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Fatty acid profile of the broiler birds' breast meat fed on citrus waste supplemented feed and bio evaluation assay of the albino rats fed on the feed with antioxidant enriched meat from the Citrus Waste

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Abstract Citrus is a major fruit crop of the Pakistan and grown on large area as Pakistan is amongst 10 major citrus producers of the world. After juice extraction major waste is lost which is more than 50% of the whole citrus fruit containing valuable antioxidants and essential fatty acids. In current study citrus waste was incorporated with the basal feed of the broilers birds and fed for 42 days. It had been observed that it has positive effects on the fatty acids profile of the broiler breast meat. The degree of unsaturation of the meat fats increased as the citrus waste proportion in the feed of the broilers was increased. Moreover, the breast meat of the birds with citrus waste effect was freeze dried and the freeze-dried meat was incorporated in the feed of the albino rats to assess the effect of antioxidant rich meat on the lipid profile of the rats. It has been observed that total cholesterol and triglycerides of the rats decreased as the meat level in the feed of the rats was increased, high density lipoproteins of the albino rats increased with the increase in meat level and meat incorporation has non-significant effects on low density lipoproteins of the rats.

Keywords: Fatty acids profile, broiler breast meat, citrus waste supplementation, natural antioxidants.

1. INTRODUCTION

Citrus fruit is major fruit crop of Pakistan and grown on large scale especially in the Sargodha division of Pakistan. Pakistan is amongst few top producers of the citrus fruit. Citrus fruit was cultivated on the land of 9 million hectare in the year 2009 and production of the citrus was 122.3 million tons which is more than any fruit in the (FAO. 2012) (Marini *et al*., 1995) observed in study that peels and seeds of the citrus fruit are good source of phenolic compounds like phenolic acids and flavonoids. The citrus waste has been used successfully as a broiler feed ingredient. The profile of the fats in the meat and the nutritional composition of the livestock can be altered by changing the nutrients in the feed system, like increase of the polyunsaturated fatty acid is possible with incorporation of natural antioxidants in the feed which is also helpful in reducing the oxidation of pigments and lipids in the fatty tissues of the meat (Arshad, *et al*., 2011). (Scaife *et al*. 1994) observed that omega 3 and omega 6 fatty acids ratio in the broiler meat tissues decreased as soybean oil supplemented to broilers feed. (Skrivan *et al*. 2000) studied the effect of different source of fats on composition of the fats of the meat. The broiler chicken which were fed on the lord as a fat source have higher contents of the saturated fatty acids. Yasin *et al.* (2012) fed the broilers with alpha tocopherol and alpha lipoic acids in addition to the basal feed and observed that feeding the antioxidants in the form of alpha tocopherol and alpha lipoic acids to the broilers significantly reduced the total lipids of the broilers in the breast and thigh meat. Yang *et al*. (2010) investigated the effect of *Morus Alba* fruits on the rats which were hypolipidemic. *Morus Alba* diets administrated at the rate of 5% and 10% in the diet of the rats for 4 weeks had been found effective in raising the high density lipoprotein. The diet also reduced serum triglycerides, total cholesterol, and serum low-density lipoprotein. Bok *et al*. (1999) studied the effect of the feed which was supplemented with the bioflavonoids of the citrus on the lipid profile of the rats. It had been found that bioflavonoids from the citrus peel lowered the levels of hepatic cholesterol and hepatic triglycerides. HDL and plasma triglycerides levels of the different groups of the rats were not different. The objectives of the current study were to see the effect of citrus waste on the fatty acids profile of the broiler meat and evaluate the meat for bio evaluation assay of the albino rats for lipid profile.

2. METHODOLOGY

A day old 225 broiler birds were purchased from Big Bird, Lahore and reared at University Farm, University of agriculture Faisalabad. The birds were divided into 5 treatments having 15 birds in each treatment, with 3 replications in each treatment and placed in separate pins. The birds were fed on feed containing 0% citrus waste, 2.5% citrus waste, 5% citrus waste, 7.5% citrus waste and 10% citrus waste for 42 days and all standards requirements for the broilers production were provided to the birds (light, ventilation, water and vaccination). On 42nd day the birds were slaughters following Halal slaughtering method and

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meat was stored at freezing temperature for further analysis.

Fatty acids Profile

Fatty acids profile of breast meat was determined by following the method of Raes *et al.* (2001) using chromatography.

Bio-Evaluation of experimental meat

Albino rats were procured from National Institute of Health, Islamabad. The efficacy study was conducted on the rats which were divided into three groups. Each group of rats was comprised of 10 rats with three replications. The individual animal groups were placed in the house which was made of stainless steel, the temperature of the house was maintained at 20 to 23ºC. The light condition in the caged were maintained for equal time of darkness and the light (12Hour each). The rats were fed for one month on experimental feed. The rats were provided with the experimental feed as described in the **(Table1).** At the end of the one month experimental period the rats were withdrawn from the feed 12 hour before the sampling. The blood samples from the inferior vena cava was collected after the anesthetizing of the rats with chloroform. The samples were collected in the EDTA coated tubes. The samples were further analyzed for total cholesterol, triglycerides, HDL, LDL. The concentration of the cholesterol in the albino rats was observed by the method of Stockbridge (*et al*. 1989) using CHOD-POP method. High Density Lipoproteins of the rats were analyzed by the method of Assmann (1979) and the low density lipoproteins of the rats were analyzed by the method of McNamara *et al*. (1990). Triglyceride concentration of the rats was estimated by the method of Anoni *et al.*, (1982).

Table 1: Diets composition of albino rats for bio-evaluation of experimental meat

Broiler meat ingredients	Group 1 (%)	Group 2 (%)	Group 3 (%)
Flour	67	67	67
Oil	5	5	5
$CWM_{7.5}$	--	20	--
CWM_{10}	--	--	20
Cholic Acid	0.5	0.5	0.5
Cholesterol	2.0	2.0	2.0
Mineral Mix	5	5	5
Vitamin Mix	1	1	1

CWM: Meat of experimental birds fed on 7.5 and 10% citrus waste (freeze dried)

3.12 Statistical Analysis

The data obtained for different parameters was analyzed statistically by applying completely randomized design (CRD) described.

3. RESULTS AND DISCUSSION

Fatty acids profile

The data regarding fatty acids profile of the broiler breast meat is presented in the **(Table 2),** which shows that that saturated fatty acid levels in the meat decreased as the citrus waste in the feed is enhanced and the levels of unsaturation in the fats increased as the citrus level in the feed in increased. The highest contents of saturated fatty acids were observed in the control treatment and the lower saturated fatty acids contents were observed in the treatments of the broiler which were fed on citrus waste supplemented feeds. Citrus waste is considered as a good source of PUFA (n-3) which may increase the content of these fatty acids in the broiler. Lopez-Ferrer *et al*. (2012) reported in a study that if poly unsaturated fatty acids are incorporated in the feed of the broiler, the same fatty acids increased in the meat of the broilers, when it was fed up to 42 days. Mourao *et al.* (2012) fed the broiler chicks with citrus pulp and dehydrated pasture. It had been observed that citrus pulp and dehydrated pasture increase the level of polyunsaturated fatty acid and as the higher concentration of these natural ingredients increase the level of the polyunsaturated fatty acids in the meat of the broiler. Skrivan *et al*. (2000) observed in study that if fats are utilized in the broiler which has MUFA, the oxidative stability of the meat improved as compared to the feed which have oils from the plant sources like sunflower and linseed oil. The diets having more MUFA are also good for human health and prevent oxidation of the low-density lipoproteins. Khan *et al.* (2015) observed in a study that feeding the antioxidants to the broilers in the feed reduced the saturated fatty acids contents in the thigh meat of the broilers. Yasin *et al*. (2012) fed the broilers with alpha tocopherol and alpha lipoic acids in addition to the basal feed and observed that feeding the antioxidants in the form of alpha tocopherol and alpha lipoic acids to the broilers significantly reduced the total lipids of the broilers in the breast and thigh meat. The investigations of all above scientists support the findings of current study.

Table 2: Fatty acids composition in the breast of the broiler meat fed on citrus waste supplemented feed

	Saturated fatty acids			Monounsaturated FA			Polyunsaturated FA		
	C16:0	C17:0	C18:0	C16:1	C17:1	C18:1	C18:2	C18:3	C18:4
CW_0	20.1a	0.27a	12.75a	1.85b	0.08b	30.45b	15.25c	0.4b	5.3ab
$CW_{2.5}$	20.1a	0.25ab	12.71a	1.88b	0.09ab	30.5b	15.5b	0.4b	5.4ab
CW_5	18.8b	0.24ab	12.6ab	1.91ab	0.095ab	31.05ab	15.75ab	0.55ab	5.5a
$CW_{7.5}$	18.2bc	0.23b	12.2b	1.93a	0.10a	31.25ab	15.92ab	0.59a	5.5a
CW_{10}	17.5c	0.21bc	12.15b	1.95a	0.11a	32.05a	16.1a	0.62a	5.6a

Table 3: Fatty acid profile showing % for the treatments of breast meat fed on citrus waste supplemented feed

Fatty acids	Treatments				
	CW_0	$CW_{2.5}$	CW_5	$CW_{7.5}$	CW_{10}
SFA	33.12a	33.06a	31.64b	30.63bc	29.86c
MUFA	32.38c	32.47bc	33.06b	33.28ab	34.11a
PUFA	20.95bc	21.3b	21.8ab	22.01ab	22.32a
UFA	53.33bc	53.77b	54.86ab	55.29a	56.43a
SFA/UFA	1.48ab	1.55a	1.5a	1.39bc	1.34c
PUFA/SFA	0.63bc	0.64b	0.68ab	0.72a	0.75a

MUFA = Mono unsaturated fatty acids, PUFA = Poly unsaturated fatty acids, UFA = Unsaturated fatty acids, SFA/UFA = Ratio of saturated and unsaturated fatty acids, PUFA/SFA = Ratio of poly unsaturated fatty acids and saturated fatty acids

Bio-evaluation of the broiler meat

Total cholesterol

The values of total cholesterol of the albino rats fed on the diet based on the antioxidant enriched meat are depicted in the **(Fig. 1)** which shows that maximum value (3.27mmole/L) of the cholesterol was observed in the group of the albino rats which were fed on the control diet (without meat with elevated level of natural antioxidants from the citrus waste) and the minimum value (2.81mmole/L) of the cholesterol was observed in the group of the albino rats which were fed on the 10%CWM feed (broiler meat reared on 10% natural antioxidants from the citrus waste). The decrease in the cholesterol of the groups with 10%CWM was probably because of the natural polyphenols in the meat of the broiler fed on citrus waste. It is clear from the mean values of the cholesterol that as the meat with the more natural compounds from the citrus waste is used the value of cholesterol in the blood serum of the rats is decreased. (Bok *et al.* 1999) fed citrus peel extract and the mixture citrus bioflavonoids to the rats and observed that plasma and hepatic cholesterol of the rats was decreased when they were fed on tangerine peel extract and the mixture of the flavonoids from the citrus. Kannappan *et al.* (2006) in an experiment observed that Cinnamon bark extract improve the glucose metabolism and lipid profile of the glucose fed albino rats.

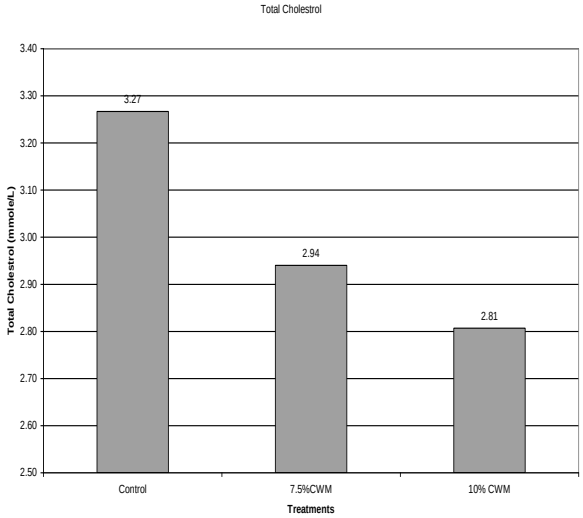

Fig 1: Total cholesterol contents of the Albino Rats fed on the broiler meat based diet with natural antioxidant

Triglycerides

The mean data for triglycerides of the albino rats fed on the meat based diet with elevated level of natural antioxidants from citrus waste presented in the **(Fig. 2)** which shows that there is decreasing trend of the blood serum triglycerides of the albino rats as the treatment of the meat in the feed of rats is increased from control to functional. The non-significance effect of the

triglycerides in the albino rats may be because of additional incorporation of the meat in the diets of the rats other than the control group. The scientific evidences showed that the cholesterol level increased as the intake of the fats increased and the high level of triglyceride and free fatty acids raise the level of lipogenesis (Kim, *et al.,* 2011). Plant polyphenols normalize the lipid abnormalities by different ways like by inhibiting the lipid absorption from the intestine, by fecal excretion using bile salts and also suppress the activity of the fat synthesis and prevent the lipid lipogenesis (Zhang *et al.,* 2012). El-Beshbishy *et al.* (2006) fed the rats with *Morus Alba* root bark fractions and observed the antioxidant status and lipid profile of the rats. Experimentally arthrosclerosis rats were made by feeding them cholesterol and coconut oil enriched diets. Methanol fraction of *Morus Alba* root bark extract when supplemented orally to the rats in the experiment @ 500mg/Kg/Day for 15 days, total cholesterol, low density lipoprotein, very low-density lipoprotein and triglycerides were reduced by 62%, 54%, 27%, and 44% respectively.

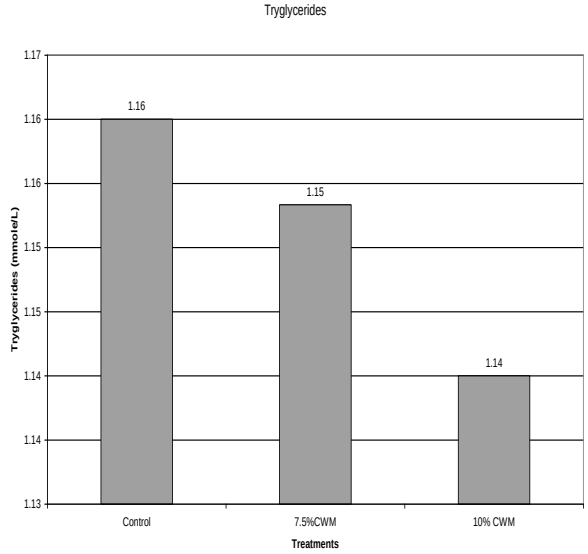

Fig. 2: Triglycerides of the Albino Rats fed on the broiler meat based diet with natural antioxidant

4.6.3 High Density Lipoprotein (HDL)

Means data for HDL of the albino rats fed on the broiler meat based diet enriched with the natural antioxidants from the citrus waste in **(Fig. 3)** shows that the values of HDL in the blood of the albino rats increased as the meat with enhanced level of natural compound from the citrus waste in the diet of the rats is used. The minimum values for HDL of the albino rats fed on the feed with broiler meat addition which was reared on citrus waste supplemented feed was observed for control group (0.83 mmole/L) which clearly shows that the meat enriched with the natural antioxidants from the citrus polyphenols improve the lipid profile of the rats and good fats increased with the addition of functional meat in the feed. (Onyeike *et al*. 2012) Michos, *et al*. (2012) observed similar results when investigating the effect of feeding juice of *Solanum Anomalum* leaf and fruit for hypolipidemic potentials in hypercholesterolemia induced albino rats by feeding the animals with extract at 30%, 60% and 90% for 28days. The results showed that high density lipoprotein was increased and low-density lipoprotein, cholesterol and triglycerides was reduced in the groups of the rats which were fed on experimental diet containing juice of *Solanum Anomalum* leaf and fruit. It is proved that as the LDL level increased the level of HDL decreased and the probability of coronary complications raised. Choi and Hwang (2005) investigated the effect of some medicinal plant on the antioxidant and lipid levels of the rats. It had been observed that the intake of the medicinal plants raised the HDL of the rats and also antioxidant enzymes of the rats fed on medicinal plant was observed more as compared to the control group of the rats.

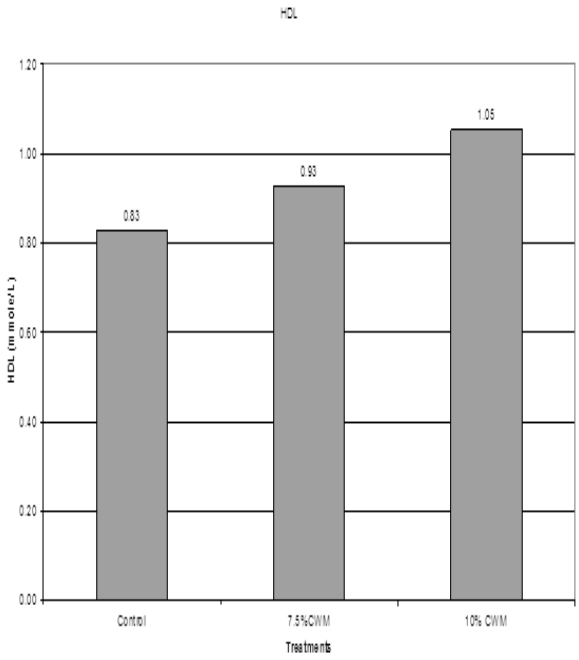

Fig 3: HDL of the Albino fed on the broiler meat based diet with Natural Antioxidant

Low Density Lipoprotein

The data regarding the analysis of variance for low density lipoprotein of the albino rats fed on the diet supplemented with broiler meat produced with the natural antioxidants from the citrus waste is presented in the **(Fig. 4)** which shows that low density lipoprotein was decreased as the meat with more level of antioxidants from the citrus waste was used. LDL

having key connection with the atherosclerosis and the cardiovascular diseases and it can combine with the macrophage receptor forming deposition of the cholesterol inside arteries (Kuo, *et al*. 2004). It has been observed that the supplemented diet to albino rats with tocotrienol rich fraction reduced triglycerides to 38-46%, Cholesterol to 48%, LDL to 39% and there was no change in the (HDL level (Minhajuddin, *et al*. 2005).

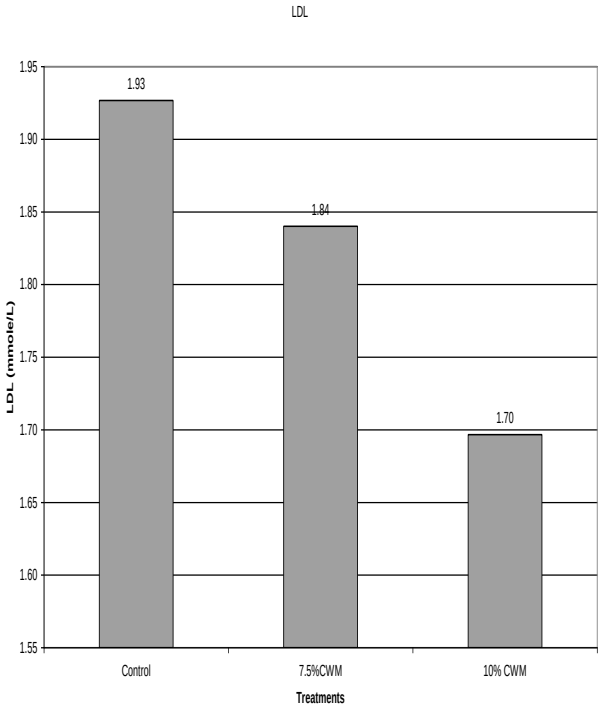

Fig 4: LDL of the Albino fed on the broiler meat based diet with natural antioxidant

4. CONCLUSIONS

The supplementation of citrus processing waste in broiler feed significantly enhanced the antioxidants status of the broiler meat which has positive effects on modulating cholesterol profile as evident from rat's model.

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REFERENCES:

Arshad, M. S., F. M. Anjum, A. Asghar, M. I. Khan, M. Yasin, M. Shahid and A. H. El-Ghorab (2011). Lipid stability and antioxidant profile of microsomal fraction of broiler meat enriched with antioxidants and natural antioxidants acetate. J. Agric. Food Chem. 59: 7346–7352.

Annoni, G. G., R. M. Bottasso, D. Ciaci, M. F. Donato and A. Tripoli. (1982). Clinical Investigation of triglycerides. J. Res. Lab. Med. 9:115-18.

Assmann. G. (1979). HDL-cholesterol precipitant. Randox Labs. Ltd. Crumlin Co. Antrim, N. Ireland. Int. 559-567.

Bok, S. H., S. H. Lee, Y. B. Park, K. H. Bae, K. H. Son, T. S. Jeong and M. S. Choi. (1999). Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-COA reductase and acyl COA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. J. Nutr. 129: 1182–1185.

Choi, E. and J. K. Hwang. (2005). Effect of some medicinal plants on plasma antioxidant system and lipid levels in rats**.** Phytotherapy Res. 9(5): 382-386.

El-Beshbishy, H. A., A. N. B. Singab, J. Sinkkonen and K. Pihlaja. (2006). Hypolipidemic and antioxidant effects of *Morus Alba L*. (Egyptian mulberry) root bark fractions supplementation in cholesterol-fed rats. Life Sci. 78: 2724 – 2733

FAO. (2012). http://www.fao.org/knowledge/goodpractices/bp-crop-systems/bp-plant-nutrients/en/

Khan, M. I., M. N. Idrees, M. S. Arshad, F. M. Anjum, C. Jo and A. Sameen. (2015). Oxidative stability and quality characteristics of whey protein coated rohu (Labeo rohita) fillets. Lipids Health Dis. 14:58-66

Kannappan, S., T. Jayaraman, P. Rajasekar, M. K. Ravichandran and C.V. Anuradha. (2006). Cinnamon bark extract improves glucose metabolism and lipid profile in the fructose-fed rat. Singapore Med. J. 47(10): 858-863.

Kim, M., E. Kim, Y. Kim, C. Choi and B. Lee. (2011). Effects of a-lipoic acid and l-carnosine supplementation on antioxidant activities and lipid profiles in rats. Nutr. Res. Practice. 5(5): 421-428.

Kuo, P. C., A. G. Damu, K. H. Lee, and T. S. Wu. (2004). Cytotoxic and Antimalarial Constituents from the Roots of Eurycoma Longifolia. Bioorg. Med. Chem.12: 537-544.

Lopez-Ferrer, S., M. D. Baucells, A. C. Barroeta, and M. A. Grashorn. (1999). N-3 enrichment of chicken meat using fish oil: Alternative substitution with rapeseed and linseed oils. Poult. Sci. 78:356–365.

Marini, D., F. Balestrieri and H Multivariate.(1995). Analysis of flavanone glycosides in citrus juices. Int. J. Food Sci. 3, 255- 264.

McNamara, J. R., J. Cohn, P. W. Wilson and E. J. Schaefer. (1990). Calculated Values for Low-density Lipoprotein Cholesterol in the Assessment of Lipid a Anormalities and Coronary Disease Risk. Clin. Chem. 36:36-42.

Mourao, J. L., 1 V. M. Pinheiro, J. A. M. Prates, R. J. B. Bessa, L. M. A. Ferreira, C. M. G. A. Fontes, and P. I. P. Ponte. (2008). Effect of dietary dehydrated pasture and citrus pulp on the performance and meat quality of broiler chickens. Poult. Sci. 87:733–743

Michos, M. D., P. R. Jared, S.P. Venday, L.L. Pamela, Rebecca F. G. and H. M. Thomas. (2012). 25-Hydroxyvitamin D deficiency is associated with fatal stroke among whites but not blacks: The NHANES-III linked mortality files. Nutr. 28(4).

Minhajuddin, M., Z. H. Beg and J. Iqbal. (2005). Hypolipidemic and Antioxidant Properties of Tocotrienol Rich Fraction Isolated from Rice Bran Oil in Experimentally Induced Hyperlipidemic Rats. Food Chem. Toxicol.43:747–753.

Onyeike, E. N., M. O. Monanu and C. N. Okoye. (2012). Changes in the blood lipid profile of wistar albino rats fed rich cholesterol diet. Annals Bio. Res. 3 (11):5186-5191

Raes, K., De Smet and D. Demeyer. (2001). Effect of double muscling in Belgian Blue young bulls on the intramuscular fatty acid composition with emphasis on Conjugated linoleic acid and polyunsaturated fatty acids. Anim. Sci. 73:253–260.

Scaife, J. R., J. Moyo, H. Galbraith, W. Michie and V. Campbell. (1994). Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers. Brit. Poult. Sci. 35: 107–118.

Skrivan, M., V. Skrivanova, M. Marounek, E. Tumova and J. Wolf. (2000). Influence of dietary fat source and copper supplementation on broiler performance, fatty acid profile of meat and depot fat, and on cholesterol content in meat. Brit. Poult. Sci. 41: 608–614

Stockbridge, H., R. I. Hardy and C. J. Glueck. (1989). Photometric determination of cholesterol (CHOD-PAP method). J. Lab. Clin. Med. 114(2):142-151.

Steel, R. G. D., J. H. Torrie and D. A. Dicky. (1997). Principles and procedures of statistics. A biometrical approach. 3rd Ed. McGraw Hill Book Co. Inc., New York.

Yasin, M., A. Asghar, F. M. Anjum, M. S. Butt, M. I. Khan, M. S. Arshad, M. Shahid, A. H. El-Ghorab and T. Shibamoto. (2012). Oxidative stability enhancement of broiler bird meats with a-lipoic acid and a-tocopherol acetate supplemented feed. Food Chem. 131:768–773.

Yang, X., L. Yang and H. Zheng. (2010). Hypolipidemic and antioxidant effects of mulberry (Morus Alba *L.*) fruit in hyperlipidaemia rats. Food Chem. Toxicology. 48(9): 2374–2379.

Zhang, W. J., E. B. Karyn, S. M. Timothy, C. L. Renee and T. M. Hagen. (2012). Dietary alpha-lipoic acid supplementation inhibits atherosclerotic lesion development in apolipoprotein e–deficient and apolipoprotein e/low-density lipoprotein receptor–deficient mice. Circulation. 117: 421-428.