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Original Research Article

Effects of *Moringa Oleifera* leaf meal inclusions on serum activities of hepatic marker enzymes and lipid profile of Anak 2000 broiler chicks

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Abstract

The study was designed to investigate the effects of Moringa oleifera leaf meal (Molm) inclusions on serum activities of hepatic marker enzymes and lipids profile of Anak 2000 broiler chicks. A total of 54 Anak 2000 broiler three weeks old chicks were randomly assigned into 3 treatments and 3 replicates of 6 birds each for the purpose of this study. Treatment 1 (control) received 0% Moringa oleifera leaf meal (Molm) while treatment 2 and 3 received 10% and 20% of Molm inclusions respectively. The treatment was terminated at the end of 6 weeks and blood sample collected, allowed to stand for one hour spun at 3000rpm for the collection of serum sample. The serum sample so collected was assayed for hepatic marker enzymes: alkaline phosphates (ALP), aspartate aminotransaminase, (AST) and alanine aminotransaminase, (ALT) activities and serum lipid profile. The result obtained showed significant (P<O.05) reduction in serum activity of AST and ALT in both 10% and 20% inclusions relative to control with no significant changes in ALP activity at 20% inclusion rate. The lipid profile on the hand showed a significant (p<0.05) reduction in serum triacylglycerol at both levels of inclusion but neither a significant (P>0.05) increase in total cholesterol nor a decrease in high density lipoprotein HDL-cholesterol at both levels of inclusion of Molm. The low density lipoprotein LDL-cholesterol was however significantly elevated at both levels of inclusion of Moringa oleifera leaf meal. The result suggests that Moringa oleifera leaf meal posses antihepatotoxic and hypotriacylglycerol effects in broiler chicks when added at 10% and 20% levels.

Keywords: Moringa oleifera, hepatic marker enzymes, lipid profile, broiler chicks

INTRODUCTION

Moringa oleifera lam, an Asian plant of high, nutritive and medicinal values belongs to the plant family Moringaceae which is known as drumstick tree in English (Rajanandh, *et al.,* 2012). A wide variety of nutritional and medicinal values have been attributed to its root, bark, leaves, flowers, fruits and seeds (Anwar *et al*., 2007; Kumar et al 2010). According to Oduru *et al.* (2008), Moringa oleifera contains 35% moisture, 7.13% crude ash, 2.25% fat, 27.51% crude protein, 9.15% crude fibre and 43% carbohydrates by chemical composition. (Onu, and Aniebo, 2011)

Olugbemi *et al.* (2010) reported that Moringa oleifera leaves contain 6.30% moisture, 27.44% crude protein, 9.13% crude fibre, 6.30% ether extract, 6.20% Ash and 44.63% nitrogen free extract by proximate composition. Scientific research confirms that Moringa oleifera leaves are a power house of nutritional values. Gram for grain weight bases comparison confirms that Moringa olifera

leaves contain 7 times the Vitamins C value in orange, 4 times the calcium in milk, 4 times the Vitamin A in carrots, 2 times the protein in milk, and 3 times the potassium in banana. However, phyto-chemical screening of this plant reveals that it contains appreciable levels of anti-nutritional factor as trypsin inhibitor 30%, cyanide 0.1%, tannins 21.19%, phytate 2.57%, saponins 1.60%, and chelate 0.4% (Ogbe and John, 2011).

A lot of medicinal values of Moringa oleifera have been reported. Prevention of early liver injury and restoration of antioxidant status by leaves extract in mice fed with high fat diet has been reported (Das *et al*., 2012). Acetaminophen induced liver injury has been prevented by the leaves extract through the restoration of glutathione level (Fakurazi *et al*., 2012). Hepatoprotective activity of Moringa oleifera leaf extract on antitubercular drug induced liver damage in rats has been reported by Pari and Kumar (2002). Moringa oleifera has been extensively studied for many potential uses which include wound healing, antineoplasia, antiinfertility, hypotensive, nalgesia, antipyretic, antiepileptic, antiinflamatory, antiulcer, antispasmodic, diurectic, hypo-cholesterolamia, antifungal, antibacterial, aphrodisiac, cholagogue, anti-oxidant, hepatoprotective, immunomodulators, cardio-tonic, and as cardiac and circulatory stimulants (Rajanandh, 2012). It has also been shown to have therapeutic values against cancer, diabeties, mellitus, rheumatoid, arthritis and other diseases (Mehta *et al.* 2003; Kuradi *et al*., 2006; Roy *et al*., 2007).

Athorosclerosis (hardening of blood vessels) and hypertriglyceridemic associated with consumption of meat rich in fat have caused emphasis to shift from meat to lean meat consumption. Broiler chicks as one of the fastest and most available producer of meat for human need can bridge this gap especially if the lipid level could be lowered. The degree of fatness in this animal is a function of genetic, environment and nutritional factors and therefore could be manipulated by varying any of these factors. Moringa oleifera leaf meal was tested for this.

The hepatic marker enzymes on the other hand signal the health status of the liver and increase serum activity indicates marked injury to the liver and may impair metabolic activities. This study was therefore set to investigate the effects of Moringa oleifera leaf meal (Molm) on hepatic marker enzymes activities and lipid profile of broiler chicks.

MATERIALS AND METHODS

Preparation of Moringa oleifera leaf meal (molm)

A total of 65.00kg of Moringa oleifera leaves were procured from Obubra prison yard in Cross River State of Nigeria. The leaves were shade dried to a constant weight by reducing the moisture content of the leaves by 7.0%. The dried leaves were macerated and milled to powder by motor- power milling machine in Apiapum market.

Ten (10) parts of this powder was thoroughly mixed with (90) ninety parts of vital starter and finisher feed to form 10% of Moringa oleifera leaf meal (Molm). The same procedure was adopted in the formation of 20% Molm but in these case (20) parts of the powder were used as against (10) parts in 10%.

Animal procurement and treatment

Anak 2000 breed of broiler chicks were obtained from Uyo in Akwa Ibom State. A total of 54 three weeks old chicks of Anak 2000 broilers were used in this study. The birds were randomly assigned into 3 treatments of 18 birds after brooding and replicated 3 times of 6 birds per replicate.

Treatment 1 (control) received (0) zero part of Moringa oleifera leaf meal (Molm) with (100) one hundred parts of vital starter and finisher rations making 0% inclusion level..

Treatments 2 and 3 received 10 parts and 20 parts of Moringa oleifera leaf meal making 10% and 20% inclusion levels. The treatment commenced on the 3rd week of life of the birds and ended on the 8th week making a total of 5 weeks of treatment.

Collection of serum samples

The treatment was terminated at the end of 8 weeks and birds were sacrificed after 12 hour of starvation. Blood sample were collected from the jugular vein using a 10 ml syringe and transferred into clean labeled sample bottles. The blood samples were allowed to stand for an hour, spurned and the supernatent decanted and collected as serum. The serum samples were assayed for hepatic marker enzymes and lipid profile.

Assay of Hepatic Marker Enzymes

The method of Thomas (1995) was used for the determination of Alkaline phosphatase activity in serum. The method is based on the photocolorimetric determination of inorganic phosphate split off from glycerophosphate with phosphotase of the blood serum in an alkaline medium. The estimation of aspartate and alanine transaminases (AST and ALT) were carried out according to the method of Reitman and Frankel, (1957) based on conversion of pyruvate to lactate in the presence of reduced nicotinamide adenine dinucleotide which was oxidized eventually. The AST was measured by monitoring the concentration of oxalocaetate hydrazone formed with 2, 4 dinitrophyenyl hydrazine. The

Table 1. Hepatic marker enzyme activities of treated broiler chick.

Marker enzymes	Dietary leaf meal inclusion level		
	0%	10%	20%
ALP (iu/L)	61.50±4.90	80.0±4.54	62.55± 4.82
AST (iu/L)	83.58±13.20^{c}	27.52±12.70^{d}	54.62±13.56^{d}
ALT (iu/L)	25.69±2.63^{a}	14.50±2.16^{b}	16.55±2.36^{b}

Values are means ± SEM. Means on the same row bearing the different superscripts are significantly (P<0.05) different.

Table 2. Lipid profile of broiler chicks treated with MoLm.

Component	Dietary leaf inclusion level.		
Lipid (mmol/L)	0%	10%	20%
Total cholesterol	3.12±0.09^{d}	3.32±0.08^{d}	3.54±0.07^{d}
HDL- cholesterol	2.05±0.06^{d}	1.82±0.05^{d}	1.95±0.04^{d}
LDL- cholesterol	0.65±0.15^{d}	1.15±0.17^{d}	1.25±0.16^{d}
VLDL- cholesterol	0.41±0.04^{d}	0.35±0.02^{d}	0.23±0.03^{d}
Triglyceride	0.85±0.01^{a}	0.76±0.02^{b}	0.51±0.03^{c}

Values are means ± SEM. Mean values on the same row with different superscripts are significantly (p<0.05) different.

ALT on the other hand was measured by monitoring the concentration of pyruvate hydrazone formed by reaction with 2, 4 dinitrophenyl hydrazine.

Lipid Assay

Component of lipid were estimated using enzymatic colorimetric diagnostic kits obtained from Randox laboratory Antrium, United Kingdom. The GPO-PAP method of Trinder (1969) was used for the determination of serum triglycerides.

Total cholesterol in serum was determined using CHOP-PAP method of Richmond (1973) and Fleggy (1973) while high density lipoprotein cholesterol (HDL) was determined using phosphotungstate precipitation method of Richmond (1973). The low density lipoprotein cholesterol (LDL) was estimated as the difference between total cholesterol and high density lipoprotein with triglyceride divide by five.

Statistical analysis

One way analysis of variance was used to test for significant differences among group of treatments and error of the mean and compared to control treatment. (Table 1, 2)

The result presented in table 1.1 shows the hepatic marker enzyme activities of (Molm) at different levels of inclusion. There was significant (P<0.05) decrease in Aspartate transaminase (27.52±12.70 and 54.62±13.56) iu/L relative to (83.58±13.20 iu/L) control treatment at both levels of inclusion of Molm. The activities of Alanine transsaminase was equally significantly (P<0.05) reduced by Molm at 10% and 20% inclusion levels. An abnormally high value of alkaline phosphatase activity was observed at 10% level of inclusion but no significant changes at 20% level relative to control treatment.

Table 2 shows the serum lipid profile of broiler chicks fed with Molm at 10% and 20% inclusion rate. The result obtained revealed a significant (P<0.05) elevation in serum LDL-cholesterol at both levels of inclusion with significant (P<0.05) decrease in serum triglyceride at 10% and 20% inclusion rate. There is however no significant (P<0.05) changes (increase or decrease) in serum total cholesterol, very low density lipoprotein cholesterol and high density lipoprotein cholesterol at both 10% and 20% inclusion rate relative to the control treatment.

DISCUSSION

Impairment of metabolic activity of the liver cell in any form will result in either nutrient over dose or metabolite deficiency all leading to pronounced health disorders. These problems can be detected by measuring the activities of hepatic marker enzymes in serum. Alanine arninotransferase (ALT) is commonly measured clinically as a part of diagnostic evaluation of hepatocellular injury to determine health states of the liver (Wang, 2012). The result of this study revealed a significant (P<0.05) reduction in serum activity of alanine aminotransferase at 10% and 20% levels of inclusion of Moringa oleifera leaf meal in broiler chicks relative to 0% control treatment. This gives credence to the report of Rajanandh *et al*, 2012) on antioxidant properties of ethanolic leaf extract of

Moringa oleifera leaves in wistar rats. This result is also in consonance with the report of (Herietta *et al*., 2012) on hepatoprotective effect of ethanolic leaf extract of Moringa oleifera in wistar rats. This result could be attributed to the protective role of detoxifying (microsomal) enzymes which are more abundant in the liver. The serum aspartate aminotransferase activity is significantly (P<0.05) reduced relative to control broiler chicks treated with 0% inclusion rates. This also supports the hepatoprotective effect of Moringa oleifera leaf meal (Anwar *et al.* 2007). The observation in this study is equally supported by Schmidt and Schmidt (1979). Although this enzyme is not liver specific yet its concentration is high. The ability of Moringa oleifera leaf meal to decrease serum activities of ALT and AST at 10% and 20% inclusion rate relative to control indicates that this leaf meal has protective effect on the liver cells.

The serum alkaline phosphatase activity in broiler chicks fed with 20% of Moringa oleoifera leaf meal (Molm) inclusion rate did not significantly (P<0.05) change. There is however, a significant (P<0.05) increase in serum activity of this enzyme at 10% Molm inclusion rate. This increase may not automatically translate to hepatocellular injury since alkaline phosphatase is a generic name for a group of relatively non-specific phosphatases which hydrolyse phoshpomonoesters under alkaline condition (Debayo, 1997). Moreover, this enzyme is widely distributed to many organs and tissues of the body such as the bone, hepatic biliary tract, kidney, spleen, pancreas and lactating mammary gland (Zilva and Pannall, 1984). Its increased serum activity may indicate injuries to any of these organs and if the liver is involved, it is localized to the biliary tract.

The result presented in table 1.2 shows serum lipid profile of broiler chicks treated with Moringa oleifera leaf meal (Molm). The result obtained indicates a significant (P<0.05) reduction in serum triacylglycerol of broiler chicks treated with 10% and 20% Moringa oleifera leaf meal (Molm) relative to the control experiment. Generally plant steroids (sterol) inhibit absorption of lip[id by the monogastric by lowering the plasma concentration of low density lipoprotein (Aattar, (2006). The observation is in consonance with the finding of Herietta *et al.* (2012) on a significant lowering of triacylglycerol by Moringa oleifera leaf extract. Moringa oleifera leaf extract contains B-sisterol and the hydroethanolic Moringa oleifera leaf extract contains 0.09% B-sisterol (Rajanandh *et al*., 2012) which may be responsible for the lowering of triacylglycerol as observed in this study. This reflects the beneficial effect of Molm in production of lean meat by broiler chicks where the feed is supplemented with 10% and 20% inclusion rates. By this finding, the quality of broiler chick meat may be improved on and the attendant nutritional values.

Although there was neither a significant (P>0.05) increase in total cholesterol, very low density lipop-rotein cholesterol nor significant (P>0.0) decrease in high density lipoprotein (HDL) yet there was a significant (P<0.05) rise in atherogenic factor (Low density lipoprotein cholesterol) LDL in both inclusion rates. The plausible explanation may be that Moringa oleifera leaf meal (Molm) enhances the activity of lipoprotein lipase (LPL) which hydrolyses very low density lipoprotein (VLDL) thereby increasing the conversion of VLDL remnant into intermediate density lipoprotein (IDL) and subsequently into low density liprotein (LDL). On the other hand, Molm, may be interfering with hepatic lipase hydrolytic activity leaving less of LDL in the liver and more in the blood stream.

This observation is however at variance with the finding of Rajanandh *et al*. (2012) who reported a significant (P<0.001) reduction in serum LDL of wistar rats treated with hydroethanolic Moringa oleifera extract. This finding does not also agree with the report of Oyewole *et al.* (2010) who reported a generalized significant (P<0.05) reduction in serum lipid of albino rats treated with Moringa oleifera leaf extract. One could also attribute the high serum LDL observed in this study to sex difference in the broiler chicks used. Freeman (1984) reported that female birds generally possess higher levels of plasma LDL 200mg/dl as against 140mg/dl for the male counterpart .Since the study employed mixed sexes, it may be that the treated groups have more female than the male birds in relation to control treament and hence the high serum LDL observed.

CONCLUSION

Moringa oleifera leaf meal (Molm) at 10% and 20% inclusion rate in broiler chicks production exhibited significant (P<0.05) decrease in serum ALT, AST and TG indicating antihepatotoxic and hypoglyceridemic effects and hence could be used in lean meat and healthy broiler chicks production.

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