2. REVIEW OF LITEATURES

2.1 Medicinal Plants

Natural feed additives of plant origin are believed to be safer, healthier and less regarded than synthetic additives (antibiotics). It was estimated that there are 250000-500000 species of plants on earth (Borris, 1996; Hashemi and Davoodi, 2010). Recent bans and restrictions on the use of animal antibiotic growth promoters stimulated interest in bioactive secondary metabolites of plant source as alternative performance enhancers (Greathead, 2003). Many scientists have searched for alternatives to antibiotics through utilization of the extracts or leaves of some of these plants (Longhout, 2000; Mellor, 2000; Wenk, 2000; Kamel, 2001; Alcicek *et al.*, 2003; Zeweil (2008) and Zeweil *et al.* 2013). The supplementation of spices and herbs could have many benefits to broilers health and performance such as having antioxidative potential (Hui, 1996), antimicrobial activity (Dorman and Deans, 2000), enhancing digestion by stimulating endogenous enzymes (Brugalli, 2003). Naidoo *et al.* (2008) demonstrated that antioxidant rich plant extracts have potential benefits in treating coccidial infections.

Herbs and herbal products are incorporated in poultry diets to replace synthetic products in order to stimulate or promote the effective use of feed nutrients which may subsequently result in more rapid body weight gain, higher production rates and improved feed efficiency. Moreover, active components of herbs may improve digestion and stimulate the immune function in broilers (Ghazalah and Ali, 2008). Steiner (2009) stated that medicinal plants and essential oils extracted from these plants are becoming more important due to their antimicrobial effects and the stimulating effects on the animal digestive systems. The microflora of the small intestine is made up mostly of lactic-acid producing bacteria (Engberg *et al.*, 2000). Lactic acid is the fermentation byproduct of lactic-acid producing bacteria and the increase in lactic acid concentrations in the poultry gastrointestinal tract that causes the pH to drop, and thus preventing the colonization of certain pathogens (Zhang *et al.*, 2003). Also, Rahimi *et al.* (2011) observed that plant extracts can increase the number of lactic acid bacteria in the ileal and ceacal contents of broilers. It has been shown that the dietary incorporation of herbs and their associated essential oils may provide beneficial effects on poultry performance and health due to the antimicrobial activity of their phytochemical components (Lee *et al.*, 2004b). However, other studies have not found positive effects of herbs and their related essential oils. These latter findings may be related to experimental conditions, such as hygiene and dietary agents (Lee *et al.*, 2003a).

2.1.1. Garlic (*Allium sativum*)

Garlic (*Allium sativum*) is the most important specie of the onion genus, *Allium* belonging to the family *Alliaceae* (Lonzotti, 2006: Eric, 2010). It is readily available and widely used around the world as it can be grown year-round (Charlson and McFerren, 2007). It is the second most widely consumed spice in the world and its popularity has been boosted by the growing awareness of its health benefits (FAO, 1992). Garlic is well known for its dietary and medicinal applications (Lawson, 1998).

Garlic consists of several organo sulfur constituents such as glutamyl cystein, sallyl cysteine, alliin, allicin and ajoene (Agarwal *et al.*, 1996). It is believed that allicin is the primary active compound responsible for inhibiting cholesterol biosynthesis (Gebhardt *et al.*, 1994) Freshly crushed garlic (*Allium sativum*) contains allicin, alliin, ajoene, diallylsulfied, dithiin, s-allylcysteine (Onu, 2010). Intact garlic bulbs contain alliin (S-allyl cysteine sulfoxide), the precursor of allicin, which is hydrolyzed by enzyme allinase upon

crushing to its active form, the allicin (S-allyl-2-propenthiosulphinate). Many studies have indicated that allicin is the most potentially active component of garlic that is responsible for its characteristic odour, flavour as well as most of its biological properties (Chowdhury *et al.,* 2002 and Heinrich *et al.,* 2004). Several reports of *in vitro* antibacterial (Indu *et al.,* 2006), antifungal (Adetumbi *et al.,* 1986; Durak *et al.,* 2002) antiviral and anti-cancer (Weber *et al.,* 1992; Durak *et al.,* 2002) activities of fresh and freeze-dried garlic extract are well documented. Essential oil of garlic has also been reported for its antioxidant potentials (Hui, 1996). Sovova and Sova (2004) have suggested possible medicinal benefits of garlic on cardiovascular system in preventing high blood pressure, accumulation of cholesterol on the vascular walls and atheriosclerosis. Prasad *et al.,* (2009) reported that garlic can prevent fat- induced hyperlipemia.

Dietary garlic paste was reported for its effectiveness in reducing cholesterol level in serum of laying hens and egg yolk (Chowdhury *et al.,* 2002). Sklan *et al.* (1992) observed depressed hepatic cholesterol concentration in chickens when 2% garlic was fed for 14 days. Similar effects of garlic were found in rats fed diets containing either cholesterol or triglyceride (Myung *et al.,* 1982). According to Konjufca *et al.* (1997), a decrease of about 28% in liver cholesterol was found in birds fed garlic, copper, and garlic–copper mixture in comparison with the control. Ademola *et al.* (2009) reported that garlic alone or in combination with ginger have no significant effect on haematological indices of broilers.

2.1.1.1. Garlic in animals Studies:

In poultry nutrition, garlic is known to result in improved growth (Onibi *et al.,* 2009; Mahmood *et al.,* 2009), inhibition of growth of pathogens in the gut (Ahsan *et al.,* 1996; Sarica *et al.,* 2005), enhanced pancreatic function (Adibmoradi *et al.,* 2006), and improved meat and carcass quality (Kim *et al.,* 2009).

Garlic can be used as a feed additive in broiler diets as it able to improve weight gain and reduce feed conversion ratio (Singh *et al.,* 1998; Avato *et al.,* 2000; Lewis *et al.,* 2003; Carrijo *et al.,* 2005; Mahmood *et al.,* 2009). This performance improving property is attributed to the antibacterial properties of allicin and ajoene. Maluf *et al.* (2008) reported that ajoene (4,5,9-trithiadodeca-1,6,11-triene 9-oxide), an organic sulphur compound, has antimicrobial properties. Therefore, feeding diets containing ajoene may inhibit the growth of entero-pathogenic bacteria, thus contributing on the balance of gut microbial populations (Harris *et al.,* 2001) and resulting in a better growth performance (Lewis *et al.,* 2003; Adibmoradi *et al.,* 2006). *In vitro* studies have shown that garlic extract has antimicrobial effects, such as antibacterial and antifungal properties (Indu *et al.,* 2006). Numerous studies reported that garlic can be used effectively to inhibit the growth of enteropathogenic bacteria, including 20 different serogroups of *Escherichia coli*, serotypes of *Salmonella*, and *Aeromonas hydrophila* (Johnson and Vaughn, 1969). Earlier *in vitro* studies have shown that garlic extract have strong antibacterial properties against *Escherichia coli*, *Salmonella typhimurium* (Johnson and Vaughn, 1969), *Vibrio cholera* (Ahsan *et al.,* 1996), *Shigella dysenteriae*, *Shigella flexneri*, *Staphylococcus epiderdimis*, *Enterobacter aerogenes* (Arora and Kaur, 1999), *Bacillus subtilis*, *Micrococcus* (Sharma *et al.,* 1977), *Clostridium botulinum* (De Wit *et al.,* 1979), and antifungal properties against *Aspergillus niger*, *Candida albicans* (Yoshida *et al.,* 1987), *C. tropicalis*, *C. acutus*, and *C. inconspicua* (Arora and Kaur, 1999).

In a study using broiler chickens, Sarica *et al.* (2005) have investigated that supplementation of 1.0 g/kg garlic meal reduced the concentrations of total aerobic bacteria and *E. coli* in the small intestine. Supplementation of garlic meal was reported to

improve proliferation of absorptive cells in the gut. Dietary garlic supplementation increased villus height, crypt depth and ratio of villus height to crypt depth (Adibmoradi *et al.*, 2006). Yason *et al.* (1987) stated that the crypt can be regarded as the villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue. An improvement in either villus height or crypt depth may lead to an increase in nutrient absorption and better utility. In a product evaluation study, it was reported that supplementing diets of laying hens with 30 g/kg garlic meal did not affect the egg flavour (Birrenkott *et al.*, 2000). However, a study using male Arbor Acre broiler chickens showed that dietary supplementation with 20 g/kg garlic husk or garlic bulb increased the thigh meat hardness and flavor scores (Kim *et al.*, 2009). Similarly, Onibi *et al.* (2009) reported that dietary supplementation of 0.5 g/kg garlic meal increased the garlic aroma but not the palatability of the meat of 7 weeks old Shaver Starbo broiler chickens. These data suggest that supplementing broiler diets with garlic meal can enhance eating quality because sensory panels found that the meat from chickens fed a garlic-supplemented diet had better texture and flavour than the meat from control diets. On the other hand, no severe toxic side effects were reported in these clinical studies, even at high dosages. Other garlic supplements do not have studies of toxicity or safety, and few have any clinical studies to confirm their efficacy (Amagase *et al.*, 2001).

Those positive effects of garlic were mainly attributed to the bioactive components of garlic, including sulfur-containing compounds such as alliin, diallylsulfides, and allicin (Amagase *et al.*, 2001), which may be altered during processing. The enzyme allicinase sequestered within garlic cell vacuoles plays a key role in reaction of the chemical substrate and can be changed by temperature, duration of drying, use of polar and nonpolar extraction solvents, and condition and period of maceration before final extraction. Therefore, the different garlic processing methods might be one of the reasons for the inconsistent results observed in various studies (Cullen *et al.*, 2005; Tatara *et al.*, 2005; Yan *et al.*, 2010). Today, there are many different garlic-based products on the market with different chemical composition (Yu et al., 1994; Velíšek *et al.*, 1997).

2.1.1.2. Effect of garlic on growth performance:

2.1.1.2.1. Live body weight and weight gain:

The use of garlic as natural feed additives in rabbit nutrition was rarely has not been substantially investigated because of the availability of low costs and proven effective synthetic growth-promoting feed additives.

The enhanced body weight by feeding mixture of garlic and ginger powder was observed in the findings of Ahmed and Sharma (1997) who reported significant increase in body weight gain of rats fed a mixture of garlic and ginger.

Onu and Aja (2011) reported an improvement in growth rate of rabbits fed garlic supplemented diets compared to the control diet. These results were in line with the findings of Ortsergu *et al.* (2008) and Ademola *et al.*, (2005) who reported an increase in weight gain of rabbits and broilers fed garlic supplemented diets respectively.

Cullen et al. (2005) and Janz et al. (2007) reported that pigs fed a garlic-treated diet through the grower-finisher period had higher average daily gain compared to the control diets. Grela et al. (1998) observed a significant improvement in daily gain with the use of an herb mixture (great nettle, garlic, wheat grass) in the diet of pigs from 25 to 105 kg.

In the study of Lewis *et al.* (2003) reported that garlic extract increased body weight gain in broilers between 7-27d. In broilers, it was reported that garlic, as a natural feed additive, improved broiler growth and decreased mortality rate (Tollba and Hassan, 2003). Improvement of broilers performance and carcass merits can be achieved by

supplementation of diets with garlic powder (Demir et al., 2003; Sivam, 2001; Amagase et al., 2001). Also, Yin and Cheng (2003) reported that garlic has been found to demonstrate improve productive performance of broiler chicks

Khan et al. (2008) reported that dietary garlic powder had better effects on layer performance. Onibi et al. (2009) concluded that powdered garlic at 0.5% level may be incorporated as a growth promoter in the ration of Japanese quail. These results also are in line with those reported by Ahmad (2005) who reported higher weight gain in broilers fed rations supplemented with garlic.

Mansoub et al. (2011) reported that garlic have positive effects on body weight gain performance of broiler chicks from 0-42 days of age. The same results were presented by Canogullari et al. (2009), Kumar (2005) and Afsharmanesh et al (2008) they reported the positive effect of garlic on broiler performance.

Khan *et al.* (2012) examined the potentials of feeding dried garlic powder to male broiler chicks (Cobb-500). One hundred and sixty, one-day-old broiler chicks were randomly allocated to four treatments. The first group fed basal diet free of garlic powder and served as control group, whiles the second, third and fourth groups fed 1, 2 and 3 g of dried garlic / Kg diet, respectively. Their results indicated that garlic powder significantly ($P \leq 0.05$) improved body weight gain in birds given 2 and 3 g garlic powder / Kg diet as compared to the group had 1 g garlic powder in their diet and control group.

Oladele et al. (2012) reported that garlic meal supplementation at 0.125% in the diet of commercial broilers improved body weight gain of broiler chickens.

Al-Kassie and Al-Qaraghul (2013) obtained that the diet with garlic extract at 35ppm/kg improved body weight gain of broilers and decreased the mortality rate when compared with control group.

Chowdhuury *et al.* (2002) added different levels of garlic to layers diet. They reported no significant effects of this supplement on growth performance.

Raeesi *et al.,* 2010 stated that garlic powder at different levels (0.5 to 3%) had no significant effect on weight gain during the first 21 days of feeding trial compared to that of control birds. However, for the period from 22 to 42 days, garlic level at 1% resulted in the highest gain weight. For the whole feeding period garlic levels of 1 and 3% significantly increased body weight gain as compared with 0.5% garlic supplemented groups but it was not significant in comparison with control group.

Bamidele and Adejumo (2012) fed growing pullets basal diet free of additives and different experimental diets containing 0.50 % garlic and 0.50 % ginger, 1.00% garlic and 0.50% ginger mixture, 1.50% garlic and 0.75% ginger mixture and 2.00% garlic and 0.75% ginger. The results showed that the mixtures had no significant ($P \leq 0.05$) effect on growth performance and are also considered non toxic as shown by the White Blood Cell (WBC) count.

2.1.1.2.2. Feed intake and feed conversion ratio:

Using pigs, Cullen *et al.* (2005) reported that the addition of garlic to the diets of grower-finisher pigs had reduced feed intake and improved feed conversion ratio as compared to the control group. Also, Janz et al. (2007) reported that pigs fed a garlic-treated diet through the grower-finisher period had the higher daily feed intake and the best feed conversion ratio compared to the control diets. Grela et al. (1998) observed a significant improvement in feed conversion ratio with the use of an herb mixture (great nettle, garlic, wheat grass) in the diet of pigs from 25 to 105 kg.

Chowdhuury *et al,* (2002) added different levels of garlic to layers diet. They reported no significant effects of this supplement on feed intake and feed efficiency.

Lewis *et al.* (2003) reported that garlic extract improved feed conversion rate (FCR) in broilers between 7-27d.

Choi et al. (2010) found that, in addition to its antimicrobial activities, garlic has been shown to increase feed palatability and thus feed intake of the chicken.

The results presented by Raeesi *et al.* (2010) reported that birds received garlic for the whole of the experimental period had higher feed intake as compared to the control group fed diet free of garlic powder. They added that supplementation of 1% garlic powder, decreased feed conversion ratio (FCR) compared with 0.5% supplemented and control group. Generally, birds received 3% garlic powder in their diet had better feed conversion ratio than the control group.

Mansoub et al. (2011) reported that garlic have positive effects on feed conversion ratio of broiler chicks from 0-42 days of age.

Khan *et al.* (2012) examined the potentials of feeding dried garlic powder to male broiler chicks (Cobb-500). One hundred and sixty, one-day-old broiler chicks were randomly allocated to four treatments. The first group fed basal diet free of garlic powder and served as control group, whiles the second, third and fourth groups fed 1, 2 and 3 g of dried garlic / Kg diet, respectively. The results showed that garlic powder did not affect feed intake. However, feed efficiency was significantly ($P \leq 0.05$) improved by birds in the groups given 2 and 3 g garlic powder / Kg diet as compared to the group had 1 g garlic powder in their diet and control group.

Oladele et al. (2012) reported that garlic meal supplementation at 0.125% in the diet of commercial broilers improved feed conversion ratio of broilers by increasing villi length, villi width and cryptal depth.

In the study presented by Al-Kassie and Al-Qaraghul (2013) obtained that the diet with garlic extract at 35ppm/kg improved feed efficiency ratio of broiler chicks when compared with control group.

2.1.1.2.3. Effect of garlic on carcass characteristics:

Using garlic powder in broilers' diet had no significant effect on performance but it influenced meat quality and carcass yield positively (Horton *et al.* (1991). Similar findings of higher dressed weight in broiler fed garlic have been previously reported by (Cross *et al.*, 2007).

Ahmad (2005) demonstrated a non-significant effect on broiler dressing percentage values due to the inclusion of garlic in the diet of broilers.

Dieumou *et al.* (2009) showed that the effects of ginger and garlic essential oils on organ weights and carcass characteristics were not affected by the treatments, except for a decrease ($P< 0.05$) in relative liver weight of birds fed garlic oil treatment compared with those given ginger oil and control.

Carcass yield was higher in birds fed garlic. Diets supplemented with 1% garlic powder had higher carcass yield than those which received 0.5 and 3 % (Raeesi *et al.* 2010). Supplementation of garlic powder in finisher diet, resulted in higher carcass yield ($p<0.001$) than those which received garlic in starter diet or for whole of the experiment. Thigh yield was also higher in birds received garlic in their starter diet than finisher diet ($p<0.001$). Breast yield was also higher in groups received garlic in their finisher diet than others ($p<0.001$). Relative weight of bursa was significantly higher in 3% supplemented groups. Relative weight of liver was higher in control and 3% supplemented groups (Raeesi *et al.* 2010). When birds fed garlic in their starter diets, they showed higher relative liver weight ($p<0.001$) (Raeesi *et al.* 2010). Relative weight of gizzard was significantly higher in control groups (Raeesi *et al.* 2010). Relative weight of spleen also

was higher, when starter diet was supplemented with garlic, while relative pancreas weight was higher when birds received garlic in starter and also for the whole of the experiment.

Mansoub *et al.* (2011) reported that garlic have positive effects on carcass traits and and blood biochemical parameters of broiler chicks from 0-42 days of age.

Khan *et al.* (2012) examined the potentials of feeding dried garlic powder to male broiler chicks (Cobb-500). The results indicated that the dressed weight was significantly ($p<0.05$) improved by birds given 2 and 3 g garlic powder / Kg diet as compared to the group had 1 g garlic powder in their diet and control group. Group given 3 g garlic powder / Kg diet had higher ($p<0.05$) antibody titre against ND (4552 ± 24), IB (5.50 ± 0.7) and IBD (4.25 ± 0.6) compared to other groups except, the group had 2 g garlic powder.

Jimoh *et al.* (2012) observed that the garlic supplement elicited significant ($P<0.05$) depression of the abdominal fat pad of the broiler birds at 1.0 g / kg supplementation level in the diets compared to the control treatment.

2.1.1.2.4. Effect of garlic on digestibility coefficients of nutrients:

Issa and Omar (2012) reported that the apparent digestibility of total tract DM, CP and EE was improved ($P < 0.05$) by the addition of the garlic powder in broiler diets compared to that in the control diet. These findings are in agreement with previous research of Hernandes *et al.* (2004) who showed that plant extract supplementation improved apparent whole tract digestibility of the nutrients.

Supplementation of garlic meal was reported to improve proliferation of absorptive cells in the gut. Dietary garlic supplementation increased villus height, crypt depth and ratio of villus height to crypt depth (Adibmoradi *et al.*, 2006). Yason *et al.* (1987) stated that the crypt can be regarded as the villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue. An improvement in either villus height or crypt depth may lead to an increase in nutrient absorption and better utility.

Oladele *et al.* (2012) reported that garlic meal supplementation at 0.125% in the diet of commercial broilers recorded the highest ($p<0.05$) mean villi length, villi width and cryptal depth indicating increased absorptive surface area.

The primary mode of action of phytochemicals as growth-promoter is attributed to the growth inhibition of harmful intestinal microflora in the gastrointestinal tract (Benjilali *et al.*, 1984; Juven *et al.*, 1994; Hammer *et al.*, 1999; Lopez *et al.*, 2005; Islam *et al.*, 2006) and by stimulating function of digestive organ, e.g. the pancreas and small intestine (Jang *et al.*, 2004). Windisch and Kroismayr (2007) reported that reduction on the population of enteropathogens results in a more stabilized microflora that will indirectly stimulate functions of digestive organs and reduce microbe-host competition for nutrients. The mechanism by which the phytochemicals exert their antimicrobial activity consists of interactions with the microbial cell membranes of microorganisms by changing permeability for cations such as H+ and K+ (Cabuk *et al.*, 2006). The antimicrobial compounds are quickly exerted by determining structural alterations of the cell envelope. Population of enteropathogen microbes which are known to less resistant to this antimicrobials activity will decreased, while many beneficial microbes, such as *Bifidobacterium* spp. and *Lactobacillus* spp. are relatively resistant (Di Pasqua *et al.*, 2007; Ouwehand *et al.*, 2010).

Another mechanism of actions which proposed for active compounds in herbal products as growth promoters are related to their oxidation-resistant activity (Faix *et al.*, 2009; Zhang *et al.*, 2009) and improvement of the immune system (Emadi and Kermanshahi, 2007; Yarru *et al.*, 2008; Najafi and Torki, 2010), thereby stimulating animal's growth.

2.1.1.2.5. Effect of garlic on blood parameters:

2.1.1.2.5.1. Effect of garlic on serum blood constituents:

The chronic effects of garlic on lipid metabolism in rats were also encouraging. The duration of these studies was at least 4 weeks. Garlic (1–4% in diet) and garlic protein administration in hypercholesterolemic rats induced by a high-cholesterol diet, significantly reduced serum cholesterol, triglyceride and LDL cholesterol (Chang and Johnson, 1980; Rajasree et al. 1999; Chi et al., 1982) but there was no effect on serum HDL. Total lipid content and cholesterol levels in liver were also decreased in rat after chronic garlic consumption. Abramoviz et al. (1999) investigated the effect of allicin as an active component of garlic on the formation of fatty streaks in aorta and lipid profile in mice. While no significant differences were observed between blood lipid profiles, the microscopic evaluation of formation of fatty streaks in the aortic sinus showed that values for mice in the allicin treated groups were significantly lower by nearly 50%.

Several groups of investigators (Jain, 1975; Betz and Weidler, 1989) studied the effects of long term (2–9 months) feeding of garlic and garlic preparations (2% garlic powder in diet) on experimental atherosclerosis induced by a high-cholesterol diet in rabbits. Most of these studies reported a statistically significant reduction in atheromatous lesions, particularly in the aorta, that averaged about 50%.

Qureshi *et al.* (1983a) indicated that male broilers fed diets containing the equivalent of 1, 2, 4, 6 and 8% garlic paste showed significant serum cholesterol reduction reached to 18, 21, 24 and 25%, respectively, as compared to the control group.

Horton *et al.* (1991) reported that supplementation of 1 g garlic powder / Kg diet resulted in non-significant effect on concentrations of cholesterol and triglyceride as compared to the control group over a 35-d growth period. Garlic has been found to reduce oxidative stress (Cavallito *et al.* 1994).

However, several clinical reports have shown that garlic has cholesterol-lowering effect in animals due to the presence of sulphur containing bioactive compounds in its homogenates. Konjufca *et al.* (1997) reported that although performance was not affected when broiler diets were supplemented with 1.5, 3 and 4.5% garlic in powder form, their serum and liver cholesterol decreased significantly.

Chowdhury *et al.*(2002) showed a linear decrease in total serum cholesterol with increasing levels of garlic (0, 2, 4, 6, 8 or 10%) in laying hens fed on wheat maize based diet.

It was reported that serum cholesterol was decreased with feeding garlic to layers (Sivam, 2001; Amagase *et al.,* 2001 and Lewis *et al.,* 2003).

Tropentag, (2008) observed that feeding of raw or sun dried garlic to broiler at 2% significantly reduced the serum lipid and are opposing present findings.

Khan *et al.* (2007) reported that cholesterol concentration significantly (P 0.05) decreased with increasing levels of dietary garlic. Also, Khan *et al.* (2012) showed a significant reduction in male broiler chicks (Cobb-500) serum cholesterol (120 ± 02), low-density lipoprotein (83±01) and triglycerides (50 ± 02) and increased in high-density lipoprotein (29 ± 01) was observed in the group had 2 g garlic powder / Kg diet compared to other groups. The difference between 2 g and 3 g garlic powder / Kg diets was however insignificant. These findings demonstrated that garlic powder can potentially be used in rabbits and broiler production to improve the immune status, growth performance and to optimize lipid profile. Some studies, however, suggested that commercial garlic oil, garlic powder and commercially available garlic extract may be hypocholesterolemic Songsanga *et al.* (2008).

Bamidele and Adejumo (2012) fed growing pullets basal diet free of additives and different experimental diets containing 0.50 % garlic and 0.50 % ginger, 1.00% garlic and 0.50% ginger mixture, 1.50% garlic and 0.75% ginger mixture and 2.00% garlic and 0.75% ginger. The results of the study revealed that garlic and ginger mixtures at the supplemented levels significantly ($p<0.01$) reduced the total cholesterol and Low-Density Lipoprotein (LDL) cholesterol of the growing pullets. The experimental diets containing 1.00% garlic and 0.50% ginger mixtures and 2.00% and 0.75% ginger mixtures had the best results for total cholesterol and LDL cholesterol of the growing pullets. Garlic has hypocholesterolemic effects on chickens through inhibition of the most important enzymes that participate in the synthesis of cholesterol and lipids (trihydroxy- tri-methyl-glutaril coenzyme A reductase, cholesterol-7-.-hydroxylase and the synthesis of fatty acids). In addition, this additive has a relatively low price (Stanacev *et al.*, 2011).

Jimoh *et al.* (2012) found that garlic supplement caused hypocholesterolemia and reduction in blood level of triglyceride of the birds. These effects were dose-dependent. It was noticed that with the increasing level of supplementation, garlic significantly ($P<0.05$) decreased serum concentration of triacylglycerol at 2 .0 and 2.5g / kg supplementation levels. Total cholesterol and low - density lipoprotein (LDL) cholesterol were not significantly ($P>0.05$) affected but high -density lipoprotein (HDL) cholesterol levels in the broilers blood slightly increased in all the groups fed garlic supplemented diets.This showed that garlic powder supplemented in the experimental diets exhibited anti-lipogenic and anti-cholesterogenic effects on the birds. This indicated that the garlic powder has modulatory effect on cholesterol and lipid metabolism and can elicit hypocholesterolemic effects.

Significant decrease ($p<0.05$) occurred in serum total cholesterol level for broiler chicks given diet with garlic extract at 35ppm/kg as compared with the control group (Al-Kassie and Al-Qaraghul, 2013).

2.1.1.2.5.2. Effect of garlic on blood hematological parameters:

Ademola *et al.* (2009) reported that the red blood cells and haemoglobin concentration of broiler chickens were not affected by dietary garlic. A reflection of the blood – thinning potential of garlic was observed as decrease in platelets values was obtained from the birds fed with the garlic supplemented diets. The result is in agreement with the early report by Apitz - castro *et al.* (1983) which stated that garlic inhibits platelets aggregation. The platelets inhibitory properties of garlic have also been reported by Foster (2008). On the other hand, Jimoh *et al.* (2012) clearly shown that haematological parameters were not significantly affected by garlic supplementation of the broilers' diets.

2.1.2. Ginger (*Zingiber Officinale*)

Ginger *Zingiber Officinale* (Roscoe) is an underground rhizome plant that belongs to the family *Zingibeaceae* and now it is considered a common constituent of diet worldwide (Sertie' *et al.*, 1991) and widely used as a spice. The genus *Zingiber* was named after the Sanskrit word *zindschi* (hornshaped) by the English botanist William Roscoe (1753-1831), in a report published in 1807 (Roscoe, 1807).

Ginger, probably, originates from South-East Asia. The ancient Greeks and Romans brought the rhizome to Southern Europe. Already in the (11th) century, it is mentioned in Anglo-Saxon veterinary pharmacopoeias and leech books. In the (13th) century, it was well known in all of Europe, and the Spanish established first plantations in the West Indies (mainly Jamaica) and in Mexico in the 16th century. Nowadays ginger is cultivated in the tropical parts of the world, from Asia to Africa, and large parts of South

and Central America; mainly in India, in southern China, Indonesia, Nepal, and Nigeria. The best quality is said to come from Jamaica (Köhler, 1887; Wichtl, 2002).

Ginger is a medicinal plant which is widely used all over the world. The main important compounds in Ginger (*Zingiber officinale*) are gingerol, gingerdiol and gingerdione which have the ability to stimulate digestive enzymes, affect the microbial activity (Dieumou *et al.*, 2009) when used in broiler diets. The pungent taste of ginger is caused by gingerol (Jolad *et al.*, 2004; Shariq *et al.*, 2011) which contains an enzyme called "zingibain" that aids digestion (Adulyatham and Owusu-Apenten, 2005). Gingerol, gingerdiol, and gingerdione possess strong antioxidant activity (Kikuzaki and Nakatani, 1996; Nakatani, 2000; Rababah *et al.*, 2004; Akhani *et al.*, 2004) reported that ginger pretreatment inhibited the induced hyperglycemia and hypoinsulinaemia. Other investigators (Sharma *et al.*, 1996) have showed that the hypolipidemic effect of ginger. Also it act as antimicrobial (Akoachere *et al.*, 2002; Jagetia *et al.*, 2003; Mahady *et al.*, 2003), and has various pharmacological effects (Chrubasik *et al.*, 2005; Ali *et al.*, 2008). Immuno-modulatory, antitumori-genic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and antiemetic properties are among the other therapeutic effects of ginger observed (Badreldin *et al.*, 2008). Powdered rhizome of ginger has long been used as traditional medicine to alleviate the gastrointestinal illnesses (Afzal *et al.*, 2001).

Ginger extracts have shown to exhibit antibacterial activity in *invitro* studies (Malu *et al.*, 2008; Indu and Nirmala, 2010). Ginger has been found to enhance pancreatic lipase activity (Platel and Srinivasan, 2000), intestinal lipase, disaccharidase, sucrase and maltase activities of rats (Platel and Srinivasan, 1996). All of these have favorable effects on gut function, which is the primary mode of action for growth promoting feed additives (Windisch et al., 2008).

2.1.2. 1. Ginger in animals studies:

In animal studies, phytochemicals in ginger rhizome have been reported to stimulate growth performance (Ademola *et al.*, 2009), and inhibit the growth of pathogenic bacteria (Smith-Palmer, 1998; Nanasombat and Lohasupthawee, 2005) of broiler chickens. Supplementing diet with ginger meal resulted in a better growth performance, as shown by the improvement to body weight (Ademola *et al.*, 2009; Zhang *et al.*, 2009), improvement of feed intake (Onimisi *et al.*, 2007), reduction of feed conversion ratio (Moorthy *et al.*, 2009), and stimulation of water consumption of broiler chickens (Onimisi *et al.*, 2007). *In vitro* studies showed that phytochemical substances in ginger rhizome inhibit the growth of *Campylobacter jejuni*, *Staphylococcus aureus* (Smith-Palmer, 1998), *Listeria monocytogenes* (Thongson *et al.*, 2004; Ekwenye and Elegalam, 2005), *Escherichia coli* (Gupta and Ravishankar, 2005; Nanasombat and Lohasupthawee, 2005), *Salmonella choleraesuis* (Nanasombat and Lohasupthawee, 2005), *Salmonella enteritidis* (Smith-Palmer, 1998; Nanasombat and Lohasupthawee, 2005), *Salmonella typhimurium* DT104 (Thongson *et al.*, 2004; Ekwenye and Elegalam, 2005), and *Klebsiella pneumonia* (Nanasombat and Lohasupthawee, 2005). Moreover, supplementation of 10.0 mg/kg ginger extract has been reported to inhibit the growth of microflora, such as *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, and *Candida albicans* (Jagetia *et al.*, 2003). Phytochemicals in ginger rhizome were also found to have antifungal properties toward various fungi including *Botrytis cinerea*, *Fusarium oxysporum*, *Mycosphaerella arachidicola*, and *Physalospora piricola* (Wang and Ng, 2005).

2.1.2. 2. Effect of ginger on poultry and animal nutrition:

2.1.2. 2. 1. Effect of ginger on body weight and weight gain:

Ahmed and Sharma (1997) reported significant increase in body weight gain of rats fed a mixture of garlic and ginger.

The study of Onu and Aja (2011) indicated that ginger could effectively be added to rabbit ration to improve of the quality of the feed and body weight gain of the weaned rabbits.

Ibrahim *et al.* (2011) reported that adding 0.75 % ginger in the diet of rabbits that received 90 % of the protein requirement, insignificantly ($P \leq 0.05$) improved the final body weight, total body weight gain and average daily weight gain compared to their control.

Using adult rat, Bhandari *et al.* (2005) reported that ethanolic ginger extract caused a decrease in body weight in adult male rat. Nammi et al. (2009) used adult male Wistar rats (140–150 g) in their studies; the rats were weight-matched and divided into six groups, each consisting of six rats. The normal- and the high-fat diet control groups received the standard diet and the high-fat diet, respectively, and treated with vehicle (1% sodium CMC) by oral gavages once daily for 6 weeks. The Z.officinale 100, 200 and 400 groups received the high-fat diet and treated, respectively, with 100, 200 and 400 mg/kg of Z. officinale extract by oral gavages once daily for 6 weeks, while the positive control group (rosiglitazone) received the high-fat diet and treated with 3 mg/kg of rosiglitazone by oral gavages once daily for 6 weeks. The obtained results showed that by the end of 3–4 weeks, Z. officinale extract treatment significantly suppressed the body weight gain by 17–23% compared with the animals fed with high-fat diet alone and this difference was more pronounced by 27–34% and gained higher significance after 6 weeks. This was contrary to the findings of Onimisi et al. (2005) who observed that ginger increased body weight when included in the diet up to 2% level in the diet.

According to El-Deek *et al.* (2002), there were no significant differences in body weight when 0.05% of ginger powder used in broiler diets (El-Deek *et al.*, 2002). The same researchers also in the trial 2 added 0.1% of ginger powder into the broiler diets but they did not find any effect on the body weight. Farinu *et al.* (2004) however reported slight improvement in the growth performance of broilers fed ginger supplements at the levels of 5, 10, 15 g/kg.

Al-Homidan (2005) reported that broilers fed 2 and 6% of ginger powder into the broiler diets did not find any effects on the final body weight when compared to the control group.

Ademola *et al.* (2009) showed that using 1 and 1.5% of ginger in broiler diets had no significant effect on the body weight, while adding 2% of ginger had significant ($P< 0.01$) negative impact on the body weight compared to the control group. They added also that mixtures of garlic and ginger significantly improved the growth of the chicks than garlic and ginger as sole agent in broiler diets.

Also according to the researchers Moorthy *et al.* (2009), adding 0.2% ginger into the broiler diets improved body weight significantly ($P< 0.05$) compared to the control group, over 42 of age.

There was no improvement in body weight of broiler chickens fed 2% of dried *Zingiber officinale* rhizomes supplement when compared to the chickens fed the control (Thayalini *et al.*, 2011). In addition, Zhao *et al.* (2011) reported that dietary supplementation of ginger powder improved laying performance.

Mohamed *et al.* (2012) reported that body weight was significantly (P< 0.05) improved by the supplementation of dietary ginger powder at levels 0.1and 0.2% compared to the control group, being 2020.83, 2075.90 and 1875 g/bird respectively during 42 days of age.

Fakhim *et al.* (2013) found that body weight gain of the chickens in the starter groups (1 to 10 day), grower groups (11 to 21 day), finisher groups (22 to 42 day) and during (1 to 42 day) of treatment was not significantly different among the experimental groups and control. This was similar to the findings of Garcia et al. (2007), Ghazaiah et al. (2007) and Tollba et al. (2007) who observed no difference in body weight gain in broilers fed with ginger extract for a period of six weeks.

Barazesh et al (2013) fed broiler chicks (Ross) diets containing different levels of ginger powder ratios of 0, 0.5, 1, 1.5 % . They observed that increasing levels of dietary ginger powder caused a significant reduction in weight gain in broilers. Through the whole experimental period the greatest weight gain value was recorded in ginger powder 0.5% group, as compared to the control and the other experimental groups.

In tilapia fish, Immanuel et al. (2009) showed that the tilapia fish species fed a basic diet with 1% ginger extract added showed a significant increase in growth compared to the tilapia fed the control diet.

In shrimp fed different concentrations of ginger added to their meals, it was found that the shrimp fed the highest concentration of ginger produced the highest weight gain compared to the shrimp fed the control diet (Venkatramalingm, Godwin-Christopher, & Citarasu, 2007; Chang et al., 2011).

Additionally, Wadikar and Premavalli (2011) showed that rodents given an appetizer containing 12% ginger juice thirty minutes prior to a meal weighed significantly more after ten days than the rats not given the ginger juice appetizer. Furthermore, broiler chickens supplemented with 1% dried fermented ginger (DFG) (Incharoen & Yamauchi, 2009) and broilers supplemented with 1.5% red ginger (Herawati, 2010) gained more weight compared to the broilers not fed any ginger.

2.1.2. 2. 2. Effect of ginger on feed intake and feed conversion ratio:

Ibrahim *et al.* (2011) reported that adding 0.75 % ginger in the diet of rabbits that received 90 % of the protein requirement, significantly ($P \leq 0.05$) decreased the feed intake as CP and DCP (g/day), but it had no significant effect on the DM, TDN and DE intakes. However, this diet containing 0.75 % ginger improved feed conversion ratio of DM, CP, DCP and TDN and DE (Kcal intake / g gain), respectively, as compared to their control.

Ginger (Zingiber officinal), a spice commonly used to treat nausea (Ghosh, Banerjeee, Mullick, & Banerjee, 2011), has also been shown to improve the appetite and food consumption in animals . Additionally, Wadikar and Premavalli (2011) showed that rodents given an appetizer containing 12% ginger juice thirty minutes prior to a meal ate significantly more food after ten days than the rats not given the ginger juice appetizer.

Fakhim *et al.* (2013) found that the mean cumulative feed conversion ratio of broilers fed with different levels of aqueous extract of ginger supplemented to drinking water show no significant differences at 11 to 21, 22 to 42 and 1 to 42 day of age, but the feed conversion ratio was significantly different between treatments at 1 to 10 days of age ($p<0.05$). Also the findings of Chowdhury *et al.* (2002) showed that sun-dried garlic non-significantly affected on the feed consumption and feed efficiency of the laying hens. Similarly, Ademola *et al.* (2004) observed such finding.

Barazesh *et al* (2013) fed broiler chicks (Ross) diets containing different levels of ginger powder ratios of 0, 0.5, 1, 1.5 %. They observed that increasing levels of dietary

ginger powder caused a significant reduction in food intake in broilers. Through the whole experimental period the lower feed intake and the best feed conversion ratio values were recorded in the group fed 1 % ginger powder as compared to the control and the other experimental groups.

2.1.2. 2. 3. Effect of ginger on carcass traits:

Ibrahim *et al.* (2011) reported that adding 0.75 % ginger in the diet of rabbits that received 90 % of the protein requirement had no significant effect on the carcass parameters. These results disagree with that obtained by Zhang et al. (2009), who noticed that inclusion of ginger in the diet increased carcass yield of broilers.

Barazesh *et al* (2013) fed broiler chicks (Ross) diets containing different levels of ginger powder ratios of 0, 0.5, 1, 1.5 % . They observed that abdominal fat in broiler chickens was decreased by increasing the level of ginger in the diet and the lowest value of abdominal fat was found in the group received 1.5 % ginger powder.

2.1.2. 2. 4. Effect of ginger on digestibility coefficients of nutrients:

Ginger rhizome has been shown to contain a high level of plant proteolytic enzyme (Thompson *et al.* 1973; Ziauddin *et al.,* 1995) that could help birds digest dirtary protein upon ingestion. Also Rao *et al.* (2003) found that ginger enhanced the activity of pancreatic chymotrypsin and amylase. They reported also that the positive influence on the activity of enzymes may have a supplementary role in the overall digestive stimulant action of ginger, besides causing an enhancement of titres of digestive enzymes in pancreatic tissue.

Researchers Incharoen and Yamauchi (2009) found that the rhizome of ginger can stimulate digestive juices such as bile, salivary, gastric, pancreatic and intestinal secretions.

Ibrahim *et al.* (2011) reported that the nutrient digestibility coefficients of the DM, OM, CP and NFE and the nutritive values as TDN and DCP were not affected by adding 0.75 % ginger in the normal diet of male growing New Zealand White rabbits. However, rabbits received 90 % of the protein requirements with 0.75 % ginger recorded the best values of the OM, CF, EE and NFE digestibility coefficients and TDN value.

2.1.2. 2. 5. Effect of ginger on blood parameters:

2.1.2. 2. 5. 1. Effect of ginger on serum blood constituents:

Thomson *et al.* (2002) and Verma *et al.* (2004) stated ginger significantly lowered rabbits serum total cholesterol, LDL, V LDL and triglycerides and raised HDL . These authors attributed their results to the reducing effect of ginger acted on cholesterol biosynthesis in the liver and may stimulate cholesterol's conversion to bile acid and increase its fecal excretion, while (Ramakrishna *et al.,* 2003) demonstrated that ginger enhanced the activity of pancreatic lipase and amylase in rats when they were directly in contact with the enzyme.

Bhandari *et al.* (2005) demonstrated that ethanolic extract of ginger produced significant decrease in serum total cholesterol and triglycerides levels and increased HDL-c cholesterol level as compared to diabetic rats, and the extract exhibit a significant lipid lowering activity and protect the tissues from lipid peroxidation. Also it caused a decrease in blood glucose, body weight and alkaline phosphatase in adult male rat.

Dieumou *et al* (2009) reported that the amount of dried ginger powder (8.4, 37, 74, 149, 300) mg cause increase weight gain, increased activity of superoxide, Glutathione peroxides and protein and reduced Molondyaldy concentration and cholesterol.

AL-Rikabi and Jawad (2013) reported that the groups of animal that received ethanolic extracted ginger showed significant decrease in serum glucose, cholesterol, AST, ALT and ALP as compared to the control group.

Hypercholesterolemic male albino rats fed diets supplemented with ginger by rates 5 and 10%. Bushuty and Shanshan (2012) revealed that, all treated rats with 5 and10% ginger resulted in significant decrease ($P<0.05$) of total cholesterol, triglyceride, LDL-c than positive control group, while HDL-c was increased. The authors showed also that liver enzymes (AST, ALT) results revealed significant decrease ($P<0.05$) in all treated rats with 5 and10 % ginger. Their study recommended the use of ginger in cholesterol patients' food.

Saeid *et al.* (2010) explored the usage of different levels of aqueous extract of ginger at concentration of 0.4 and 0.6%, respectively, on the lipid profile of the broiler chickens. He found that serum cholesterol level was significantly lower in the 0.4 and 0.6% aqueous extract of ginger ($p<0.05$) than control.

Ginger administration to diabetic and cholesterol fed rats in the study of Al-Azhary (2011), showed marked but not significant change of blood glucose, total cholesterol, LDL and HDL-cholesterol. This may be consistent with earlier studies (Bordia *et al.*, 1997; Verma *et al.*, 2004) but conflicting with others (Fuhrman *et al.*, 2000; Al-Amin *et. al*, 2006) which indicated a significant lowering effect of ginger on the previously mentioned parameters. Al-Azhary (2011) also reported a significant decrease of triglycerides was induced by ginger administration to diabetic cholesterol fed animals. This effect was also reported in the study of Fuhrman *et al.* (2000) but in concomitant with a significant decrease of plasma cholesterol. This later study attributed the decrease of cholesterol to the reduction of cellular cholesterol biosynthesis induced by ginger, but didn't explain the way of lowering effect of ginger on triglyceride levels. In the study of Al-Azhary (2011), ginger lowered lipid peroxiadation and elevated plasma total antioxidant capacity and blood reduced glutathione concentration, the finding which strongly confirms the antioxidant properties of ginger reported in previous investigations. Ginger has been reported to have a lowering effect on lipid peroxidation by influencing the enzymatic blood level of superoxide dismutase, catalase, and glutathione peroxidase (Ahmed *et al.*, 2000). It has been also shown that ginger reduces cellular oxidation and scavenges superoxide anion and hydroxyl radicals (Cao *et al.* 1993, Krishnakantha and Lokesh 1993). Similarly, Siddaraju and Dharmesh (2007) reported that ginger free phenolic and ginger hydrolysed phenolic fractions exhibit free radical scavenging activity. The antioxidative activity of ginger was attributed to scavenging superoxide anion and hydroxyl radicals by some ginger compounds such as gingirols, shogaols and some related phenolic ketone derivatives (Adhikari *et al.*, 2007; Ali *et al.*, 2008).

Other studies suggested that the response to ginger components depends on its dose concentration (Ghayur *et al.*, 2005; Siddaraju and Dharmesh, 2007).

Zhao *et al.* (2011) reported that dietary supplementation of ginger powder improved serum and egg yolk antioxidant status and enhanced dietary oxidation stability in a dose-dependent manner.

Hussein (2012) reported that biochemical's parameters in mice (blood glucose, serum cholesterol, LDL) showed significant decrease ($p<0.01$), while (HDL) appeared significant increase by injection of ginger extract for four weeks when compared with control group. On the other hand, study of kidney function showed significant decrease in (urea, uric acid, and creatinine) when compared with control group.

2.1.2. 2. 5. 2. Effect of ginger on hematological parameters:

Ademola *et al.* (2009) reported that the red blood cells and haemoglobin concentration of broiler chickens were not affected by dietary ginger.

Bamidele and Adejumo (2012) found that birds fed experimental diets containing 1.00% garlic and 0.50% ginger mixture (27.50%), 1.50% garlic and 0.75% ginger mixture (26.67%) and 2.00% garlic and 0.75% ginger mixture (26.25%) had significantly ($p<0.001$) higher values for packed cell volume than those fed the control diet (18.00%). Those fed 0.50% garlic and 0.50% ginger mixture (24.00%) was slightly different from those on other treatments. Birds on diet containing 1.00% garlic and 0.50% ginger mixture had the highest value (27.50%). Values for haemoglobin concentrations had similar trend, with birds on control diet having the least mean value (5.98 g/dl), while those on diet containing 1.00% garlic and 0.50% ginger mixture had the highest mean value (9.22 g/dl). There was no significant ($p>0.05$) difference across the treatments for white blood cell counts. However, birds on 0.50% garlic and 0.50% ginger mixture had the least value (4300.00 x 103/mm3), while those on diets containing 1.00% garlic and 0.50% ginger mixture had the highest numerical mean value (5000.00 x 103/mm3).

2. 1. 3. Ginseng (*P.ginseng*)

Ginseng, the root of Panax species, is a well-known folk medicine. It has been used as traditional herbal medicine in China, Korea and Japan for thousands of years and today is a popular and worldwide used natural medicine. Ginseng (*P.ginseng*) has a traditional reputation as a tonic and drug of longevity in Asia, where it has also long been used to treat a variety of diseases, including cancer and diabetes, as well as various infections; at present, it has been characterized as an adaptogen or anti-stress agent, exerting a non-specific normalizing effect on body functions. However, while the attention of the herbal industry has focused almost exclusively on ginsenosides, almost all investigations of immunomodulatory effects of ginseng have involved crude extracts of *P. ginseng* (see later) and polysaccharide fractions of Panax species (Yun *et al*, 1993; Gao *et al*, 1996).

Ginsenosides, also termed Panaxosides, are triterpene saponins regarded as the main active constituents of Panax species; hydrophilic bioactive polysaccharides are held responsible for anti-complementary and anti-tumor activities and the ginsenoside content of ginseng can vary depending on the Panax species, the plant age, the part of the plant, the preservation method, the season of harvest and the extraction method (Phillipson and Auderson, 1984; Gao *et al.*, 1991 and Nah *et al.*, 1995).

The basic structure of ginsenosides is similar. They consist of a gonane steroid nucleus with 17 carbon atoms arranged in four rings. The characteristic biological responses for each ginsenoside are attributed to the differences in the type, position and number of sugar moieties attached by glicosidic bond at C-3 and C-6 **(Byun *et al.*, 1997).**

2. 1. 3. 1. Effect of ginseng on animals studies:

Osfor (1995) fed Panax ginseng powder extract at 2 mg and 4 mg/ Japanese quail bird daily. His results found that Panax ginseng increased egg number per hen, average egg weight and hatchability .It slightly improved the efficiency of feed utilization and increased the levels of serum total protein, alkaline phosphatse and aspartate amino transferase (AST).The extract deceased significantly the levels of albumin, total lipids, triacylglycerols, cholesterol and glucose, and had no effect on body weight and the levels of serum urea and creatinine.

Jang *et al.* (2007) fed laying hens diets consisted of basal diet (CON), 2.5% fermented wild-ginseng culture by-product replaced lupin in basal diet (WG1) and 5.0% fermented wild-ginseng culture by-product replaced lupin in basal diet (WG2). Through the 6-wk feeding trial, egg production was significantly increased in WG1 and WG2 treatments compared to CON ($P<0.05$). Egg weight was significantly higher in WG2 than CON ($P<0.05$). WG1 resulted higher yolk color than CON ($P<0.05$). Albumen height and

Haugh unit were significantly improve in WG1 compared to WG2 (P<0.05). Red blood cell was significantly lower in WG2 than CON (P<0.05). LDL-cholesterol was significantly decreased in CON compared to WG2 (P<0.05). In conclusion, fermented wild-ginseng culture by-product could improve egg production and egg weight in laying hens.

Chrastinova *et al.* (2009) reported that the application of *Eleutherococcus senticosus* (30g/100kg diet) reduced the mortality and improved feed conversion ratio and average daily weight gain of fattening rabbits. (P<0.05). The antimicrobial effect of *Eleutherococcus* extract was observed against coagulase-positive staphylococci, *Staphylococcus aureus*, *Clostridium*-like species, similarly to results presented by Simonová *et al.* (2008). Also, Simonová *et al.*(2008) found that slaughter parameters and the quality of meat were practically the similar in control and rabbits given *Eleutherococcus senticosus* (30g/100kg diet) .

2. 1. 3. 2. Effect of ginseng on immunity:

2. 1. 3. 2. 1. Effect of ginseng on immunity in rats:

2. 1. 3. 2. 1. 1. Effect of ginseng on cell-mediated immunity in rat:

Song *et al.* (1997) found that ginseng treatment significantly reduced bacterial load and the number of mast cells in the lungs. The down-regulated specific humoral immunity in the ginseng-treated group and the fact that athymic rats have a severely compromised T-cell-mediated immune reactivity due to the absence of the thymus might suggest an activation of innate immunity after ginseng treatment. It is therefore considered that ginseng has promising potential as a natural medicine for stimulation of the immune system in patients with chronic *P. aeruginosa* lung infections.

Song *et al.* (1998) tested the effects of treatment with ginseng (*Panax ginseng*) on blood polymorphonuclear leukocyte (PMNs) chemiluminescence and serum specific antibody responses in a rat model of chronic *P. aeruginosa* pneumonia. In the ginseng-treated group, the macroscopic lung pathology was milder and the percent in the cells collected by bronchoalveolar lavage was lower than in the control group. These results suggested that ginseng treatment leads to an activation of PMNs and modulation of the IgG response to *P. aeruginosa,* enhancing the bacterial clearance and thereby reducing the formation of immune complexes, resulting in milder lung pathology.

Furthermore**,** Shin *et al.* (2002) cited that phagocytic activity was induced in ginsan-treated macrophages compared to the control. The expression of major histocompatibility antigen CD14 and 1-Ab on murine peritoneal macrophages was increased by the treatment with ginsan, while the expression of CD11b was decreased. These results suggested that ginsan has an immunopotentiating effects on macrophages.

2. 1. 3. 2. 1. 2. Effect of ginseng on humoral immunity in rat:

Kim *et al.* (1990) mentioned that extracts have been induced messenger RNA expression of interleukin-2 (IL-2), interferon gamma (IFN), interleukin-1 and granulocyte-macrophage colony-stimulating factor as well as lymphokine-activated killer cells and CD8+ cells.

Scaglione *et al. (*1996) proved that a significant decline in the frequency of colds and flues in the ginseng treated group. Also, antibody levels in response to the vaccination rose higher in the treated group than in the placebo group. Extracts of ginseng (*Panax quinquefolium*) enhanced macrophage Fc receptor expression (Shin *et al.,* 1997). Moreover**,** Nakajima *et al.* (1998) mentioned that red ginseng also increased the production of interleukin 1 beta, which is known to play important roles in immunity and

inflammation as well as increasing the production of tissue plasminogen activators, which suppress the formation of thrombin in the blood coagulation and fibrinolysis mechanisms.

Park *et al.* (2001) cited that an acidic polysaccharide isolated from the ethanol-insoluble and water-soluble fraction of *Panax ginseng* C. A. Meyer produced high output of nitric oxide synthase (iNOS) in treated female BALB/c mice intraperitoneally. Wang *et al.* (2001) mentioned that extracts have been shown to have immunomodulatory effects. Also, Shin *et al.* (2002) have isolated polysaccharide fraction of *Panax ginseng* (ginsan) and examined its effect on the function of murine peritoneal macrophages. When macrophages were treated with ginsan, cytotoxic activity against B16 melanoma cells was significantly induced. In addition, the levels of cytokines, including tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), IL-6 and Interferon-gamma (IFN-gamma) were increased and the production of reactive oxygen/nitrogen components such as nitric oxide (NO) and hydrogen peroxide (H2O2) was enhanced. **Assinewe *et al.* (2002)** mentioned that aqueous extracts of *P.quinquefolius* root (1-100 micro/ml) macrophage TNF release.

2. 1. 3. 2. 2. Effect of ginseng on immunity in human:

Scaglione *et al.* (1990) showed that blood samples drawn from the volunteers received 100 mg of aqueous ginseng extract or 100 mg of standardized ginseng extract, every 12 hours for 8 weeks revealed an increase in chemotaxis of polymorphonuclear leukocytes, the phagocytic index, and the total number of T_3 and T_4 lymphocytes. After 4 and 8 weeks of ginseng therapy, increased their T_4:T_3 ratio and the activity of natural killer cells.

Tomoda *et al.* (1993) tested that isolated two acidic polysaccharides, named ginsenan PA and ginsenan PB, whose detailed structures were determined. Both polysaccharides displayed similar reticuloendothelial system (RES) activation, anti-complementary, and alkaline-phosphatase-inducing activity. Also, Liu *et al.* (1995) mentioned that isolated saponin from *P. ginseng*, ginsenoside Rg1 was found to stimulate proliferation of lymphocytes and to increase the fluidity of lymphocyte membrane in the elderly. Furthermore, Kim *et al.* (1996) found that ginseng had radio protective effect against γ-ray-induced DNA double strand breaks in cultured murine spleen lymphocytes.

Scaglione *et al.* (1996) demonstrated that daily administration of 100 mg of ginseng for 12 weeks enhanced the efficacy of polyvalent influenza vaccine. The patients who received ginseng had a lower incidence of influenza and colds, higher antibody titers, and higher natural killer cell activity levels. Furthermore, it was found that ginseng promoted apoptosis in renal interstitial fibroblasts and thus could affect renal interstitial fibrosis (Zhang *et al.*, 1998). Similarly, Awang (1999) cited that Rg1 stimulated proliferation of lymphocytes and increased the fluidity of lymphocyte membrane in the old age. Moreover, Klein *et al.* (2000) mentioned that ginseng enhances production of macrophages, B and T cells, natural killer cells and colony–forming activity of bone marrow.

Wang *et al.* (2001) found these extracts of ginseng containing polysaccharides and oligosaccharides have been shown to enhance immune responses such as immunoglobulin production by lymphocytes and natural immune responses by peritoneal exudates macrophages. They have also been found to enhance anticomplementary and reticuloendothelial system activities. Also, Ock *et al.* (2002) noted that red ginseng powder may have some immunomodulatory properties associated with CD3 and CD4 activity in patients with advanced gastric cancer during post-operative chemotherapy.

2. 1. 3. 2. 3. Effect of ginseng on immunity of cows:

Hu *et al.* (2001) found that after the end of treatment with an extract from the root of *Panax ginseng* CA Meyer at a dose of 8 mg/kg body weight per day for 6 days, the numbers of *Staph. Aureus- infected* quarters and milk somatic cell counts SCC tended to decrease in ginseng-treated cows. Phagocytosis and oxidative burst activity of blood neutrophils were significantly increased 1 week after ginseng treatment, The number of monocytes in ginseng-injected cows was significantly higher 1 week post-treatment than pre-treatment, and the number of lymphocytes was significantly higher than pre-infusion at 2 and 3 weeks after ginseng treatment. The present findings indicate that ginseng treatment can activate the innate immunity of cows and may contribute to the cow's recovery from mastitis. In addition, Hu *et al.* (2003) mentioned that addition of ginsenoside (Rb_1) resulted both in significantly higher antibody production and lymphocyte proliferation in response to PWM, Con A and *Staph. aureus* antigens in heifers and had safe adjuvant, effect when used for immunisation against *Staph. aureus* in dairy cattle mastitis vaccine.

2. 1. 3. 2. 4. Effect of ginseng as an adjuvant in vaccines:

Saponin-based adjuvant has the unique ability to stimulate the cell-mediated immune system, as well as to enhance antibody production, and has the advantage that only a low dose is needed for adjuvant activity (Oda *et al.*, 2000). Interestingly, the vaccines supplemented with ginseng favored IgG2. The vaccines used in the evaluations varied in their immunogenic potency. However, after the addition of ginseng the less immunogenic vaccine proved to be as potent as the better one without ginseng. Thus, the use of ginseng as a co-adjuvant provides a simple, safe and cheap alternative for improving the potency of aluminium hydroxide adjuvant vaccines (Rivera *et al.*, 2003). Also, Aifang *et al.* (2005) concluded that ginsenosides have strong adjuvant effects at a dose of 0.5 or 1.0 mg when mixed with *E. tenella* recombinant 5401 antigen, and has a potential as an adjuvant in chicken vaccine.

Vaccines containing the ginseng-fraction Rb1 induced serum-detectable amounts of IL-4 and IL-10 as early as 24 h after primary injection that was confirmed in sera collected at 24 and 72 h post re-vaccination. Five weeks after booster, immune lymphocytes was still producing large amounts of cytokines including IFN- gamma, IL-2, IL-4, IL-10 and TNF-alpha (Rivera *et al.*, 2005). Furthermore, the results showed that astragalus plus ginseng Chinese herbal medicinal ingredients could significantly raise antibody titer in rabbits. The Chinese herbal medicinal ingredients could markedly promote lymphocyte proliferation and enhance antibody titer in chickens to NDV, which was similar to oil adjuvant (Jun-ling *et al.*, 2006).

2.1.3. 3. Mechanism of action of ginseng saponins:

The mechanisms of immune-stimulating action of saponins have not been clearly understood, but many explanations have been put forward. Saponins reportedly induced production of cytokines such as interleukins and interferons that might mediate their immunostimulant effects (Jie *et al.*, 1984 and Kensil, 1996).

It is likely that they interact with antigen-presenting cells to induce many of these responses (Barr *et al.*, 1998). The incorporation of the saponins into cell or endosomal membranes might expose the incorporated antigen to cytosolic proteases. The effects of saponins at the intestinal level may also need attention, given its presence in some common dietary ingredients. Also several important systemic infections gain access to the body via the intestinal route. It has long been considered that a single dose of orally administered sub-unit vaccine would be the most useful means of protecting against these disorders (Bloom, 1989; McGhee *et al.*, 1992). Oral vaccines are easy and economical to administer and avoid the hazards of routes involving needles. More importantly, induction of effective

immunity at a mucosal site can only be achieved by immunisation via a mucosal route. There is evidence that saponins may increase the immune response by increasing the uptake of antigens from the gut and other membranes (Francis *et al.*, 2002).

2. 1. 3. 4. Effect of ginseng as anti-stress (Adrenal gland):

The root of *Panax ginseng* C.A. Meyer has been reported to have an anti-stress action. The saponin-rich fraction of ginseng greatly reduced the secretion of catecholamines from bovine adrenal chromaffin cells stimulated by acetylcholine (Ach.), (Kudo *et al.*, 1992). Kim *et al.* (2003) reported that intraperitoneally administered ginseng total saponin or a few specific saponins suppress the corticosterone level in stressed rat plasma. Asian ginseng improves adrenal function and increases blood pressure and energy. Asian ginseng produced a noticeable increase in energy and concentration over a 6 week period. *Panax ginseng* has been shown to have a strong anti-stress effect, reducing high cortisol levels and bringing the ratio of cortisol to DHEA to a more healthy balance (Tode *et al.*, 1999 and Rai *et al.*, 2003).

2. 1. 3. 5. Ginseng antioxidant effect:

The antioxidant activity of ginseng was associated with both the ginsenosides and the flavonoid constituents (Sonnenborn and Proppert, 1991). Kim (1992) found that the ginsenosides protected pulmonary vascular endothelium against free-radical-induced injury. Mice given ginseng extract or ginsenosides Rb_1 and Rg_2 orally during passive avoidance response tests showed an improvement in learning ability which was negatively influenced by stress (Wagner *et al.*, 1994).

Voces *et al.* (1999) studied that the effects of prolonged treatment with the standardized *Panax ginseng* extract G115 on the antioxidant capacity of the liver. For this purpose, rats that had received G115 orally at different doses for 3 months and untreated control rats were subjected to exhaustive exercise. A bell-shaped dose response on running time was obtained. The results showed that the administration of G115 significantly increased the hepatic glutathione peroxidase activity (GPX) and reduced glutathione (GSH) levels in the liver.

The antioxidant activities of *P. ginseng* also help explain its DNA-preserving qualities with respect to chemical carcinogens and inflammation. Ginseng extracts have been shown to scavenge reactive oxidative molecules (ROS) (Zhang *et al.*, 1996 and Liu *et al.*, 2003) as well as attenuate lipid peroxidation. The importance of the antioxidants contained in foods is well appreciated for both preserving the foods themselves and supplying essential antioxidants in vivo. Often, the term is used to describe chain-breaking inhibitors of lipid peroxidation as free radicals generated in vivo damage many targets as lipids, including proteins, DNA and small molecules.

2. 1. 3. 6. Effect of ginseng on blood constituents:

Lin (1995) cleared out that liver-protectant activity of ginseng has been demonstrated in vitro and in vivo. Intraperitoneal administration of ginseng extracts and decreased aspartate aminotransferase (AST) and alanine aminotransferase (ALT), thereby demonstrating a liver- protectant effect. Voces *et al.* (1999) indicated that hepatic transaminase levels, ALT (Alanine-amino-transferase) and AST (Aspartate-amino-transferase), 48 h after the exercise were reduced indicating a clear hepatoprotective effect related to the administration of the standardized *Panax ginseng* extract G115.

Qureshi *et al.* (1983) mentioned that the ginsenosides are considered to be the active agents for the suppression of cholesterogenesis and lipogenesis, added ginseng at a rate of 0.25% for 4 weeks in the feed of broilers, significantly reduced both cholesterol and triglycerides in broiler serum. Moreover, Muwalla and Abuirmeileh (1990) cited that the

mechanism of the hypocholesterolemic action of ginseng involves the suppression of cholesterol biosynthesis.

A number of studies have shown that saponins from different sources lower the serum cholesterol levels in a variety of animals including humans (Southon *et al.* 1988; Harwood *et al,.* 1993; Potter *et al.,* 1993; Matsuura, 2001). Saponins also reduced the more harmful LDL-cholesterol selectively in the serum of rats, gerbils and humans (Potter *et al.* 1993; Harris *et al.* 1997; Matsuura, 2001). Oral administration of ginseng extract at 125 or 250 mg/kg/day produced statistically significant reductions in total cholesterol and triglyceride concentrations in the blood 3 days after hepatoctomy, the total cholesterol response appeared to be dose-related (Cui *et al.,* 1998). On the other hand, Manal *et al.* (1999) mentioned that the levels of cholesterol and triglycerides in liver under these conditions showed a similar pattern as that of serum, confirmed those recorded above. Ginseng administration failed to exert any significant protection from the remarkable hypercholesterolemia or atherosclerosis associated with the cholesterol- enriched diet. However, Yang *et al.* (1999) found that when aerobic exercise combined with ginseng total cholesterol and triglycerides (TG) decreased, and HDL-C increased.

It can be concluded that several dietary saponins do have a hypocholesterolaemic action. Since cholesterol binding takes place in the intestinal lumen, factors such as quantity of saponins and cholesterol, and the presence of other ligands of both compounds may play a role and these may have caused the observed discrepancies among the various results. Knowledge of the nature of the interaction between the particular saponin and cholesterol, and the nature of the cholesterol moieties and other ligands in the diet are essential to arrive at an effective dietary dose of that particular saponin that could have a significant hypocholesterolaemic effect (Francis *et al.,* 2002).

2. 1. 3. 7. Effect of ginseng on hormones:

Yamammoto (1977) said that the drug further stimulated spermatogenesis in rat, and rabbit testes, and increased the motility and survival of rabbit sperm outside the body (Kim, 1976). Thus the ginseng saponin was found to act on the hypothalamus and /or hypophysis primarily and stimulated ACTH secretion which resulted in increased synthesis of corticosterone in the adrenal cortex (Hiai *et al.,* 1979). The ginsenosides, which appear to be the active components, are thought to depress blood prolactin levels, thereby increasing libido (Owen, 1981). Also, Bahrke and Morgan (1994) reported that *Panax ginseng* produced adoserelated increase in serum testosterone levels and American ginseng reduced the plasma level of prolactin hormone in rats.

Choi and Seong (1995) found that in one clinical study, 90 patients with erectile dysfunction were treated with ginseng saponins (600 mg orally per day). Treatment improved rigidity, tumescence, and libido, but not the frequency of coitus.

Bespalov *et al.* (2001) demonstrated that bioginseng, panaxel and panaxel-5 administered to male rats for 7 days considerably stimulated the production of the thyroid hormones, thyroxin and tri-iodothyronine this mechanism may relate to the ant carcinogenic action of ginseng, it is known that decreased thyroid hormone activity stimulates the development of some tumers in humans and experimental animals (Rao,1996).

Murphy and Lee (2002) found that both ginseng species may have direct actions on the anterior pituitary gland and /or on the hypothalamic dopaminergic mechanisms.

2. 1. 3. 8. Antifungal, virucidal and anti- protozoal activity of ginseng saponins:

Saponins have high toxicity against fungi (Delmas *et al.* (2000) and Wang *et al.*, 2000). Fungicidal activity against Trichoderma viride was previously used as an identification method for saponins.

Some saponins have been shown to be capable of deactivating viruses; for example, purified saponin mixture from Maesa lanceolata (Sindambiwe *et al.*, 1998). Maesasaponins with 21, 22 diacylation had virucidal activity (Apers *et al.*, 2000). The triterpenoid saponin oleanolic acid inhibits HIV-1 virus replication probably by inhibiting HIV-1 protease activity (Mengoni *et al.*, 2002). Triterpenoid and steroid saponins have been found to be detrimental to several infectious protozoan such as Plasmodium falciparum (Traore *et al.*, 2000), Giardia trophozoites (McAllister *et al.*, 2001) and Leishmania species (Delmas *et al.*, 2000 and Plock *et al.*, 2001). The toxicity of saponins to protozoan seems to be widespread and nonspecific and is obviously the result of their detergent effect on the cell membranes.