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Supplementation of alpha lipoic acid on growth performance, nutrient digestibility, mineral availability and lipid peroxidation level in broiler chicken fed with animal fat

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Abstract
The aim of the present study was to determine the effects of alpha lipoic acid supplementation on growth performance, nutrient digestibility, mineral availability and lipid peroxidation level in broilers fed diet with animal fat. The trial was conducted using eighty day-old VenCobb commercial broiler chicks which were randomly assigned into two dietary treatment groups with four replicates of ten chicks each. The birds in both the control (T_1) and treatment group (T_2) were fed with diet containing 5% animal fat, while T_2 was supplemented with 100 mg of Alpha lipoic acid. The birds were fed with standard broiler starter ration up to 4 weeks of age and finisher ration up to 6 weeks of age as per BIS (1992) specifications. Alpha lipoic acid supplementation did not have any effect on body weight (g), Feed consumption (g) and feed conversion ratio (%), availability of nutrients and minerals, however, decreased the serum MDA concentration, which is one of the most common indicators of lipid peroxidation. In conclusion, the present study revealed that supplementation of alpha lipoic acid has no effect on growth performance, nutrient and mineral utilisation, but reduces the lipid peroxidation level, thereby improves the quality of meat.

Keywords: Broilers, alpha lipoic acid, animal fat, growth performance and nutrient utilisation, lipid peroxidation

Introduction
Poultry industry is one of the fastest growing fields in agricultural sector with an annual growth rate of 13% per annum. In the intensive feeding system of poultry sector, usage of fat become one of the natural components of the concentrate feed, as an additive for increasing the energy value as well as to improve the tastiness and consistency of feed. (Jeffri *et al.*, 2010) [17]. Commonly, Vegetable oils are commonly used as a fat source in broiler diets which is costlier, in turn increases the production cost of feed. (Azman *et al.*, 2004) [4] In recent years, Animal fats are available at cheaper rates compared to vegetable oils, due to the development of rendering technologies. (Mohammedi *et al.*, 2011) However, the usage level and type of animal fat influence the carcass fat composition and cause oxidative stress in chicken. This fat deposition causes negative impact to the consumers who are more concern about their health. (Buyse *et al.*, 2001) [7] Dietary modification is one of the practical and preferred ways to manipulate fat deposition and to reduce oxidative stress in chicken. Recently Alpha Lipoic acid a vitamin like substance has been shown to stimulate energy metabolism and act as potent biological antioxidant (Zhang *et al.*, 2009) [30]. Thereby, Alpha Lipoic acid has gained much interest as a feed additive for combating fat deposition and oxidative stress.

Lipoic acid (LA) also known as thioctic acid (1,2-dithiolane-3-valeric acid) is a naturally occurring compound in microorganisms, plants and animals. It is described as universal antioxidant since it can combat oxidative stress by quenching a wide variety of reactive oxygen species. (Winiarska *et al.*, 2008) [29]. Dihydrolipoic acid (DHLA), the oxidized form of LA is involved in the recycling of other antioxidants in the body such as glutathione, vitamin C, coenzyme Q_{10} and vitamin E. Both Lipoic acid and DHLA were able to chelate a wide variety of metals which are associated with increased production of free radicals. Additionally, Lipoic acid generally complex with lysine, called lipoamide, which functions as an essential co-factor in the mitochondrial dehydrogenase complexes which catalyse the oxidative decarboxylation of α-keto acids that plays a major role in energy metabolism (Marangon *et al*., 1999) [20].

The previous studies mostly focussed on Lipoic acid supplementation on growth performance and oxidative stress aspects in poultry. The trails also have positive impact on the antioxidation effect in poultry. However, no research trials have conducted on nutrient digestibility and mineral availability in poultry. Therefore, the aim of the present study was to evaluate the effects of Alpha Lipoic acid on growth performance, nutrient digestibility, mineral availability and lipid peroxidation level in broilers fed with diet containing animal fat.

Materials and methods

The present experiment was conducted at at Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala using eighty-day old unsexed commercial broiler chicks (VennCobb) and the birds were randomly allotted to two treatment groups (T_1 and T_2) with four replications of ten chicks each in separate pen. The dietary treatments were control group (T_1) fed with standard broiler chicken ration as per BIS (1992) specifications with 5 per cent animal fat and the second treatment group (T_2) was supplemented with alpha lipoic acid at 100 mg/kg diet containing 5 per cent animal fat. The ingredient and chemical composition of the broiler rations are presented in Table 1 and 2.

The birds were fed with starter ration up to 4 weeks of age and finisher ration up to 6 weeks of age. Feed and clean drinking water were provided *ad libitum* and the birds were maintained under identical management conditions, throughout the experimental period. The body weight and feed intake of broilers was recorded at weekly intervals to study the growth pattern in two dietary treatments. Feed conversion ratio (kg of feed consumed/kg body weight gain) was calculated based on the data obtained on body weight gain and feed intake. At the end of the experimental period, five birds from each treatment were fasted overnight, slaughtered and dressed as per the standard procedures. (BIS, 1973).

Table 1: Ingredient Composition of Broiler Starter and Finisher Ration, %

Ingredients	Broiler starter rations, %		Broiler finisher rations, %	
	T1	T2	T1	T2
Maize	40	40	48.5	48.5
Soybean meal	41.4	41.4	32.89	32.89
Wheat bran	9	9	9	9
Animal fat	5	5	5	5
Dicalcium phosphate	2	2	2.1	2.1
Calcite	1.79	1.79	1.8	1.8
DL-methionine	0.14	0.14	0.04	0.04
Choline chloride	0.1	0.1	0.1	0.1
Trace mineral mixture	0.01	0.01	0.01	0.01
Supplements	0.31	0.31	0.31	0.31
Salt	0.25	0.25	0.25	0.25
Total	100	100	100	100
To 100kg of the above mixture following are added				
α-Lipoic acid (mg/kg)	_	100	-	100

Trace mineral mixture containing Manganese sulphate-60 g, Zinc sulphate-50 g, Ferrous sulphate-40 g, Iodide-2 g, Copper-5 g, Cobalt-2 g and Selenium-0.3 g. Supplements containing B complex vitamins, Vitamin AB_2D_3K, Toxin binder, Coccidiostat and Liver supplement.

Metabolism trial

A metabolism trial of three days duration was conducted after the feeding trial, by randomly selecting one bird from each replicate. Birds were housed in individual metabolism cages with all facilities for feeding, watering and excreta collection to determine the digestibility of nutrients and percentage retention of minerals of the experimental diets. Excreta were collected for three consecutive days for over 24-hour period using total collection method as described by Summers *et al.* (1992). Excreta was daily collected from each bird were weighed and representative samples were taken after thorough mixing. The total amount of feed consumed was also recorded.

The chemical composition of experimental rations and faecal samples was determined as per the AOAC, 2012 [1]. Minerals such as Ca, Mg, Cu and Fe were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer AAS Model 400) after wet digestion, using nitric acid and perchloric acid (2:1). Phosphorus contents of both feed and faecal samples were analyzed by colorimetry (ANSA method, AOAC, 2012) [1] using spectrophotometer (Spectronic 20D$^+$, spectronic instruments, USA). From the data obtained from total intake and outgo of nutrients during the metabolism trial, digestibility of nutrients and nitrogen retention was calculated. The serum lipid peroxidation level was determined by the method of Fraga (1988) [12]. Data collected on various parameters were statistically analyzed by Completely Randomized Design (CRD) as described by Snedecor and Cochran (1994) [24] using SPSS 16.0 (2008) [26]. Means were compared by Independent Samples t Test.

Table 1: Chemical Composition of Broiler Starter and Finisher Rations*

Parameters	Broiler starter ration	Broiler finisher ration
Dry matter, %	86.83	87.10
Crude protein, %	23.25	20.14
Ether extract, %	5.48	5.73

Crude fibre, %	4.38	4.16
Nitrogen free extract, %	57.47	62.09
Total ash, %	9.42	7.88
Acid insoluble ash, %	1.90	1.25
Mineral Composition		
Calcium, %	1.41	1.37
Total phosphorus, %	1.23	1.13
Magnesium, %	0.37	0.35
Iron, ppm	90.82	81.80
Copper, ppm	19.92	16.65
Calculated Values		
Metabolisable energy, kcal/kg	2805	2900
Lysine, %	1.27	1.07
Methionine, %	0.34	0.31

* On dry matter basis

Ethical approval

The experiment was carried out according to the National regulations on animal welfare and
Institutional Animal Ethical Committee.

Results and discussion

The results on cumulative weekly body weight (g), Feed consumption (g) and feed conversion ratio are presented in Table 2. Throughout the experimental period, Alpha lipoic acid supplementation did not show any effect on body weight, feed consumption and feed conversion efficiency compared to the control group. In a similar study conducted by Hamano (2002, 2012) [14, 15], also couldnot notice any effect bodyweight of birds by lipoic acid supplementation at 100 ppm in broilers. Likewise, Srilatha *et al.* (2010) [27] when lipoic acid fed at 20, 40 or 60 ppm, Chen *et al.* (2011) [10] at 100, 200 and 300 ppm did not show any effect on the feed intake of broilers. While, Zhang *et al.* (2009) [30] could not observe any effect on feed conversion ratio by supplementing diet with lipoic acid at various levels (0, 300, 600 and 900 ppm) for 42 days. Maddock *et al.* (2003) [19] supplemented weanling pigs with lipoic acid (8 and 15 mg/kg body weight) in feed and found no significant influence in the feed to gain ratios. In contrast to the present study, Sohaib *et al.* (2012) [25] noticed that the alpha lipoic acid supplementation increased the body weight gain in broilers. Whereas, Diaz-Cruz *et al.* (2003) [11] found reduced feed intake and improved the feed conversion ratio in broilers as a result of lipoic acid supplementation. In contrast, Han *et al.* (2011) observed poor feed conversion ratio in rats fed with 125 and 250 mg of lipoic acid/kg body weight compared to those fed with 0, 12.5 or 25 mg of lipoic acid/kg body weight.

Table 2: Cumulative body weight gain (g), cumulative feed consumption (g) and Feed conversion ratio (%) of birds maintained on two dietary treatments

Week	Cumulative body weight gain†, g		P value	Cumulative feed consumption†, g		P value	Cumulative feed conversion ratio†		P value
	T_1	T_2		T_1	T_2		T_1	T_2	
1	73.15 ± 4.76	73.65 ± 1.19	0.92	100.74 ± 3.85	102.56 ± 2.94	0.72	1.39 ± 0.06	1.40 ± 0.06	0.91
2	273.16 ± 13.55	281.16 ± 6.13	0.61	438.90 ± 13.46	457.39 ± 23.33	0.51	1.62 ± 0.11	1.63 ± 0.08	0.97
3	633.19 ± 21.45	655.68 ± 9.01	0.37	1027.68 ± 31.70	1070.65 ± 42.71	0.45	1.63 ± 0.11	1.63 ± 0.06	0.98
4	1112.25 ± 26.45	1130.90 ± 16.46	0.57	1854.99 ± 64.34	1905.56 ± 55.28	0.57	1.67 ± 0.08	1.69 ± 0.05	0.92
5	1545.54 ± 39.09	1568.49 ± 14.09	0.60	2860.85 ± 93.53	2884.05 ± 67.61	0.84	1.86 ± 0.09	1.84 ± 0.03	0.82
6	2031.47 ± 64.62	2051.75 ± 13.95	0.76	3976.49 ± 103.61	4063.46 ± 78.52	0.52	1.97 ± 0.10	1.98 ± 0.03	0.91

The reasons for the discrepancies on body weight, feed intake and feed conversion ratio between the studies were speculative but may be due to the different levels of α-lipoic acid used, age at which α-lipoic acid is added to diet, species, sex and broiler genotype, chick weight or basal diet composition.

The data on nutrient availability (%) and nitrogen balance (g/day) of two experimental rations are presented in Table 3. On statistical analysis of data, there is no significant difference (P>0.05) between the treatment and control groups. Similar to the results of the present study, Attia *et al.*, 2016 didnot find any effect on dry matter, ether extract, crude fibre and nitrogen free extract digestibility supplemented with Vitamin - E at 150 mg/kg diet in laying hens. Likewise, Crude protein and fat digestibility was not affected by vitamin-E supplementation in broilers at 200 mg/kg feed (Acikgoz *et al.*, 2010) and (Brenes *et al*., 2008) [8]. In contrast, Chae *et al.*, 2006 observed that, supplementation α-Tocopherol Acetate at 100 & 200 mg/kg feed significantly improved the digestibilities of crude protein, fat & gross energy compared to control. Similarly, Lohakare *et al*., 2004 [18] also found that addition of vitamin - E either in feed or water improved the digestibilities of crude protein, fat & gross energy.

Table 3: Nutrient availability (%) and nitrogen balance (g/day) of two rations

Parameter	Nutrient availability†		P value
	T_1	T_2	
Dry matter (%)	68.23 ± 3.21	68.00 ± 1.47	0.61
Crude protein (%)	58.72 ± 2.92	55.98 ± 2.78	0.20
Ether extract (%)	73.85 ± 3.29	71.35 ± 2.31	0.85
Crude fibre (%)	34.19 ± 3.06	33.86 ± 1.08	0.60
Nitrogen free extract (%)	79.18 ± 3.08	79.59 ± 0.90	0.90
Energy efficiency (%)	70.24 ± 3.04	70.03 ± 1.31	0.46

Nitrogen balance (g/day)	2.35 ± 0.09	2.39 ± 0.21	0.62

† Mean of four values with SE

The percentage availability of calcium (Ca), phosphorus (P), magnesium (Mg), copper (Cu) and iron (Fe) as influenced by two dietary treatments are presented in Table 4. There is no difference (P>0.05) between the treatment groups on the availability of minerals. The per cent availability of Ca, P, Mg, Cu and Fe (%) for T1 & T2 were 55.22 & 54.11, 55.31 & 54.57, 54.17 & 55.69, 53.96 & 47.88, 47.95 & 46.38, respectively. In agreement with the results of present study, Rayani *et al*., 2017 [27] found that vitamin –E supplementation at 125 and 250 ppm did not have any effect on mineral availability of Calcium, Phosphorus and zinc in broiler chicken.

Table 4: Mineral availability of two experimental rations, %

Parameter	Mineral availability†, %		P value
	T_1	T_2	
Calcium	55.22 ± 3.29	54.11 ± 3.82	0.83
Phosphorus	55.31 ± 6.65	54.57 ± 2.11	0.91
Magnesium	54.17 ± 6.56	55.69 ± 2.15	0.83
Copper	53.96 ± 5.81	47.88 ± 3.83	0.41
Iron	47.95 ± 5.79	46.38 ± 1.07	0.79

† Mean of four values with SE

The Serum malondialdehyde (MDA) concentration (nmol/ml) of birds maintained on two dietary treatments are shown in Table 5 and MDA values were significantly (p<0.05) reduced in alpha lipoic acid supplemented group. In agreement with the results of the present study, Srilatha *et al*., 2010 [27] noticed that supplementation of Alpha lipoic acid at 80mg/kg feed significantly reduced the serum MDA level in broiler chicken. Similarly, Lipoic acid supplementation in feed reduced the serum MDA concentration at 200 & 300mg/kg (Chen *et al*., 2011) [10] and at 100, 600 & 900 ppm (Zhang *et al*., 2009) [30] in broilers. Generally, measurement of MDA concentration is one of the most commonly used method to measure the degree of lipid peroxidation (Halliwell & Chirico, 1993) [13]. Higher MDA concenterations reveals higher amount of lipids, which will deteriorate the quality the meat. In this study, supplementation of lipoic acid, indicated less peroxidation by producing lower amount of MDA levels. This may be due to the antioxidant activity either by directly scavenging Reactive oxygen species or by recycling endogenous antioxidants such as Vitamins C, coenzyme $Q_{10,}$ glutathione (Busse *et al*, 1992) [6]. In addition, alpha lipoic acid may have an indirect effect on oxidative stress by chelating redox-active transition compounds such as iron and copper (Ou *et al*. 1995) [22].

Table 5: Serum Lipid peroxidation level of two dietary treatments

Parameter	Lipid peroxidation level †		P value
	T_1	T_2	
MDA (nmol/ml)	2.13 ± 0.14^a	1.64 ± 0.12^b	0.03

a, b – Means bearing different superscripts within the same row differ significantly (P<0.05)
† Mean of five values with SE

Conclusion

The results of the present study indicated that alpha lipoic acid supplementation did not affect the body weight, feed consumption, feed conversion ratio, availability of nutrients and minerals, however, decreased the serum MDA concentration compared to control group. Supplementation of alpha lipoic acid reduced the lipid peroxidation level, thereby improves the quality of meat.

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Competing interests: The authors declare that they have no competing interests.

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