added to the resultant solution and the volume was made up to 10.0ml with water. The optical density was read at 500nm against a blank set at zero. The glucose concentration in the samples was then calculated from a glucose calibration curve (see Table 1) which was also run at the same time with the glucose analysis.

Table 1: Glucose calibration curve.

	Conc. Mg/dl	Stock mg/dl	Made up benzoic acid (ml)	Absorbance
1	0.00	0.00	50.00	0.0
2	25.00	2.50	50.00	0.03
3	50.00	5.00	50.00	0.05
4	75.00	7.50	50.00	0.09
5	100.00	10.00	50.00	0.14
6	150.00	15.00	50.00	0.20
7	200.00	20.00	50.00	0.26
8	250.00	25.00	50.00	0.33
9	300.00	30.00	50.00	0.46

3.4.2. Estimation of liver glycogen content

Liver samples (200mg) were rinsed with ice-cold saline and then solubilized by incubating with 2ml of 30 % potassium hydroxide at 55⁰C for 30 minutes. The solubilised liver tissue (0.2ml) was placed on ice bath and then neutralized with 0.2ml of 1.0M HCL, 0.8ml of water and 2.0ml anthrone reagent (0.2g anthrone / 100ml of 95% H₂So4). The mixture was then incubated at 100⁰C for 10 minutes. Absorbance was measured at 620 nm and the liver glycogen content (mg glycogen/g tissue) was calculated using glucose standard curve.

3.4.3. Enzymatic determination of total cholesterol

The cholesterol concentration was estimated by thoroughly mixing 10µl of serum or standard solution (to yield calibrated cholesterol concentration) with 1.0ml of kit reagent (Chemo Lab (Fismes, Marne, France) refs.87656). The mixed solutions were allowed to stand for 5 minutes at 37⁰C prior to absorbance reading at 500nm.

The cholesterol concentration was calculated using the following formula:

$$\text{Sample concentration} = (\text{Abs}_{\text{sample}}/\text{Abs}_{\text{standard}}) \times \text{standard concentration}$$

Table 2: Proximate analysis of rat chow and the fresh ground seeds (%)^a.

Materials	Control	T seed	A seed
Dry matter	88.00± 0.10	95.10±0.10	91.50±0.00
Lipids	2.20±0.10	2.90±0.10	1.40±0.09
Protein	19.10±0.10	11.80±0.11	9.60±0.01
Ash	4.60±0.10	4.50±0.00	4.90±0.03
Total Carbohydrates	62.00±2.03	75.80±3.21	75.60±2.23

^aData are presented as the mean of four observations ± standard error mean.

Total carbohydrate = 100-(sum of percentages of moisture, ash, protein and lipids) (Monia et.al., 2006), T: tamarind; A: avocado

Table 3: Proximate analysis of the basal diet containing tamarind seed

Materials	0% T	2% T	4% T	8% T
Dry matter	88.00±0.20	88.00±0.10	88.8±0.20	88.3±0.60
Lipid	2.30±0.30	2.20±0.30	2.20±0.30	2.20±0.30
Protein	19.10±0.10	19.10±1.10	19.10±1.20	19.10±1.30
Ash	4.60±0.10	4.60±0.10	4.60±0.10	4.60±0.01
Total Carbohydrates	62.00±2.00	62.10± 1.50	62.90±2.10	62.40±1.80

Each value is the mean of four observations ± standard error mean.

Total carbohydrate = 100-(sum of percentages of moisture, ash, protein and lipids) (Monia et al., 2006)

Nitrogen was corrected by adding soy milk (Nutisoy Inc), T: tamarind seed.

Table 4: Proximate analysis of the high sucrose diet containing tamarind seed

Materials	0% T	2% T	4% T	8% T
Dry matter	87.80±0.20	87.70±0.10	87.90±0.20	87.80±0.60
Lipid	2.20±0.30	2.10±0.30	2.20±0.30	2.2±0.30
Protein	19.10±0.10	19.10±1.10	19.10±1.00	19.10±1.40
Ash	4.60±0.30	4.60±0.10	4.60±0.20	4.60±0.00
Total Carbohydrates	61.90±2.00	61.90± 1.50	62.80±2.10	62.10±1.80

Each value is the mean of four observations ± standard error mean.

Total carbohydrate = 100-(sum of percentages of moisture, ash, protein and lipids) (Monia et al., 2006)

Nitrogen was corrected by adding soy milk (Nutisoy Inc), T: tamarind seed.

Table 5: Proximate analysis of the basal diet containing avocado seed.

Materials	0% A	2% A	4% A	8% A
Dry matter	88.00±0.10	88.50±0.10	88.00±0.20	88.10±0.60
Lipid	2.20±0.10	2.20±0.20	2.20±0.30	2.30±0.50
Protein	19.10±0.40	19.30±1.10	19.10±1.20	19.20±1.40
Ash	4.60±0.20	4.60±0.50	4.60±0.30	4.50±0.60
Total Carbohydrates	62.10±2.00	63.20±1.70	62.60±1.80	62.70±2.10

Each value is the mean of four observations ± standard error mean.

Total carbohydrate = 100-(sum of percentages of moisture, ash, protein and lipids) (Monia et al., 2006)

Nitrogen was corrected by adding soy milk (Nutisoy Inc), A: avocado seed.

Table 6: Proximate analysis of the high sucrose diet containing Avocado.

Materials	0% A	2% A	4% A	8% A
Dry matter	87.80±0.10	87.90±0.10	87.60±0.20	88.10±0.60
Lipid	2.20±0.10	2.20±0.20	2.20±0.30	2.30±0.50
Protein	19.10±0.40	19.20±1.10	19.20±1.20	19.10±1.40
Ash	4.60±0.20	4.60±0.50	4.60±0.30	4.50±0.60
Total Carbohydrates	62.00±2.30	62.20±1.60	62.4±1.80	62.50±2.10

Each value is the mean of four observations ± standard error mean.

Total carbohydrate = 100-(sum of percentages of moisture, ash, protein and lipids) (Monia et al., 2006)

Nitrogen was corrected by adding soy milk (Nutisoy Inc), A: avocado seed.

3.4.4. Statistical Analysis.

Data were analyzed using paired t test. Mean values were obtained by averaging independent measurements. Data were presented as mean ± standard error mean.

Difference between control and experimental groups were considered significant at

$p < 0.05$

4.0 RESULTS

4.1. Feed intake and Body weight gain of SD rats fed BD containing tamarind seed (Experiment 1).

The rats in the control consumed feed at 63 ± 4 g/kg BW/day during the first week of feeding and this figure reduced gradually to 23 ± 10 g/kg BW/day by the end of week four. Inclusion of 2%T into BD resulted in reduced FI to 38 ± 15 g/kg BW/day ($p < 0.05$) during week 1 of feeding whereas BD containing 4 and 8%T were not different from control. Intake of diets containing T during the next 3 weeks was different from control except at 4 and 8%T in week 2, and 2%T in week 3 ($p < 0.05$).

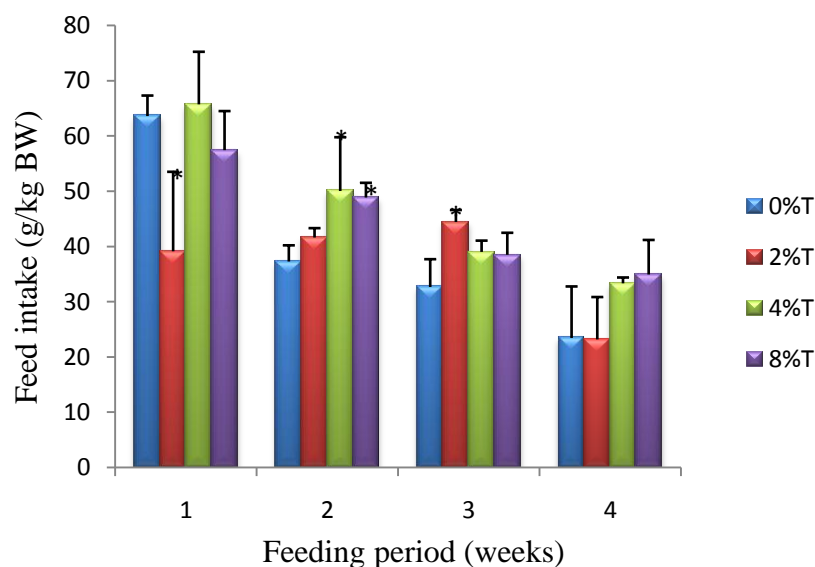


Figure 4.1: Feed intake of SD rats offered BD containing tamarind seed. Values are presented as mean \pm SEM (n=4).*: $p < 0.05$ compared to control.

All animals had a marginal increase in body weight of between 15-47g during the 4 weeks of feeding. The increase in body weight was linear and similar (25-47g) for rats which consumed 0, 2 and 4%T whereas rats offered 8%T showed the least increase in bodyweight (15g). However when expressed over initial body weight (IBW, Table 7), rats fed with control feed had the lowest BWG; 57 ± 15 g/kg BW whereas those offered tamarind had higher BWG (105 ± 27 ($p < 0.05$), 69 ± 22 ($p > 0.05$) and 71 ± 38 ($p > 0.05$) g/kg BW for 2, 4, and 8%T respectively).

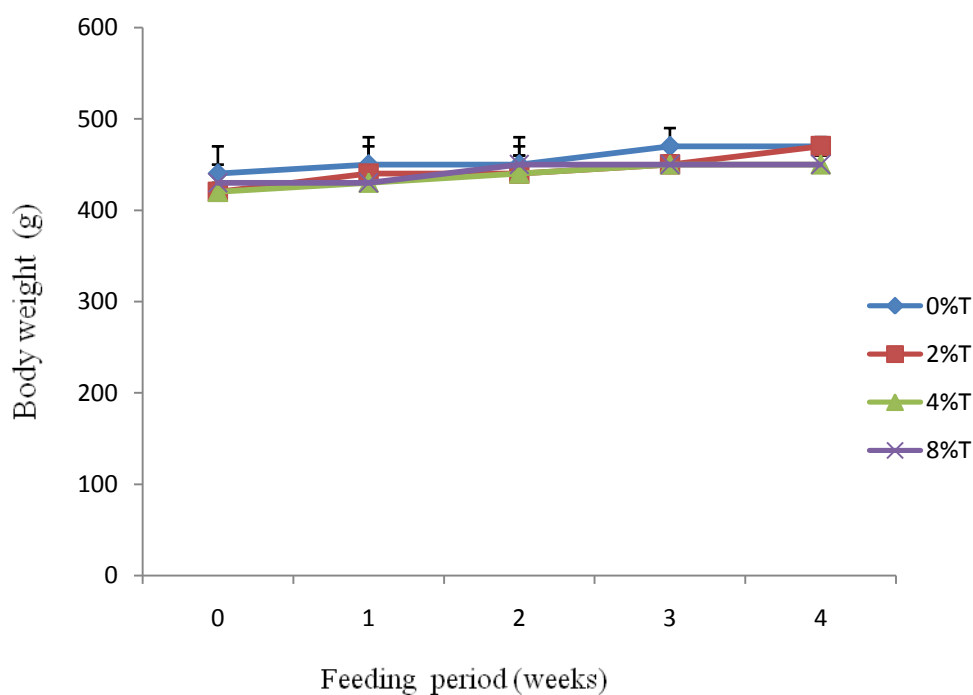


Figure 4.2: Body weight of SD rats offered BD containing tamarind seed. Values are presented as mean \pm SEM ($n=4$). There was no significant differences between the treated groups and control.

Table 7: Average Feed intake per day and body weight gain of SD rats offered BD containing tamarind seed (Experiment 1).

Parameters (g/kg BW)	0%T	2%T	4%T	8%T
<i>Feed intake</i>				
SD offered BD	39.30±8.60 ^a	37.10±4.80 ^a	47.00±7.10 ^a	44.90± 5.10 ^a
<i>Body weight gain</i>				
SD offered BD	57.00±15.00 ^a	105.00±27.00 ^b	69.00±22.00 ^b	71.00±38.00 ^{ab}

Data are expressed as mean ± SEM. Within row, values having different superscripts are significantly different, $p < 0.05$. T: tamarind seed; SD: Sprague Dawley rat; BD: basal diet.

4.2. Feed intake and body weight gain of SD rats offered HSD containing tamarind seed (Experiment 2).

Average daily FI of SD for all diets, except for 2%T, in the first week ranged 50-60g/kg BW. The SD FI of 0, 4 and 8%T reduced to 33-48 g/kg BW during the subsequent 3 weeks of feeding. SD offered 2%T had lower ($p < 0.05$) initial FI of about 30g/kg BW that stayed for the first 3 weeks of feeding but this increased to 44g/kg BW during the 4th week of feeding.

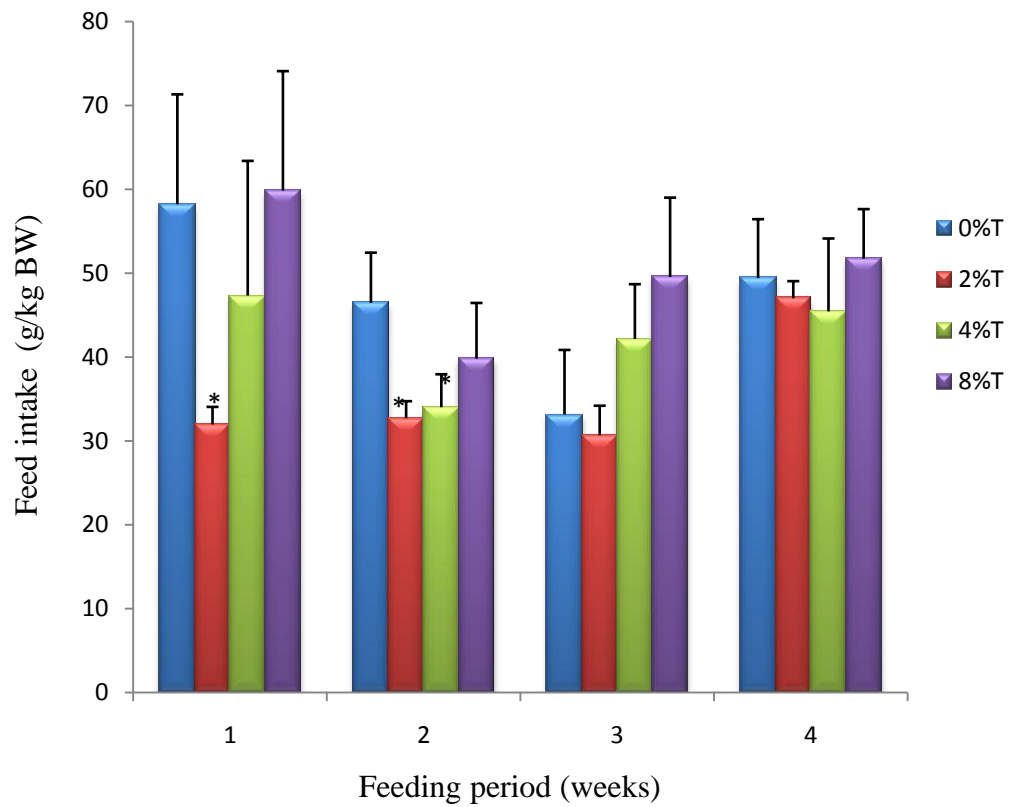


Figure 4.3. Feed intake of SD rats offered HSD containing tamarind seed. Values are presented as mean \pm SEM (n=4). *: $p < 0.05$ compared to control.

All rats increased in body weight during the feeding trial when compared with their initial body weight. The difference in initial body weight and final body weight of the rats was in the range of 20 to 40 % (see Figure 4.4). The inclusion of T from 2 to 4% into the diet reduced average BWG (g/kg BW). The BWG of control (282±36g/kg BW, Table 8) was higher than the experimental groups, 2, 4, and 8%T (155±22, 118±36, 156± 26g/kg BW respectively; $p<0.05$).

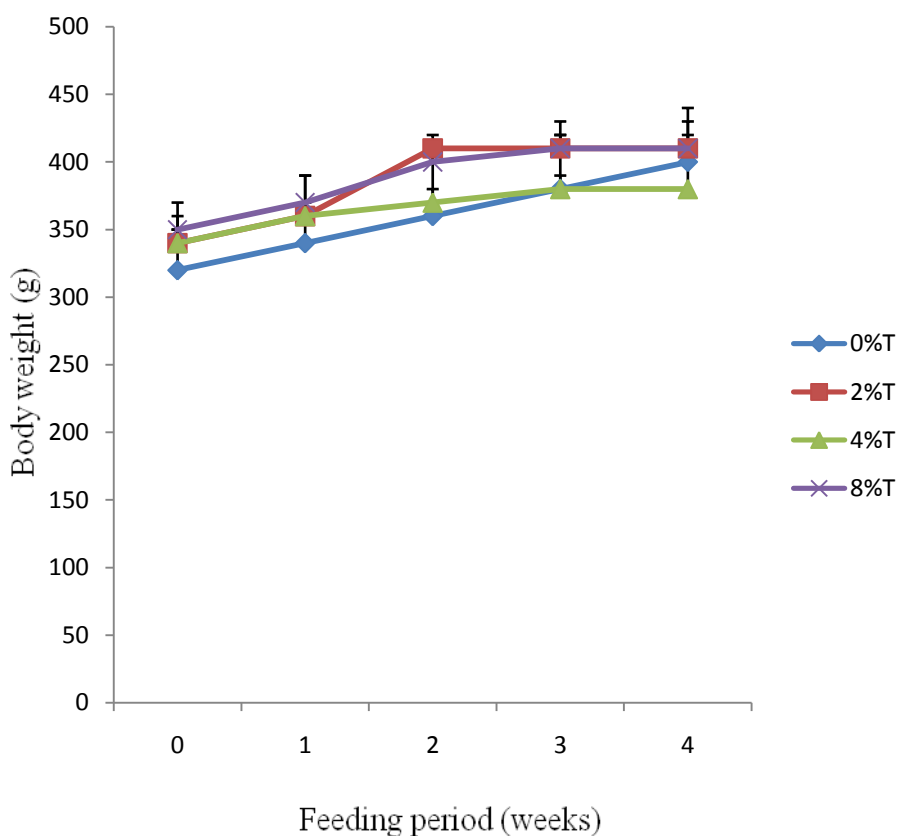


Figure 4.4. Body weight of SD rats offered HSD containing tamarind seed. Values are presented as mean \pm SEM (n=4). No significant difference was observed between control and treated rats.

Table 8: Average Feed intake per day and body weight gain of SD rats offered HSD diet containing T (Experiment 2).

Parameters (g/kg BW)	0%T	2%T	4%T	8%T
<i>Feed intake</i>				
SD offered HSD	46.80±5.20 ^a	35.60±3.80 ^b	42.20±2.90 ^a	50.20±4.10 ^a
<i>Body weight gain</i>				
SD offered HSD	282.00±36.00 ^a	155.00±22.00 ^b	118.00±36.00 ^b	156.00±26.00 ^b

Data are expressed as mean ± SEM. Within row, values having different superscripts are significantly different, $p < 0.05$. T: tamarind; SD: Sprague Dawley rat; HSD: high sucrose diet.

4.3. Feed intake and body weight gain of SHR offered BD containing tamarind seed (Experiment 3).

FI of all SHR increased during the first two weeks of feeding but reduced towards basal values by week 4 (see Figure 4.5). The highest FI per day was seen in SHR offered control feed on day 21 of the feeding trial. The experimental groups consumed less than the control with an average intake of 52.8 ± 4.8 , 50.7 ± 4.2 and 44.6 ± 3.7 ($p < 0.05$) g/kg BW for 2, 4 and 8%T respectively compared to control (58.8 ± 7.1 g/kg BW, Table 9).

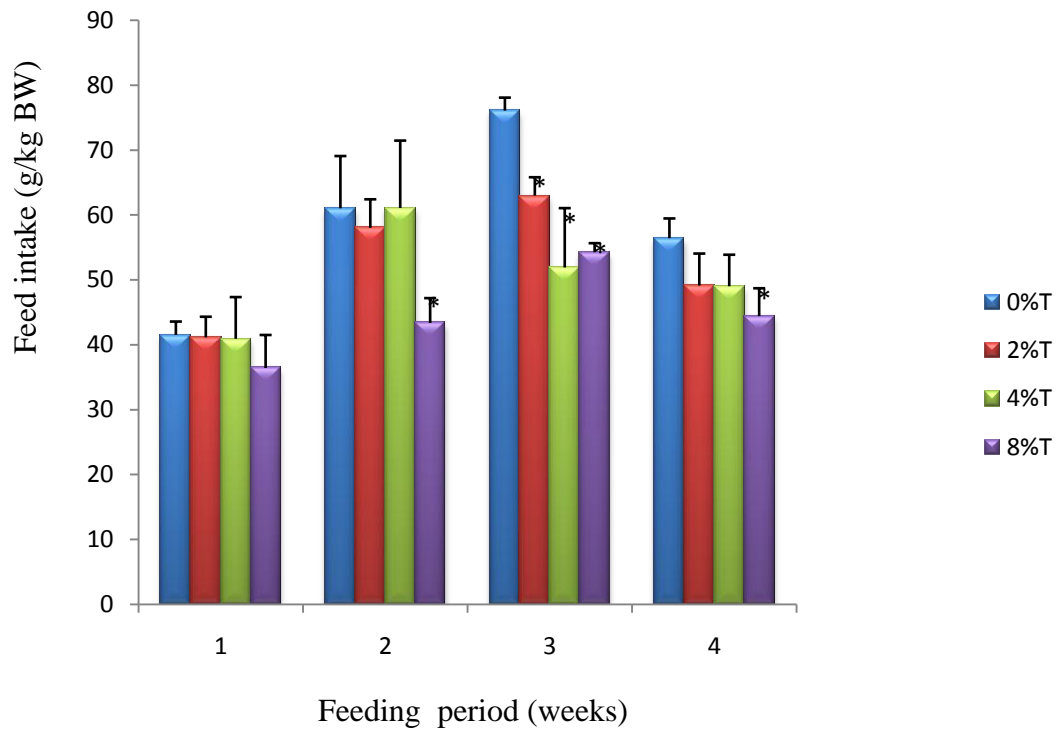


Figure 4.5. Feed intake of SHR offered BD containing tamarind seed. Values are presented as mean \pm SEM (n=4).*: p<0.05 compared to control.

SHR rats offered BD showed that 4%T gained more weight than the rest of the group (see Figure 4.6) with all rats showed an increase in body weight within 15-25% by the end of the feeding trial. However, increasing the inclusion of T from 2 to 8% into the diet reduced average BWG (g/kg BW). The average body weight gain by the control (134 ± 21 g/kg BW) was higher than the experimental groups except for 4%T (2, 4, and 8%T were 95 ± 18 ($p<0.05$), 146 ± 30 , 95 ± 18 ($p<0.05$) g/kg BW respectively; see Table 9).

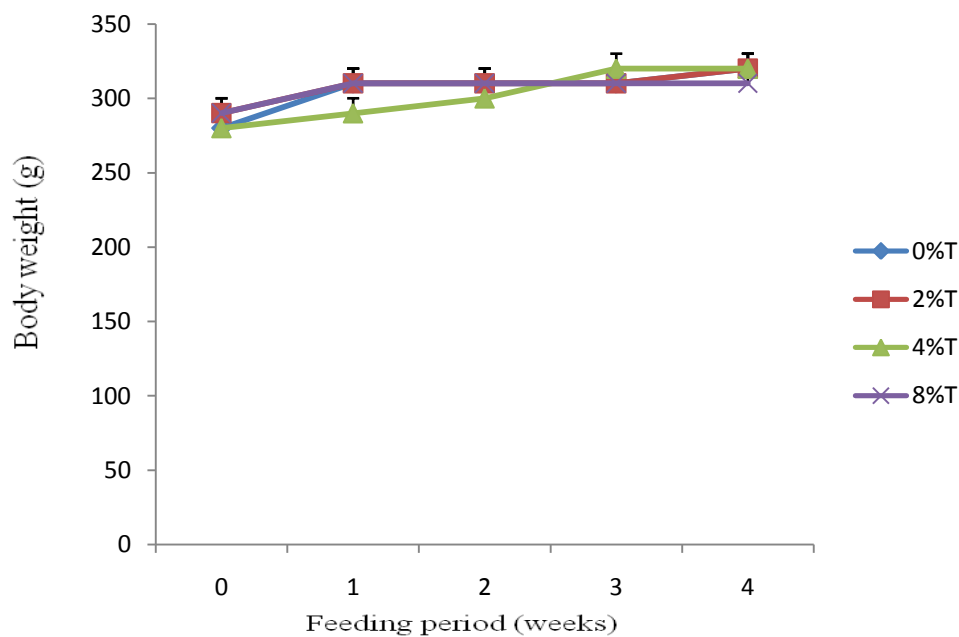


Figure 4.6. Body weight of SHR offered BD containing tamarind seed. Values are presented as mean \pm SEM (n=4). No significant difference was observed between control and treated rats.

Table 9: Average Feed intake per day and body weight gain of SHR offered BD containing T (Experiment 3)

Parameters (g/kg BW)	0%T	2%T	4%T	8%T
<i>Feed intake</i>				
SHR offered BD	58.80±7.10 ^a	52.80±4.80 ^a	50.71±4.20 ^a	44.60± 3.70 ^b
<i>Body weight gain</i>				
SHR offered BD	282.00±36.00 ^a	155.00±22.00 ^b	118.00±36.00 ^b	156.00±26.00 ^b

Data are expressed as mean ± SEM. Within row, values having different superscripts are significantly different, $p < 0.05$. T: tamarind; SHR: spontaneously hypertensive rat; BD: basal diet.

4.4. Digestibility of the diets containing tamarind seed in rats in experiment 1-3.

BD was more digestible than when sucrose was also included (Figure 4.7). BD was also more digestible when consumed by SD than by SHR. The inclusion of T did not affect the digestibility of BD by SD and BD by SHR. However, increased inclusion of T in the diet increased digestibility of HSD by SD ($p < 0.05$) with values at 8% T inclusion (80.5%) not different to that of BD (0% T; 81.3%) consumed by SD.

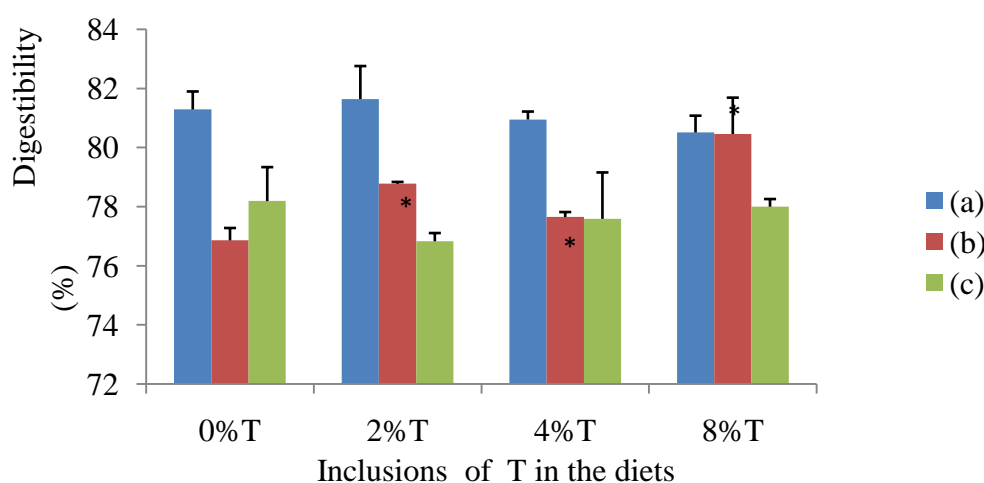


Figure 4.7: The digestibility of feed containing tamarind seed offered to SD and SHR. (a), (b), and (c) represent SD offered BD, SD offered HSD and SHR offered BD respectively. Values are presented as mean ± SEM (n=4).*: $p < 0.05$ compared to control.

4.5. Feed conversion efficiency (FCE) of rats offered diets containing tamarind seed in experiment 1-3.

SD offered BD showed the least FCE ($27 \pm 5\%$) followed by SHR offered BD ($14 \pm 4\%$) and SD offered HSD ($5.3 \pm 0.2\%$; see Figure 4.8). The inclusion of 2%T increased the FCE up to 50% in SD fed BD compared to control ($p < 0.05$). Higher inclusion of 4 and 8%T resulted in similar FCE with control. The inclusion of T into BD lowered FCE of SHR at 2%T. Similar results were obtained in FCE of SD fed HSD which decreased by nearly 100% when 4 or 8%T was included in the diet.

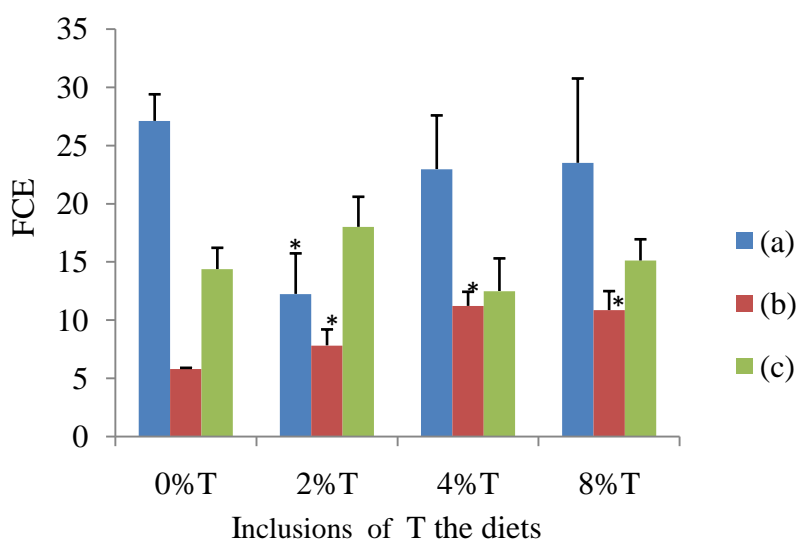


Figure 4.8: FCE of rats fed BD or HSD containing tamarind seed. (a), (b) and (c) represents SD fed BD, SD fed HSD and SHR fed BD respectively. Values are presented as mean \pm SEM (n=4). *: $p < 0.05$ compared to control.

4.6. The protein efficiency ratio (PER) of diets containing tamarind seed in rats in experiment 1-3.

The PER of SD fed BD ranged between 0.2 and 0.5 (see Figure 4.9). Inclusion of 2%T resulted in the highest PER (0.54 ± 0.20) compared to control ($p < 0.05$). SD fed HSD had the highest PER (0.90 ± 0.03) but the inclusion of T reduced PER with lowest values of (0.48 ± 0.05 and 0.50 ± 0.07) were recorded in rats which consumed 4 and 8%T in BD ($p < 0.05$). SHR fed BD in the absence of T was 0.38 ± 0.05 and the addition of T increased PER only at 4%T (0.48 ± 0.10).

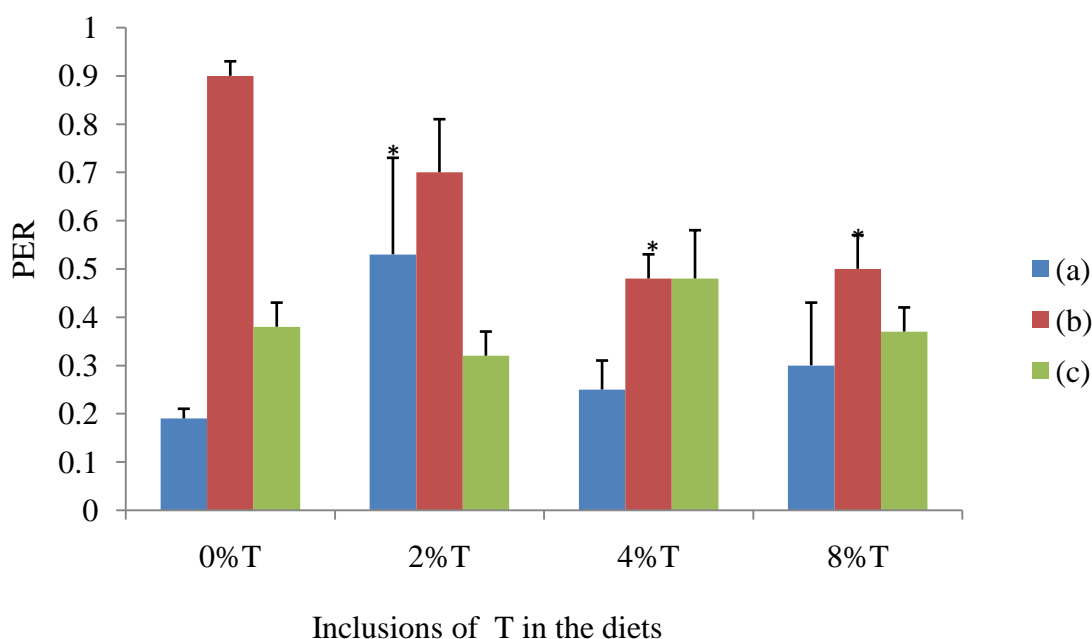


Figure 4.9: The PER of feed containing tamarind seed offered to SD and SHR. (a), (b) and (c) represents SD fed BD, SD fed HSD and SHR fed BD respectively. Values are presented as mean \pm SEM (n=4).*: $p < 0.05$ compared to control.

4.7. Serum cholesterol concentration of rats offered diets containing tamarind seed in experiment 1-3.

SD fed with BD had blood cholesterol of 0.79 ± 0.04 g/l (see Figure 4.10). The blood cholesterol level was lowered ($p < 0.05$) with the inclusion of T at all levels in BD. SD fed HSD had lower cholesterol (0.47 ± 0.05) than those fed BD. The addition of T however had no further lowering effects on blood cholesterol except at the highest (8%) inclusion of T (0.31 ± 0.13 g/l; $p > 0.05$). SHR had the highest blood cholesterol (1.19 ± 0.05 g/l). The addition of T tended to result in the lowering of blood cholesterol but a significant effect was only seen at 2%T inclusion (0.91 ± 0.14 g/l).

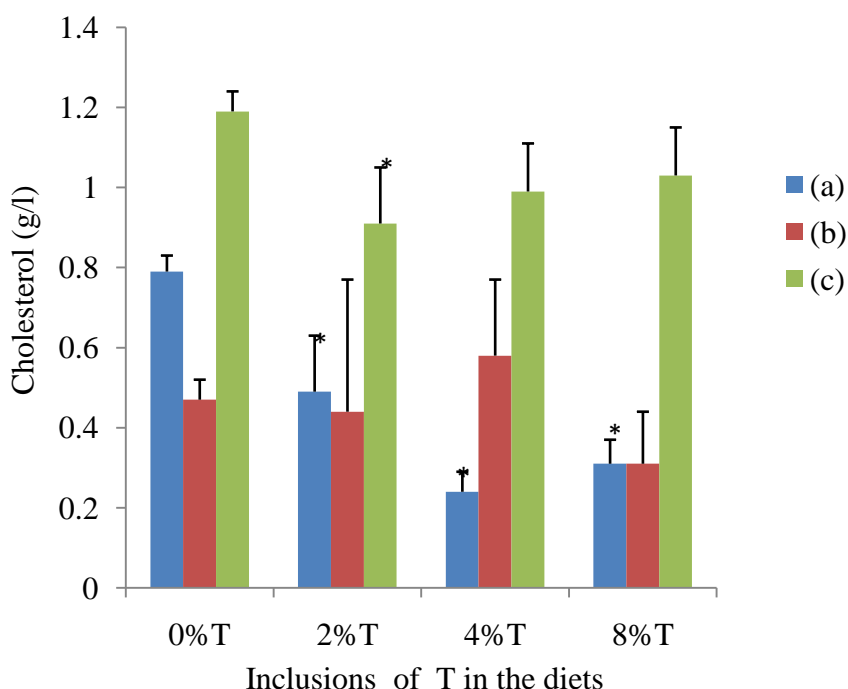


Figure 4.10: Serum cholesterol levels of SD and SHR fed on diets containing tamarind seed. (a), (b) and (c) represent SD offered BD, SD offered HSD and SHR offered BD respectively. Values are presented as mean \pm SEM (n=4) *: $p < 0.05$ compared to control (0%T).

4.8. Serum glucose levels of rats offered diets containing tamarind seed in experiment 1-3.

Rats (SD or SHR) fed on BD had lower serum glucose (40-95mg/dl) than SD fed on HSD (130±30mg/dl; see Figure 4.11). The addition of T did not affect serum glucose levels of BD-fed rats except for SHR which showed reduced serum glucose of 50±3mg/dl ($p<0.05$) at 4%T inclusion. Inclusion of 2 and 4% T in HSD resulted in increased serum glucose level (158±31; $p>0.05$ and 250±44; $p<0.05$ mg/dl respectively) whereas rats fed 8%T were not different from control.

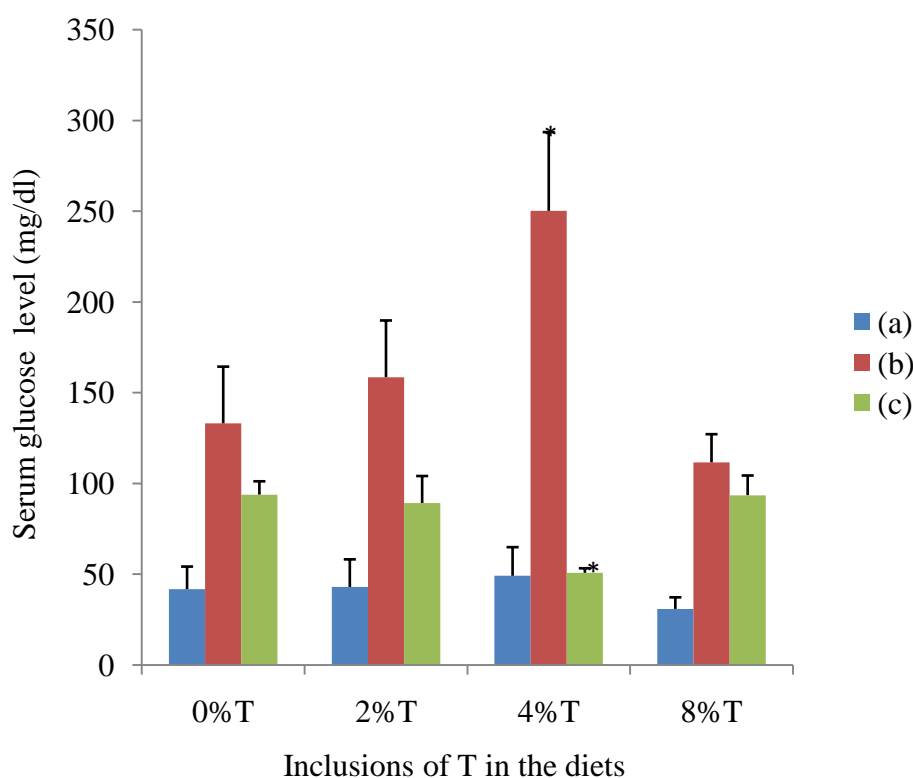


Figure 4.11: Serum glucose levels of SD and SHR rats fed on diets containing tamarind seed. (a), (b) and (c) represents SD offered BD, SD offered HSD and SHR were offered BD. Values are presented as mean \pm SEM (n=4) *: $p<0.05$ compared to control (0%T).

4.9. Liver glycogen content of rats offered diets containing tamarind seed in experiment 1-3.

Rats fed with diet unsupplemented with T showed similar liver glycogen content (0.95-1.27 mg/g). Incorporation of increasing amount of T resulted in linear increase of glycogen storage in livers of SD fed BD with maximal level (3.43 ± 0.55 mg/g, $p < 0.05$) recorded in BD containing 8%T. There was also evidence of increased liver glycogen storage with increasing T inclusion for SD fed HSD and SHR fed BD but the effect appeared to be saturated at 4 and 8%T inclusion in the diets.

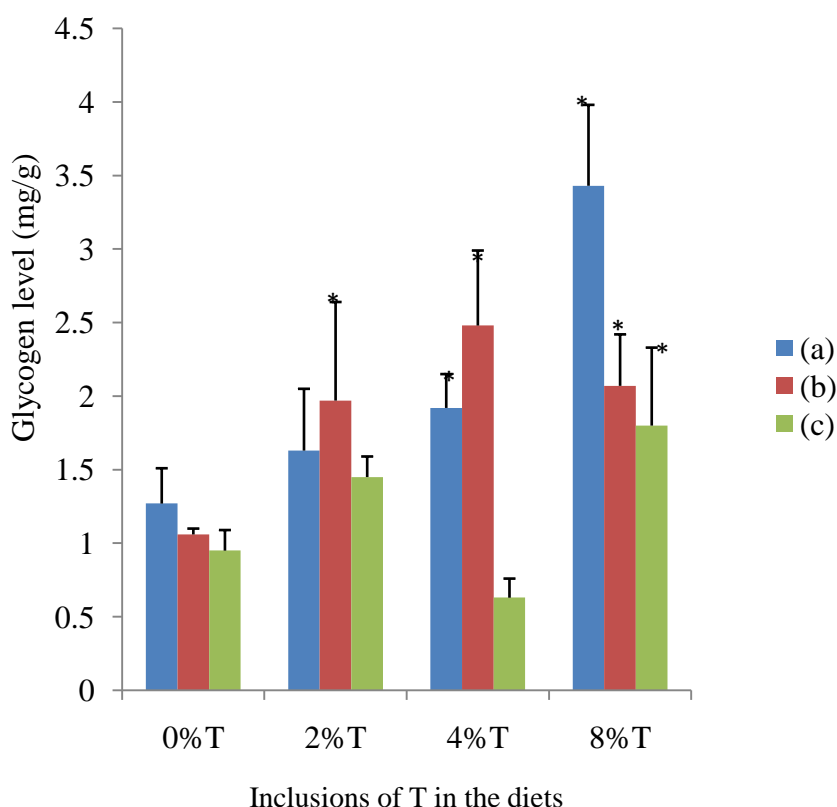


Figure 4.12: Liver glycogen levels of SD and SHR fed on diets containing tamarind seed (a), (b) and (c) represents SD offered BD, SD offered HSD and SHR were offered BD respectively. Values are presented as mean \pm SEM (n=4). *: $p < 0.05$ compared to control.

4.10. Feed intake and Body weight gain of SD rats offered BD containing avocado seed (Experiment 1)

The FI of SD fed on control diet reduced gradually from 66 to 26g/kg BW during the 28 days feeding period (see Fig. 4.13). The inclusion of the seed to the diet tended to increase the FI of experimental groups when compared with the control during the feeding period. The inclusion of 2 and 4%A at first week increased the FI to about 74- 85g/kg BW except for 8%A where FI was lower than the control. Lowest FI (28-38 g/kg BW) was recorded on week 4 of feeding for all dietary treatments.

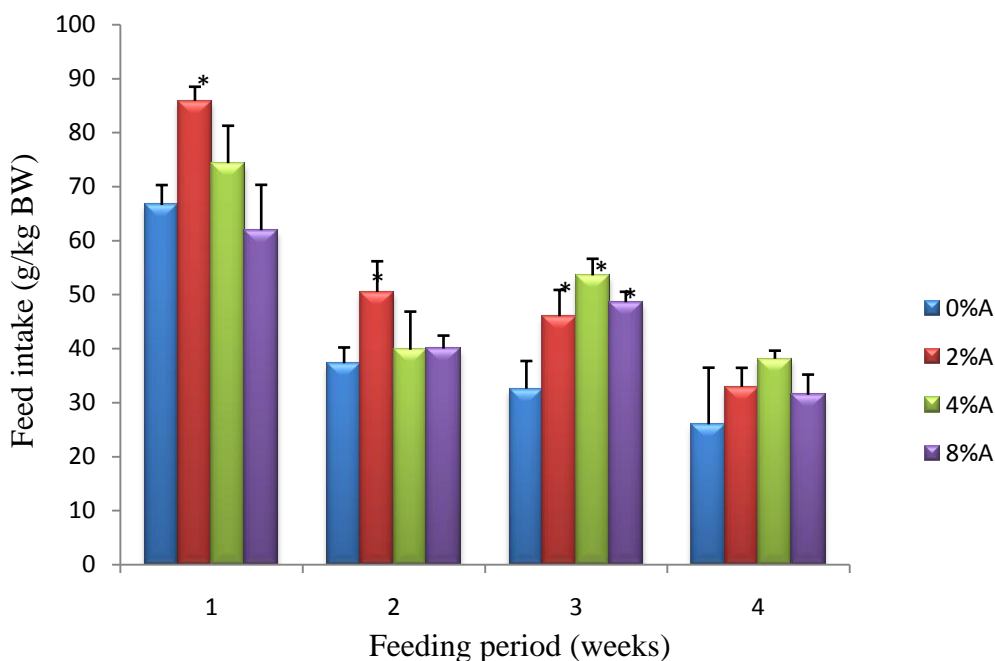


Figure 4.13: Feed intake of SD rats offered BD containing avocado seed. Values are presented as mean \pm SEM (n=4). *: p<0.05 compared to control.

All rats had 5-10% body weight gain during the feeding period (see Fig 4.14). However when expressed as average body weight gain (g) during the entire feeding period to initial body weight (kg IBW), it was found that the inclusion of A resulted in increased average body weight gain compared to control ($56.8 \pm 15 \text{g/kg IBW}$). Nevertheless, increasing inclusion of A from 2 to 4 and 8% into the diet tended to reduce average body weight gain from 93.16 to 85.77, and 58.47g/kg IBW respectively (see Table 10).

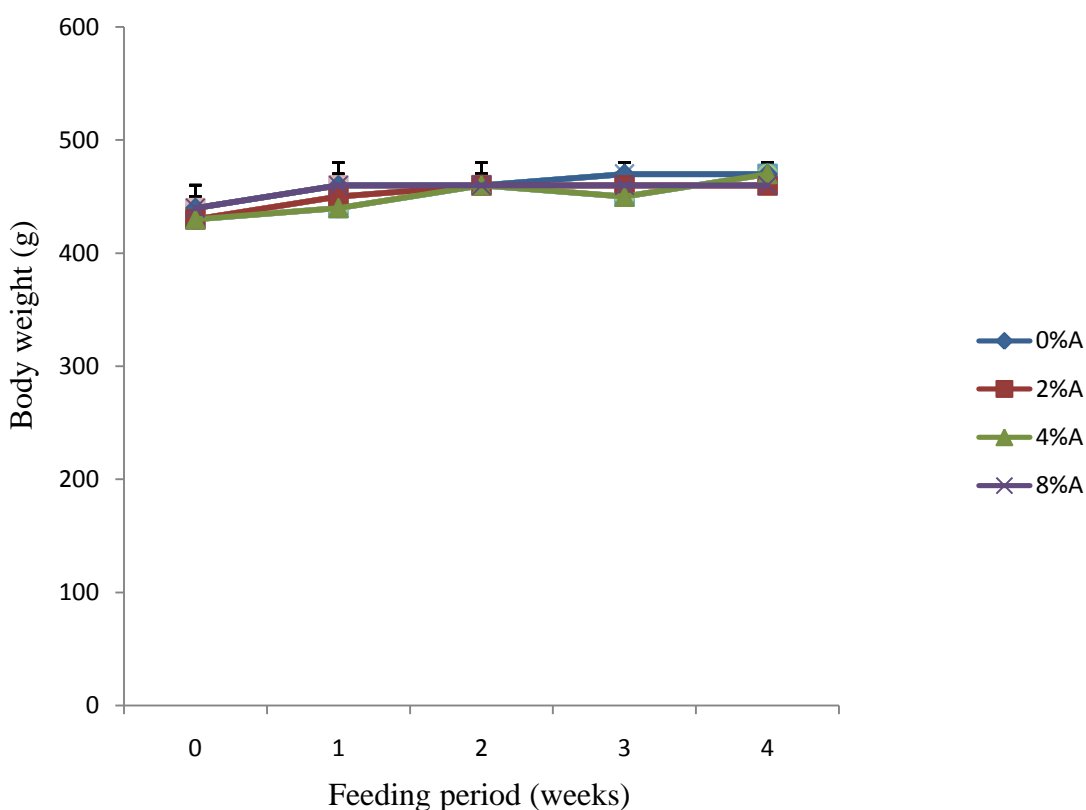


Figure 4.14: Body weight of SD rats offered BD containing avocado seed. Values are presented as mean \pm SEM (n=4). No significant difference was observed between the treated groups and control.

Table 10: Average feed intake per day and body weight gain of SD rats fed BD containing avocado seed for 4 weeks (Experiment 1).

Parameter (g/kg BW)	0% A	2% A	4% A	8% A
<i>Feed intake</i>				
SD offered BD	39.30±8.60 ^a	53.80±11.30 ^a	51.50±8.40 ^a	45.50± 6.50 ^a
<i>Body weight gain</i>				
SD offered BD	56.80±15.00 ^a	93.20±3.00 ^b	85.80±22.00 ^a	58.50± 15.00 ^a

Data are presented as the mean of four observations ±standard error. Means in the same row with different superscripts differ significantly ($p < 0.05$). A: avocado; SD: Sprague Dawley rats; BD; basal diet.

4.11. Feed intake and body weight gain of SD rats offered HSD containing avocado seed (Experiment 2).

The FI of the control reduced gradually from 62 to 33g/kg BW in the first three weeks of the experiment (see Figure 4.15). The inclusion of 2%A in the diet resulted to higher intake by the SD in the first and third week of the experiment, while 4 and 8%A had lower FI in the first two week of the experiment when compared to control. The inclusion of A tended to lower the average feed intake during the feeding period except at 2%A where the intake was higher than control during week 1 and 3. Average FI during the entire feeding trial showed a reduced FI for 8%A (40.05g/kg BW) compared to control (0% A, 46.81g/kg BW, see Table 11).

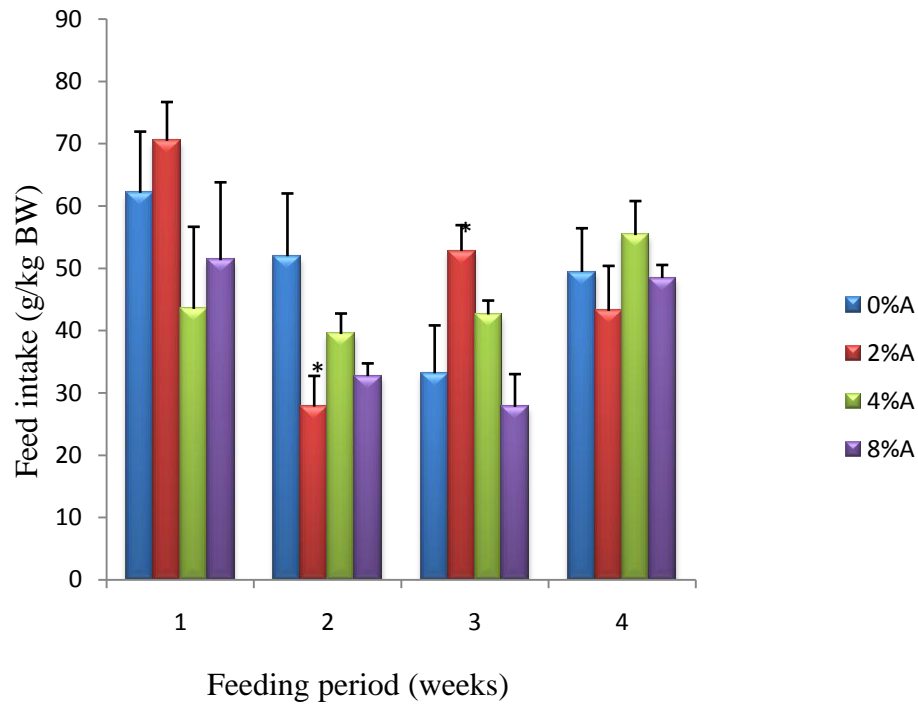


Figure 4.15: Feed intake of SD rats offered HSD containing avocado seed. Values are presented as means \pm SEM (n=4). *: $p < 0.05$ compared to control.

The body weight of rats during the feeding trial is as presented in Fig. 4.16. The gain in weight when expressed to the initial body weight range from 15 to 25%. Inclusion of A lowered the average body weight gain of the experimental groups. Control rats (0%A) had the highest BWG (281.97 ± 36.39 g/kg IBW) followed by 4, 2 and 8%A (200.03 ± 22.61 , 174.34 ± 75.47 and 151.53 ± 24.83 g/kg IBW respectively see Table 11).

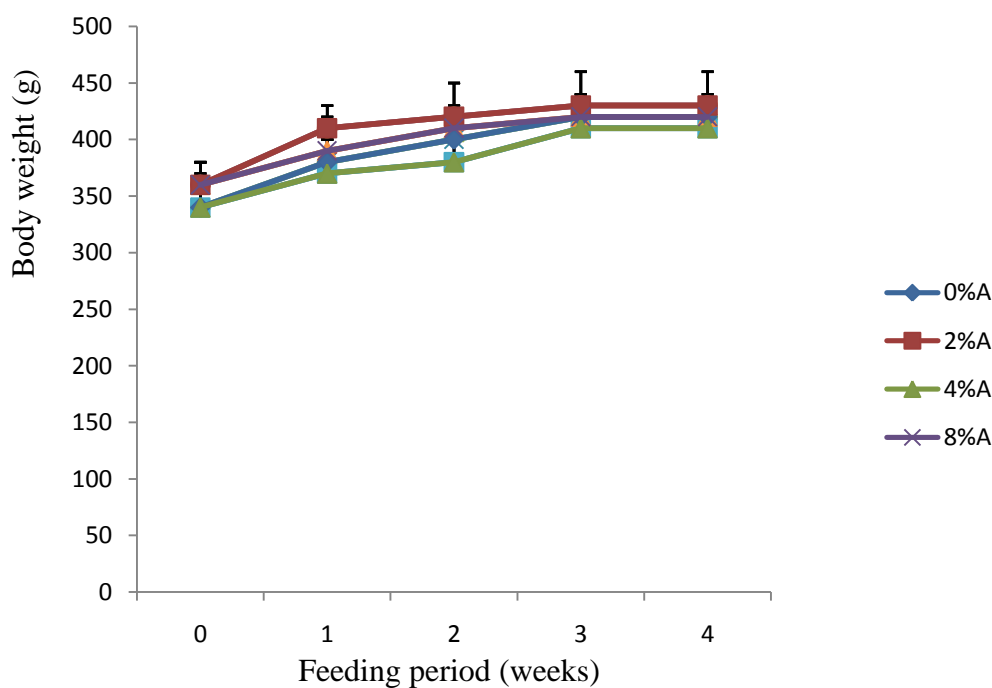


Figure 4.16. Body weight of SD rats offered HSD containing avocado. Values are given as mean \pm SEM. No significant difference was observed between control and the treated groups.

Table 11: Average feed intake and body weight gain of SD rats fed HSD containing avocado seed for 4 weeks (Experiment 2).

Parameter (g/kg BW)	0%A	2%A	4%A	8%A
SD offered HSD	46.80 \pm 5.20 ^a	48.60 \pm 8.90 ^a	45.20 \pm 3.50 ^a	40.10 \pm 5.80 ^a
SD offered HSD	281.90 \pm 36.00 ^a	174.30 \pm 75.00 ^{ab}	200.00 \pm 23.00 ^b	151.30 \pm 53.00 ^b

Data are presented as the mean of four observations \pm standard error. Means in the same row with different superscripts differ significantly ($p < 0.05$). A: avocado; SD Sprague Dawley rats; HSD: high sucrose diet.

4.12. Feed intake and Body weight gain of SHR offered BD containing avocado seed (Experiment 3).

The FI of the control increased gradually from 42 to 76g/kg BW (80% increase) during the first three weeks before reducing to 53g/kg BW in week 4 of the feeding period ($p<0.05$). The FI of feed containing A was similar for all groups in week 1 of the feeding trial but increased by only 12-40% for the next three weeks. The average FI over the period for the experimental groups 2, 4 and 8%A were 49.45, 49.51 and 47.85 g/kgIBW respectively, which were lower than control (58.75g/kg IBW; see Table 12).

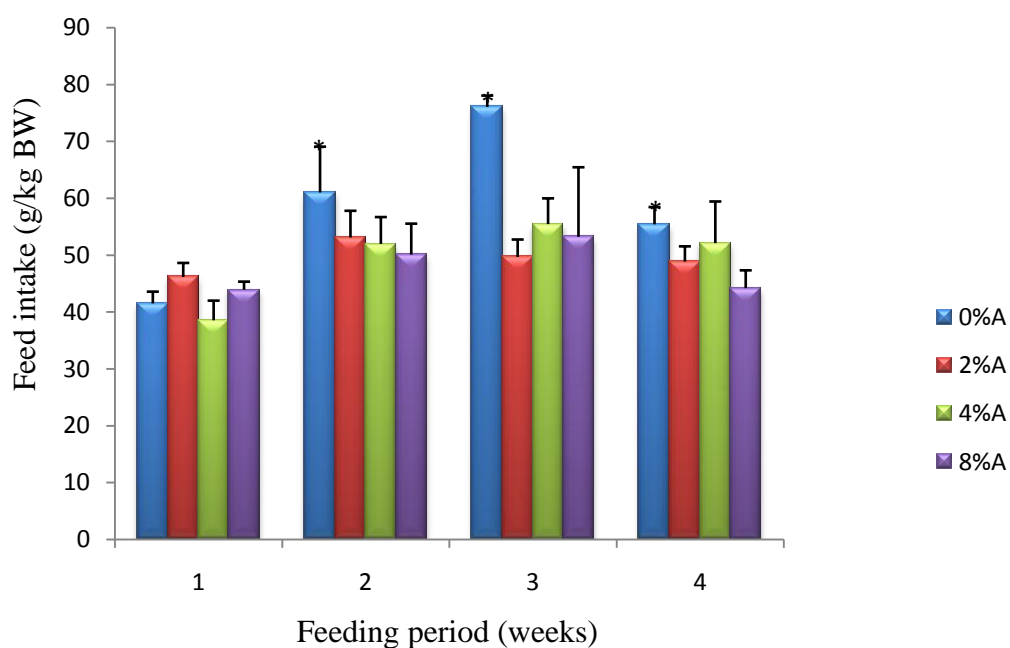


Figure 4.17: Feed intake of SHR offered BD containing avocado seed. Values are presented as mean \pm SEM (n=4). *: $p<0.05$ compared to control.

The body weight gain of SHR ranged between 15 and 20% during the feeding period with rats offered control diet (0%A) gained more than rats offered experimental diets (see Figure 4.18). The average body weight gain (g) to initial body weight (kg IBW) during the feeding trial reduced with increasing inclusion of A in the diet. Control rats had average body weight gain of 134.06 ± 20.53 g/kg IBW whereas 2 to 4 and 8%A fed rats had average body weight gain of 126.44, 118.30 and 112.15g/kg BW respectively (see Table 12).

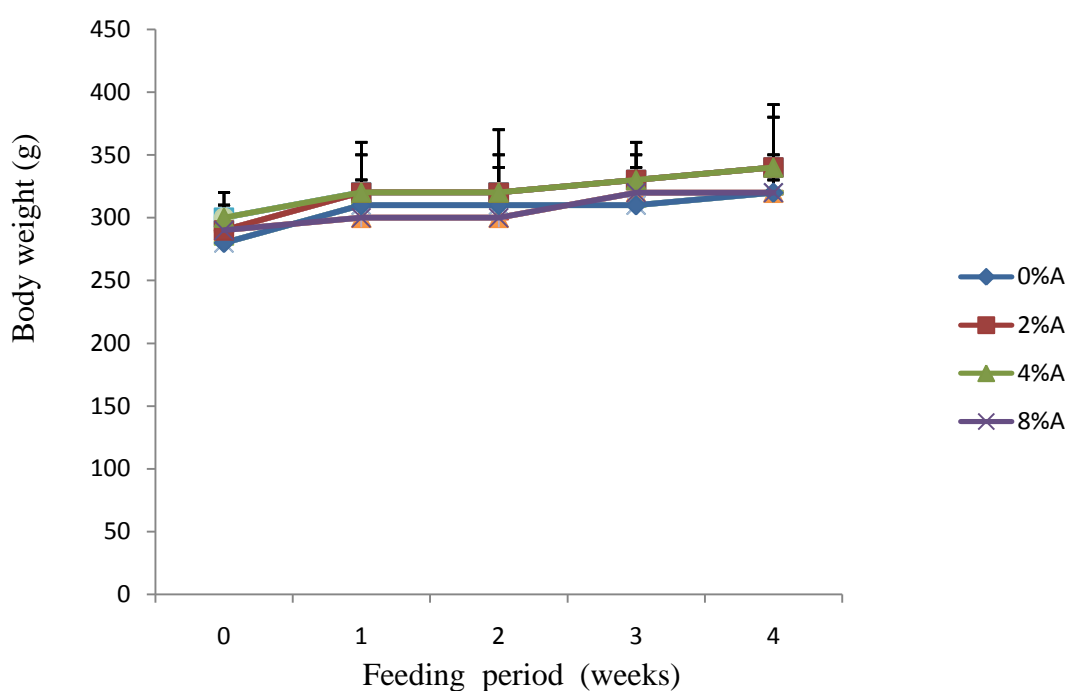


Figure 4.18. Body weight of SHR offered BD containing avocado seed. Values are presented as mean \pm SEM (n=4). No significant differences were observed.

Table 12: Average feed intake per day and body weight gain of SHR fed BD containing avocado seed for 4 weeks (Experiment 3)

Parameter (g/kg BW)	0% A	2% A	4% A	8% A
<i>Feed intake</i>				
SHR offered BD	58.80±7.10 ^a	49.50±1.40 ^a	49.50±3.80 ^a	47.90± 2.30 ^a
<i>Body weight gain</i>				
SHR offered BD	134.10±21.00	126.40±22.00	118.30±19.00	112.2±25.00

Data are presented as the mean of four observations ±standard error. Means in the same row with different superscripts differ significantly ($p < 0.05$). A: avocado; SHR: spontaneously hypertensive rats; BD; basal diet.

4.13. Effect of avocado seed on the digestibility of diets in experiment 1-3.

BD consumed by SD (81.3%) was more digestible ($p < 0.05$) than HSD consumed by SD (76.9%) and BD consumed by SHR (77.0%; see Figure 4.19). The addition of A into both BD and HSD did not affect the digestibility of the diets

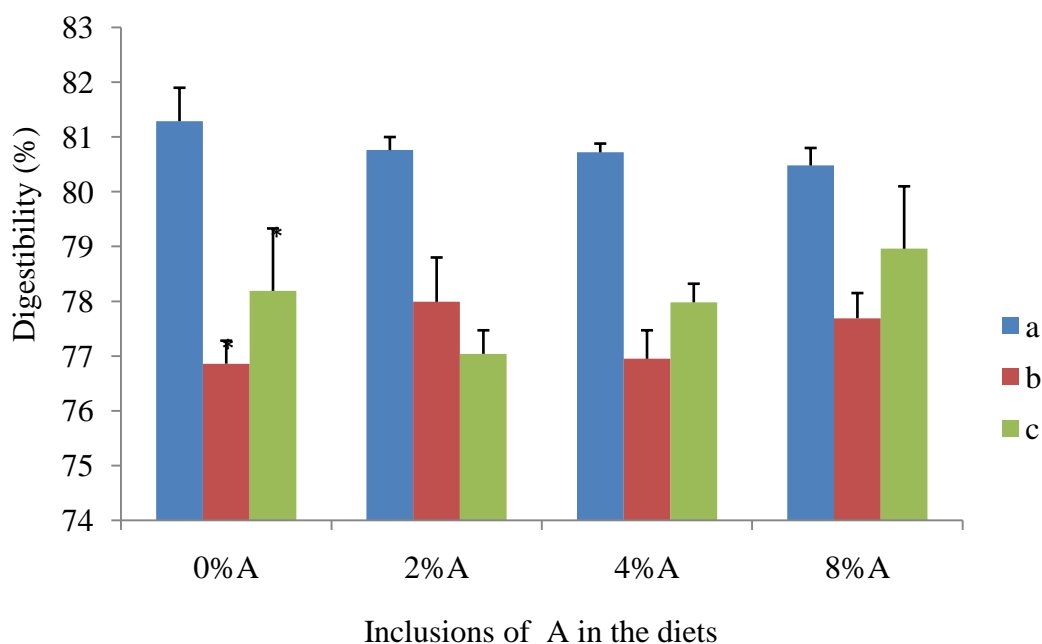


Figure 4.19: The digestibility of feed containing avocado offered to SD and SHR. (a), (b), and (c) represents SD offered BD, SD offered HSD and SHR offered to BD respectively. Values are presented as mean ± SEM (n=4). *: $p < 0.05$ when SD offered BD was compared to SD offered HSD and SHR offered BD.

4.14. Effect of avocado seed on feed conversion efficiency (FCE) of diets in experiment 1-3.

The FCE was best in SD offered HSD (5.8) whereas the FCE of BD was better when consumed by SHR (14.4) than by SD (27.1; see Figure 4.20). The FCE of BD consumed by SD improved when A was included at 2 (16.8± 1.0) and 4% A (15.5±1.6) compared with control (0% A, 27.1±2.3; $p<0.05$). The inclusion of A did not improve the FCE of BD and HSD when offered to SHR and SD respectively.

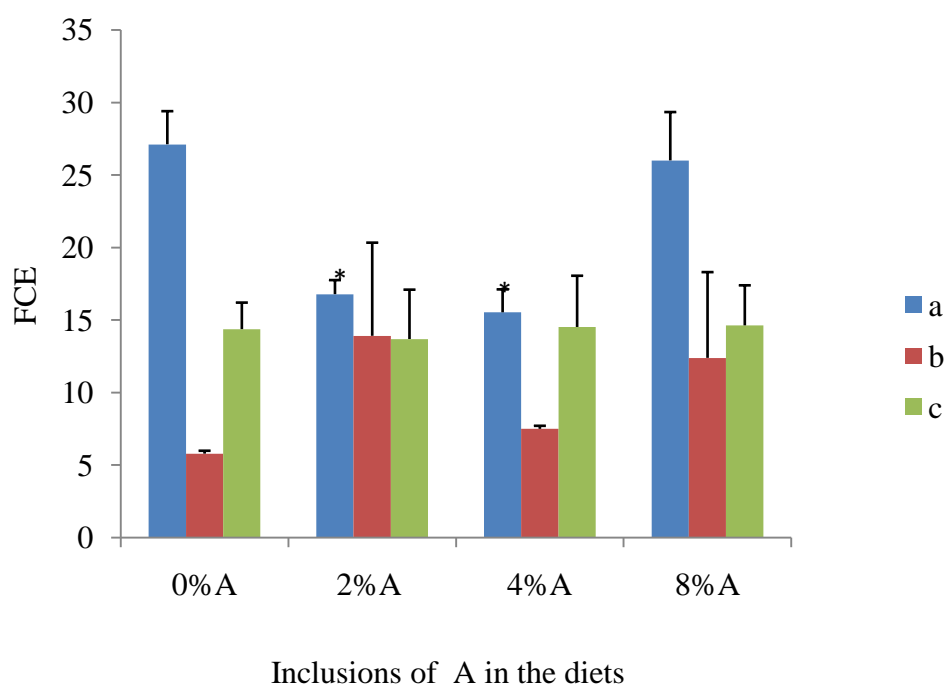


Figure 4.20. The FCE of diets containing avocado offered to SD and SHR. (a), (b), and (c) represents SD offered BD, SD offered HSD and SHR offered BD respectively. Values are presented as mean ± SEM (n=4). *: $p<0.05$ compared to control.

4.15. Effect of avocado seed on protein efficiency ratio (PER) of diets in experiment 1-3.

PER of HSD consumed by SD was the highest followed by BD consumed by SHR and BD consumed by SD (0.19, see Figure 4.21). The PER of the diet was only improved ($p < 0.05$) for BD consumed by SD when A was included at 2 and 4% (0.31 and 0.40 respectively). Inclusion of A at all 3 levels did not cause any change in the PER of SHR offered BD whereas PER of SD offered HSD reduced to between 0.53 and 0.68 ($p < 0.05$).

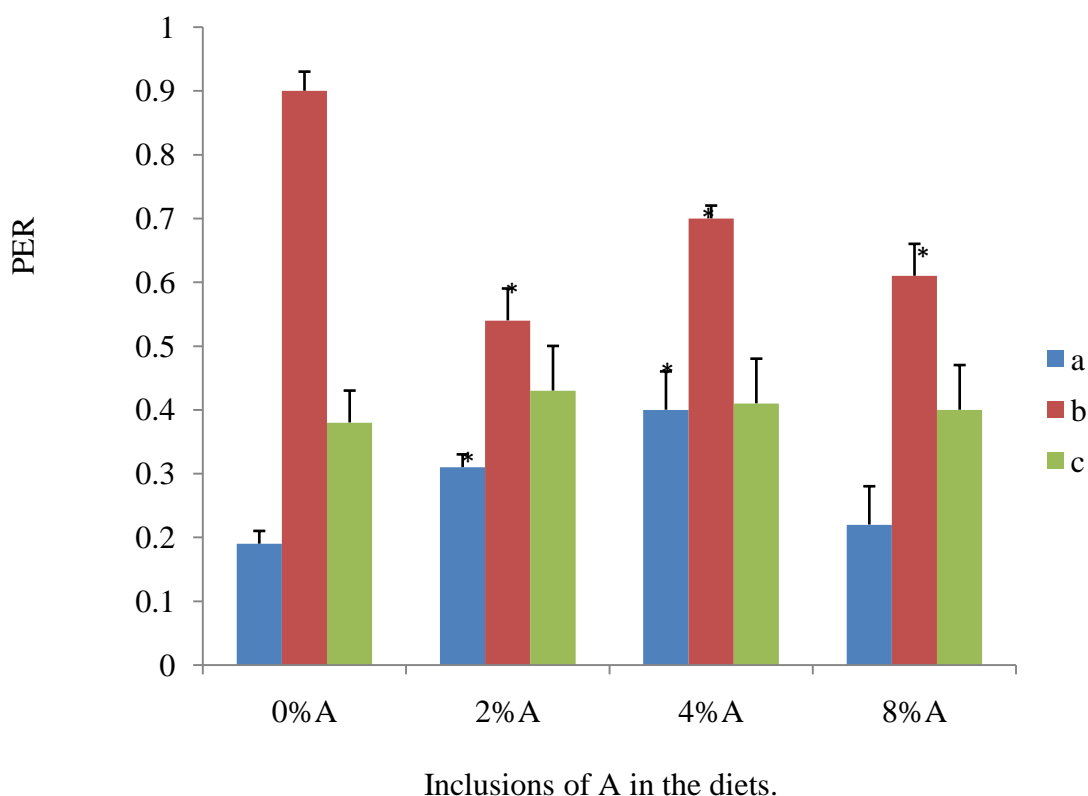


Figure 4.21. The PER of feed containing avocado offered to SD and SHR. (a), (b), and (c) represents SD offered BD, SD offered HSD and SHR offered BD respectively. Values presented as mean \pm SEM (n=4). *: $p < 0.05$ compared to control.

4.16. Serum cholesterol concentration of rats offered diets containing avocado seed in experiment 1-3.

The blood cholesterol was highest in SHR fed BD ($1.18 \pm 0.30 \text{g/l}$) followed by SD fed BD ($0.79 \pm 0.04 \text{g/l}$) and SD fed HSD ($0.47 \pm 0.02 \text{g/l}$). There was an increased reduction ($p < 0.05$) in blood cholesterol of SHR fed BD with increased incorporation of A in the diet. Maximal reduction was achieved at 4% A ($0.27 \pm 0.03 \text{g/l}$). The inclusion of A lowered the blood cholesterol of SD fed BD with significant effect ($p < 0.05$) occurred at 2% A ($0.27 \pm 0.05 \text{g/l}$) and 4% A ($0.48 \pm 0.07 \text{g/l}$) compared to control. The inclusion of A increased the blood cholesterol level of SD offered HSD with significant effects seen at 4 and 8% A (0.63 ± 0.04 and $0.82 \pm 0.09 \text{g/l}$ respectively).

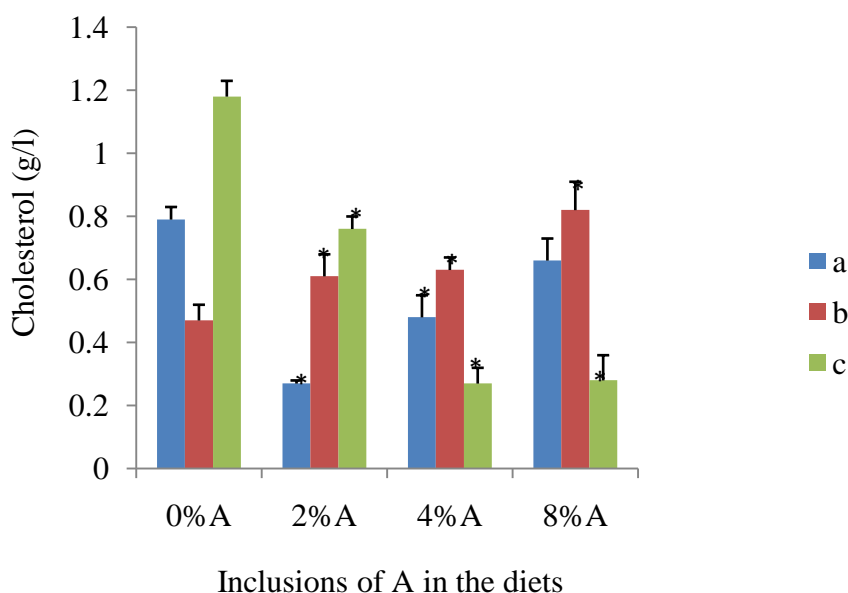


Figure 4.22. The serum cholesterol of SD and SHR rats fed on diets containing avocado seed. (a), (b), and (c) represents SD offered BD, SD offered HSD and SHR offered BD respectively. Values are presented as mean \pm SEM (n=4) *: $p < 0.05$ compared to control.

4.17. Effects of avocado seed on serum glucose concentration of rats offered diets in experiment 1-3.

SD offered BD (41.7 ± 12.5 mg/dl); had the lowest blood glucose than SHR offered BD (93.82 ± 7.34 mg/dl) and SD offered HSD (133.08 ± 31.23 mg/dl; see Figure 4.23). Inclusion of A in BD reduced SD blood glucose at 2%A (21.35 ± 2.29 mg/d; $p < 0.05$) whereas no significant effects ($p > 0.05$) at any level of A inclusion in HSD were observed in SD. The addition of A into BD did not lower but rather increased the blood glucose level of the SHR (values at 4 and 8%A $p < 0.05$).

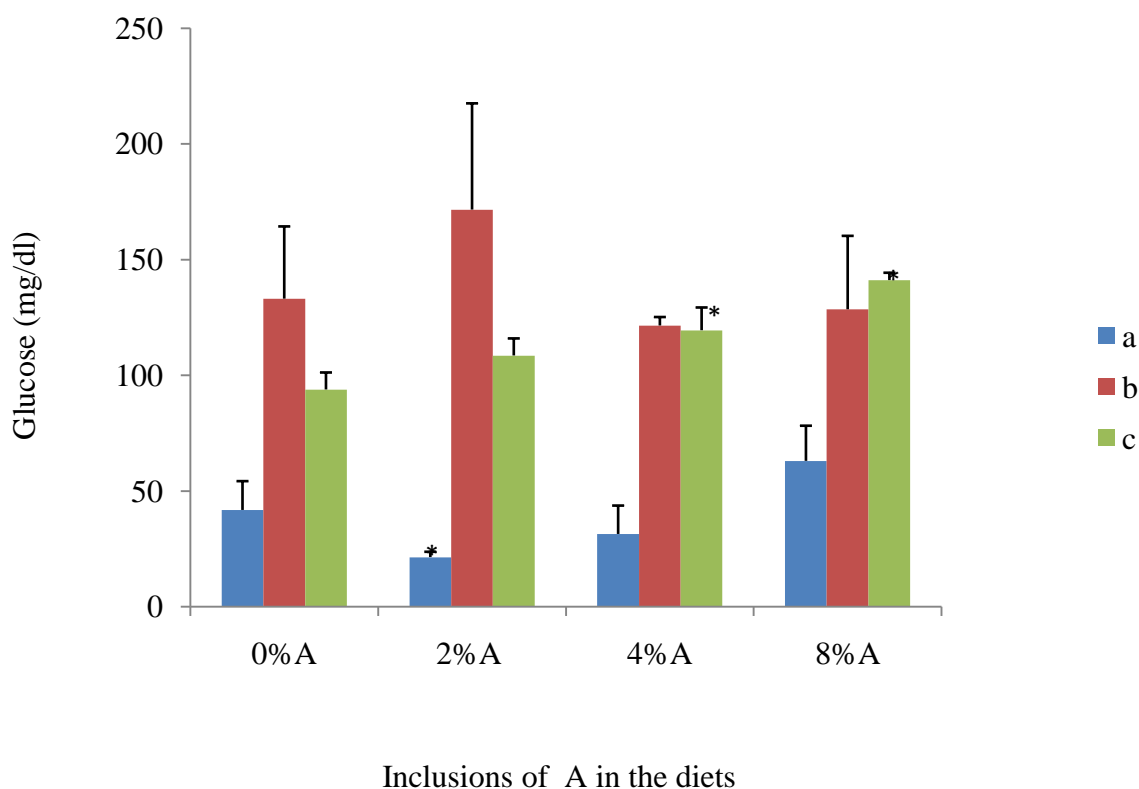


Figure 4.23: The serum glucose concentration of SD and SHR fed on diets containing avocado seed. (a), (b), and (c) represents SD offered BD, SD offered HSD and SHR offered BD respectively. Values are presented as mean \pm SEM (n=4). *: $p < 0.05$ compared to control.

4.18. Effects of avocado seed on liver glycogen content of rats offered diets in experiment 1-3

The liver glycogen content of rats offered BD or HSD was not different ($p > 0.05$) to each other (values ranged from 0.9 – 1.3 mg/g). Increased liver glycogen content attributed to A inclusion in the diets occurred only at 8%A in SD fed BD whereas all avocado seed inclusion levels increased ($p < 0.05$) glycogen storage in SD fed HSD and SHR fed BD.

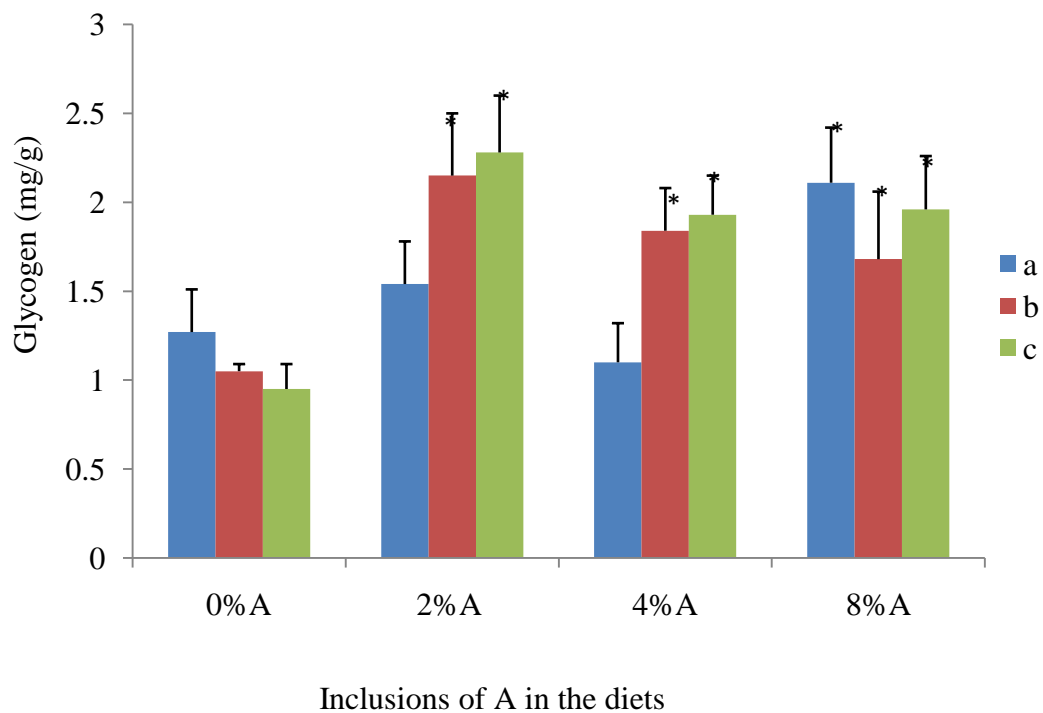


Figure 4.24. The liver glycogen level of SD and SHR rats fed on diets containing A. (a), (b), and (c) represents SD offered BD, SD offered HSD and SHR offered BD respectively. Values are presented as mean \pm SEM (n=4). *: $p < 0.05$ compared to control.

5.0 GENERAL DISCUSSION

5.1 INTRODUCTION

Seeds in general are good sources of carbohydrate and lipids (Imafidon and Okunrobo, 2009). In most instances edible seeds such as groundnut, wheat, corn and rice are readily consumed as food but seeds of fruits like tamarind and avocado contain comparatively less carbohydrate and fat and thus are not palatable and not readily digestible (Olaeta et al., 2007; Batta et al., 2000). In addition, these seeds contain phytochemicals in the form of phytosterols and tannins which may be hazardous if consumed in large amounts (Olaeta et al., 2007). Attempts have been made to include small proportion of these seeds in compounded feed to cows (Garry, 2008), broilers (Aengwanich et al., 2009) and sheep (Bhatta et al., 2000) in which it was demonstrated that these seeds exhibit some antibiotic properties and at low concentration could be beneficial on the production performance of lactating cows.

The present studies explored the promising values of seed components like the lipid and carbohydrate as source of energy, protein as well as bioactive phytochemical to stimulate growth and metabolism. The inclusion of tamarind and avocado seeds was shown to affect feed intake, body weight gain, feed digestibility, protein efficiency ratio and feed conversion efficiency of rats. Other parameters also shown to be affected to varying extents include the blood glucose, blood cholesterol and the liver glycogen content. These observations are discussed further in the following sections.

5.2. Tamarind inclusion in feed on feeding and growth performance in rats.

5.2.1. Effects on feed intake and body weight gain.

Feed intake can be affected by diet palatability, flavour and odour (Jacela et al., 2010) whereas body weight gain is determined by the energy and nitrogen content of food consumed (Tran-Duy et al., 2008). Previous attempts on tamarind seed inclusion in the diet showed lower feed intake in cow (Bhatt et al., 2000) and pig (Mahto, et al., 2010). This has been suggested to be due to the presence of tannin (Gu et al., 2003) in seed, which in general could cause feed avoidance as the % inclusion of seed in the compound feed increased. In the present studies the inclusion of 2-8% tamarind seed in the diets was shown not to affect the feed intake of the SD rats during the period which is agreement with Kumar and Bhattacharya (2008). Nevertheless, there were some variations (10-40%) in the quantity of feed consumed (g/kg BW) per day between control and treatments during the trial and this occurred possibly due the differences in adaptations of the rats to test diets (Shi et al., 1997). In general these variations did not result in significant change in feed intake over the period in normal SD rats, but in SHR animals, the negative effect of the T secondary compounds (tannins) resulted in lowering voluntary feed intake after two weeks of feeding possibly by decreasing palatability of the ration because of tannin astringent effects on the oral cavity (Yang et al., 2001).

The inclusion of T had no effect on body weight gain of SD fed BD although there were some variations in body weight gain between the treated and control which may be attributed to the differences in animal's efficiency in converting absorbed nutrients into body mass (Makni et al., 2008). Previous studies showed that rats fed on high carbohydrate meal (e.g. sucrose) gain more weight than rats fed on normal rat

chow (Hansen et al., 2008) which was also seen in rats fed on HSD in the present study. However the apparent beneficial effect of sucrose on body weight gain was lowered when T was included in the diet. Tannins in these feed may have acted as anti-nutritive factors since it was demonstrated that this plant's secondary compound lower absorption and post digestive assimilation of nutrients into body mass in animals (Simon et al., 1990; Andres et al., 1996). In SHR fed with BD, the inclusion of T lowered the body weight gain of the rats when compared with control and this may be explained by the reduced intake of feed by the SD rats fed with BD containing T (see Table 7).

5.2.2 Effects on digestibility, feed conversion efficiency (FCE) and protein efficiency ratio (PER).

Digestibility is described as the difference in feed intake and fecal excretion in relation to feed intake (Makni et al., 2008). Thus when consumption of feed is high and fecal excretion is high, the value of digestibility is low i.e. feed is not properly digested and/or absorption is impaired. The values of digestibility from the present studies ranged from 78-85% which are comparable to previous report using normal rats (Monia et al., 2006) and hypercholesterolemic rats (Makni et al., 2008). There was no evidence of diarrhoea during the experiment. In the absence of T, BD had higher digestibility than HSD ($p>0.05$) when offered to SD (Figure 4.5). In addition SD digested BD better than could SHR. The inclusion of T has no effect on the digestibility of diets consumed by SD or SHR. To date there are no reports which indicated the negative effects of T inclusion in diet on digestibility. The addition of T in HSD may however provide additional beneficial effect of the secondary compounds of T (i.e. tannins) which was reported to improve on protein digestibility at low concentration (Bressani et al., 1983).

FCE is defined as a measure of an animal's efficiency in converting feed mass into increased body mass, which is expressed as the mass of the food eaten divided by the body mass gain over a specified period of time (Makni et al., 2008). Low values of FCE implies high efficiencies and vice versa. At 0%T the lowest FCE was recorded in BD-fed SD followed by BD-fed SHR and HSD-fed SD. This shows that the same diet may have different effect on animals of different physiological status and that the same animal may perform differently on different diet (i.e. SD vs SHR and BD vs HSD respectively) as reported in several earlier studies (Shi et al., 1997; Imafidon, 2010). At 2% inclusion of T, SD showed increased FCE but this decreased as the inclusion of T increased towards 8%.

The improved FCE observed in the present studies support earlier reports that the addition of tannins in feed improve animal growth performance in domestic animals such as sheep (Amlan and Jyotisna, 2010), cow (Bhatta et al., 2000), chicken (Mahmood et al., 2008) and pigs (Mahto et al., 2010). Such a positive effect of tannin however occurred at low (2%) but not at high (4 and 8%) T inclusion. This suggest the anti-nutritive effects of tannins in T is greater at a higher doses than lower doses which is in agreement with earlier studies (Bhatt et al, 2000) which showed growth and performance was lowered in animals at higher doses than lower dose of tannins.

FCE of SD was higher when fed with HSD than when fed with BD (Figure 4.6) and this occurred despite the fact that the digestibility of HSD was lower than BD when fed to SD. Sucrose is a simple carbohydrate and is easily digested (Lombardo et al., 1996) and once absorbed in the form of glucose and fructose (Ushijima et al., 1991) can be easily stored in various energy depots including glycogen in the liver (see section 5.34). The beneficial effect of sucrose on FCE was reduced in the presence of T.

T contains many secondary compounds including phenolic compounds and phytosterols (Engel and Schubert, 2005). The phenolic compounds, in particular tannins, have been shown to impair macro nutrient utilization by forming tannin-protein complexes with various digestive enzymes responsible for the formation of products absorbable by the small intestine (Andres et al. 1996). Such conditions compromise the digestibility of macronutrients (Silanikove et al., 2001) and subsequently FCE (Dykes and Rooney, 2007).

The effect of T on FCE consistently increased with increasing inclusion of T in the feed, although not as drastic as that seen in BD-fed SD (Figure 4.6). The presence of sucrose may thus play crucial role in minimizing the negative effects of tannins on FCE in the present studies, possibly by its capability to enhance the affinity and selectivity of tannins binding to proteins (Thomas et al., 1987). FCE was also higher in SHR than in SD when both were offered BD. In contrast to SD, SHR did not show significant changes in FCE upon the elevated inclusion of T. This can be explained by the concomitant higher feed intake and body weight gain in SHR at 0%T than at 2, 4 and 8% T (see Table 5). Thus, although FCE was not affected by the increased T inclusion in the diet, the negative effect of high T may still be observed on the feed intake and body weight gain.

Protein efficiency ratio relates the body weight gain over the protein consumed with the implication that a high PER value indicates an efficient feed as a protein source (Monia et al., 2006). At 0%T, the lowest value of PER was observed in SD fed with BD followed by SHR fed with BD and SD fed with HSD. There was evidence of improved PER in BD-fed SD but it occurred only at 2%T (Figure 4.6). Further increment of T

inclusion in the diet had only minimal effects on improving PER. Inclusion of tannin in diets has been reported to increase digestibility of protein and this enhancement could be explained by the formation of tannin-protein complexes in the stomach (Andres et al. 1996). The formation of these complexes was reported to reduce feed and protein degradation (Hervas et al., 2003) and even internal gastro internal parasitism (Gatechew et al., 2007). Taken together tannins could improve feed intake and the proportion of feed protein available for lower digestive tract digestion and absorption. Absence of improved PER at higher T inclusion in the diet could have happened as a result of negative effect of tannins at higher concentration (Barry and Manley, 1986). In this regard there could be a limit of T inclusion in the diet before the elevation of secondary compounds in T exerts their negative effects on growth performance.

There was no effect on PER of SHR when fed with BD. The highest PER seen in HSD-fed rats compared to BD-fed rats can be attributed to the high body in relation to feed intake (see Table 5). The T inclusion reduced the PER of the HSD-fed rats which is in agreement to the negative effects of T on FCR (Figure 4.8) as described earlier. In summary T inclusion in the diet did not have a profound effect on PER improvement. In fact T inclusion in the diet may even reduce PER when the diet also contains sucrose.

5.3 The effect of tamarind seed on serum cholesterol level in rats.

The cholesterol content in animals is the sum total of high density lipoproteins (HDL), low density lipoproteins (LDL), and very low density lipoproteins (Pearson et al., 2003). Cholesterol is required to build and maintain membranes such that low level of cholesterol can be detrimental (John et al., 2007). The total cholesterol content estimation shows the amount of cholesterol present. The rats blood cholesterol ranged from 0.5g/l to 1.2g/l when fed diet not containing T. High total cholesterol

content (>2.5g/l) indicates hypercholesterolemia while values less than 2.0g/l is considered normal (American Heart Association. 2006). The inclusion of T in the diet reduced blood cholesterol in SD and SHR fed BD, with significant effect achieved at all level in SD and 2%T in SHR respectively. The lowering of blood cholesterol as a result of T inclusion in the diet may be due to the phytochemicals present. T seed contains phenolic compounds (Aengwanichet al., 2009) such as phytosterols (Engel and Schubert, 2005) in a concentration of 590mg/kg dry weight (Leroux et al., 2002).

These phytosterols, in particular the beta-sitosterols, are known to induce a decrease in plasma lipoprotein and cholesterol levels (Ikeda et al., 1998), by decreasing the cholesterol solubility and absorption across the intestinal barrier (Heinmann et., 1993; Wasan et al., 2001). This lowering effect is based on the fact that the higher phytosterols hydrophobicity are more readily mixed with bile salt and acid micelles (Piironen et al., 2000) than can animal cholesterol (Elkin et al., 2009) resulting in the excretion with the faeces a greater part of unabsorbed cholesterol, particularly the low density lipoprotein (Pouteau et al., 2003).

SD fed HSD showed mixed response of blood cholesterol levels with elevation occurred at 4%T ($p<0.05$) in contrast to lowered blood cholesterol at 2 and 8% inclusion. Such discrepancies may be attributed to animal variations in responses to the presence of T in the diet. SD fed with HSD are expected to be hypercholesterolemic as demonstrated in other reports of animals fed on high sucrose diet (Hansen et al., 2008)

5.4 The effect of tamarind seed on serum glucose level.

Blood sugar concentration in the body has a range of 64.8-104.4mg/dl in a normal person (American Diabetes Association. 2008b). The blood glucose is the

primary source of energy in the body obtained from carbohydrates breakdown (Kaye et al., 2002). Elevated or reduced blood glucose occurred as a result of certain conditions like illness, stress, surgery or the intake of a particular substance (Daly et al., 1998). Phytosterols can have blood glucose lowering capacity (Monia et al., 2006) and for tamarind it was suggested to achieve this directly by inhibiting intestinal absorption of glucose (Beppu et al., 2006).

In the present study the inclusion of T did not affect the glucose content of SD fed with BD and SHR fed with BD except at 4%T in SHR, where the serum glucose level was reduced when compared to control. This suggests that T may not disrupt normal glucose metabolism when the diet does not contain excess carbohydrate. T however appeared not to be a strong suppressor of blood glucose elevation due to excess carbohydrate in the diet (i.e. HSD-fed SD) because the inhibition occurred only at 8%T but not at 2 and 4%T inclusion. It could be possible that at higher T inclusion glucose absorption may be affected by other T secondary component which is reported to be dose dependent (Engel and Schubert, 2005).

The effects of T on blood glucose may also be dependent on the physiological conditions of the animals (Roske et al., 1990). The difference in response of these rats reflects the important of several facets of blood glucose homeostasis. Phytochemicals in the form of phytosterols (Delaney et al., 2004), polyphenols (Naczki et al., 2004) and flavonoids (Yao et al., 2004) may cause an increase in the pancreatic secretion of insulin from the beta cells or its release from bound form (Standley, 2004). The effects on insulin may indirectly enhance increased peripheral utilization of glucose (Erah et al., 1996). In addition the intestinal enzymes may also be inhibited by polyphenols (Monia et al., 2006).

5.5 The effect of tamarind seed on liver glycogen content.

Glycogen is stored in the liver or muscles (Miwa and Suzuki, 2002) and functions as secondary energy storage in animals (Saladin, 2007). Glycogen is reconverted back to glucose by glycogenolysis in low energy state (Pedersen et al., 2008). Glycogen storage could be enhanced by various processes such as increased glucose intake or use of additives or compounds like the phytosterols which could enhance glycogen storage (Monia et al., 2006). In the present studies the inclusion of T had positive effects on glycogen storage. Glycogen storage in the liver induced by T occurred in dose dependent manner. This improved liver glycogen storage could be attributed to the presence secondary compounds in T. Phytosterols for instance increases pancreatic secretion of insulin which lowers glucose and enhances glucose incorporation into glycogen in the liver for regulation of blood glucose (Monia et al., 2006). It may be suggested that in HSD-fed rats the lower T inclusion (2 and 4%) may not be sufficient to suppress elevated blood glucose but are effective enough to enhance deposition of glucose into glycogen.

5.6. Avocado inclusion in feed on feeding and growth performance in rats

5.6.1 Effects on feed intake and body weight gain of rats

Animals responds differently to different diets (Clements and Wainwright, 2007; Imafidon, 2010). In the present studies the inclusion of 2-8% avocado seed in the diets had no effect on feed intake of the rats during the trial. The small differences in daily feed intake (g/kg BW) between treatments and control (5-10%; $p>0.05$) may be regarded as adaptation responses of the rats to different test diets. However the adaptation response to the inclusion of A seed in rat chow diet may not be a good explanation to the profound effects of either increase (Salazar et al.,

2005), decrease (Devalaraja et al., 2011; Imafidon, 2010) or no effects (Imafidon et al., 2009) on growth and performance. The presence of tannin (e.g. 2.45gkg⁻¹; Agostini, 1995) in avocado seed, (as also mentioned in tamarind seed see section 5.2.1) may partially influence the feeding and growth performances, particularly when the effects appeared to be dose dependent (see Figure 4.20).

The inclusion of A in BD fed to SD did not affect the body weight gain but however the increased body weight gain observed in the treated group could be attributed to the increased feed intake in the group (Lee et al., 2003). In HSD experiment, the inclusion of T lowered the body weight gain which suggests that there is an impaired assimilation of nutrients (reduced feed efficiency) from ingested feed when A was added. This phenomenon was also observed in tamarind supplementation studies (See section 5.2.1).

Control animals in BD-fed SHR had higher feed intake and gained more weight than those which consumed A. The presence of A could have lowered the feed intake which resulted to lower weight gain. This supports the earlier report that the negative effect of secondary compounds of A (tannins) could lower intake by decreasing palatability of the ration because of its astringent effect on the oral cavity (Yang et al., 2001), as was also seen in tamarind seed studies (see section 5.2.1).

5.6.2 Digestibility, feed conversion efficiency (FCE) and protein efficiency ratio (PER) of feed containing avocado seed.

The digestibility of the diets supplemented with avocado seed in the experiment ranged from 77-82% which are comparable to the values obtained in tamarind supplementation studies (see section 5.2.2). In the absence of T, BD had higher digestibility than HSD ($p>0.05$) when offered to SD (Figure 4.19) and SD digested BD

better than could SHR. The inclusion of T has no effect on the digestibility of diets consumed by SD or SHR.

At 0%T the lowest FCE was recorded in BD-fed SD followed by BD-fed SHR and HSD-fed SD, and these results are similar to the result obtained when tamarind seed was used instead. At 2 and 4% inclusion of T, SD showed increased FCE but this decreased at 8%. This improved FCE is in agreement to the positive effect of tannins as reported in the tamarind supplementation studies (see section 5.2.2) This positive effect of tannin however occurred at low (2 and 4%) but not at high (8%) T inclusion, suggesting the anti-nutritive effects of tannins may cause detrimental effects on growth performances at high concentration.

FCE of SD was higher when fed with HSD than when fed with BD (Figure 4.20) which is comparable to those seen in tamarind seed experiment section (5.2.2). The beneficial effect of sucrose on FCE in HSD was also reduced in the presence of A which could also have resulted due to the presence of tannins in the avocado seed (Thomas et al., 1987). This supports the earlier report on activities of tannins on sucrose as described earlier in section (5.2.2).

The maximum lowering effect of A on FCE was recorded at the highest inclusion of (8%) (see Figure 4.20). This also supports our earlier report that sucrose may reduce the negative effects of tannins on FCE as described earlier in section (5.2.2). FCE was also higher in SHR than in SD when both were offered BD. In contrast to SD, SHR did not show significant changes in FCE upon the elevated inclusion of A. Avocado seed inclusion may thus be regarded as not detrimental to feeding and growth performances when the inclusion rate was as high as 8%.

The lowest value of PER was observed in SD fed with BD followed by SHR fed with BD and SD fed with HSD, this is comparable to the results obtained with tamarind seed experiment. PER was improved in BD-fed SD at the inclusion of A, although at highest inclusion the effect of A was minimal. The presence of tannin may again play its role in enhancing PER as described earlier in section (5.2.2). The inclusion of A seed which reduced the PER of the HSD-fed rats is in line with the negative effects of A on FCR (Figure 4.19). In general, the inclusion of A improved on PER of BD when offered to SD but was reduced at presence of sucrose as described earlier in section (5.2.2). There was no effect on PER of SHR when fed with BD.

5.7. The effect of avocado seed on serum cholesterol level in rats.

The rats blood cholesterol ranged from 0.5g/l to 1.2g/l when fed diet not containing A. The inclusion of A in the in the diet reduced blood cholesterol in SD fed with BD, with significant effect achieved at 2 and 4%T. The lowered cholesterol levels may be attributed to phytosterols contained in A (e.g. 826mg/kg; Gebhardt and Thomas 2002).

This supports the earlier report on phytosterol-cholesterol lowering ability in rats as described earlier (see section 5.2.3).

There was an increase in the cholesterol levels of SD fed with HSD containing A. At 2 and 4% inclusion, the increase in cholesterol was similar but at 8% the cholesterol level increased drastically. These changes may be attributed to animal variations in responses to the presence of A since it is also expected that SD fed with HSD contains high cholesterol (Hansen et al., 2008). This was also seen in tamarind seed experiment as described earlier (see section 5.3).

5.8. The effect of avocado seed on serum glucose level.

The inclusion of T did not affect the glucose content of SD rats except at 2% A in SD fed with BD, where the glucose level was lowered when compared to control. In SHR fed with BD, it appeared that the inclusion increased the glucose level. The increase in glucose level may also be attributed to animal variations in response to the presence of A although it is expected that animals fed on diets containing phytosterols had lower blood glucose (Monia et al., 2006). This effect may also be dependent on the physiological conditions of the animals (Roske et al., 1990), as described in tamarind seed experiment see section (5.4) since it was not seen in SD rats.

5.9. The effect of avocado seed on liver glycogen content.

The inclusion of A in rat diet enhanced the glycogen storage in the liver. In SD fed with BD the greatest effect was at the highest inclusion while in SD fed with HSD and SHR fed with BD, the highest effect was at 2%A inclusion. This illustrates the sensitivities of rats to the phytochemicals in A seed to be dependent on the physiological status of the animals as described earlier in tamarind seed experiment (see section 5.5).

5.10 Possible implications of avocado or tamarind on human nutrition.

Traditional practices of using plant seeds to cure diseases (Anapaula et al., 2007) gave an insight to a possibility of the seed serving as a source of nutrients to stimulate growth and metabolism (Monia et al., 2006), since most plant seeds are good source of protein, fiber, carbohydrates and phytochemicals (Maria et al., 2004). In recent times plant seed are used to supplement natural food product especially when a particular food substance is required (Monia et al., 2006). Most of these seed contains phenolic compounds such as the tannins (Bhatta et al., 2000) and phytosterols (Beppu et al.,

2006). In addition they also contains small amount of hydrogen cyanide which are less harmful and are reduced significantly by cooking or heating (Emmy et al., 2010).

In present studies, it was observed that tamarind or avocado seed supplemented diet could modulate glucose and cholesterol metabolism, and enhance glycogen storage in the rat liver. It was suggested that this modulation could have resulted due to the presence of the phytosterols (Monia et al., 2006). The inclusion of the seeds in the diet may partially influence the feeding and growth performance in rats particularly when the effects appeared to be dose dependent. It was concluded that this positive effect of the seed on growth and performance could have resulted to the presence of tannins (Simon et al., 1990; Andres et al., 1996).

Avocado has the potential to be further considered as a good food supplement in managing hypercholesterolemia and hyperglycemia. This is especially so since avocado seed contains abscisic acid (ABA) (Maria et al., 2004) which can ameliorate the symptoms of type II diabetes because it targets peroxisome proliferator-activated receptor gamma in a similar manner as the thiazolidinediones class of anti-diabetic drugs (Bassaganya-Riera et al., 2010). It remains to be seen whether the effective use of avocado seeds in this regard is also accompanied by the desirable minimal or absence of side effects.

The ability of plant seeds to lower cholesterol and glucose in rats has also been experimented in the humans. Imafidon (2010) reported that plant seed such as mistletoe could lower the blood glucose and cholesterol level in human. The author also suggested that the cholesterol and blood glucose levels were lowered due to the presence of phytosterols. Similarly, David and Shirley (2005) also reported that phytosterols

obtained from plant seeds could to lower cholesterol levels in human. Extracts of avocado pulp have been shown to reduce blood cholesterol levels, blood glucose, and risk of prostate and breast cancer in human (Imafidon and Amaechina, 2010). The present studies have thus provided insights in the possible dietary uses of avocado and tamarind seeds in people with hyperglycemia and/or hypercholesteremia.

6.0 CONCLUSIONS

The results of the present investigation has explored the promising values of seed components like the phytosterols and tannins as source of energy, protein as well as bioactive phytochemical to stimulate growth and metabolism. The inclusion of the T or A in the diet may partially influence the feeding and growth performance particularly when the effects appeared to be dose dependent. The T or A inclusion also lowered the cholesterol levels of the rats although there were mixed response in the cholesterol levels when sucrose was added since it was expected that rats fed on high sucrose diet are hypercholesterolemic. The inclusion of T or A did not have a profound effect on blood glucose but the presence of the seeds could suppress high blood glucose especially when sucrose was added. The presence of the T or A improved liver glycogen storage capability of the rats. It could be concluded that the T or A can be used to modulate carbohydrate and lipid metabolism and enhancement of liver glycogen storage capacity rats. The present studies have thus provided insights in the possible dietary uses of avocado and tamarind in people with hyperglycemia and/or hypercholesteremia.