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Full Length Research Paper

Effects of dietary fat, vitamin E and zinc supplementation on tibia breaking strength in female broilers under heat stress

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The objective of this experiment was to characterize the relationship between dietary fat and antioxidant supplementation, on tibia breaking strength (TS) and performance parameters in broiler chickens under heat stress. The chicks with a similar body weight were equally assigned to one of the two controlled-environment chambers from 21 to 56 days of age. The birds were fed diets as follows: 1) basal diet supplemented with 5% saturated fatty acid; 2) basal diet supplemented with 5% unsaturated fatty acids (2% canola oil plus 3% fish oil); and 3) the second diet supplemented with antioxidants (100 IU vitamin E and 50 mg/kg zinc); that birds received these 3 diets in two temperatures (22°C and 32°C). Results showed that feed conversion ratio was not influenced by antioxidant and fat supplementation but antioxidants could improve body weight gain and feed conversion ratio significantly; although, higher feed intake was observed (P < 0.05). High environmental temperature showed deleterious effects, including: reduction of feed intake and live weight and increase of feed conversion ratio. Characterization of tibia bone showed that, the higher environmental temperature decreased length and width of tibia, ash and its strength, significantly (P < 0.05).

Key words: Broiler, heat stress, fat, zinc, vitamin E, femur breaking strength.

INTRODUCTION

Heat stress has deleterious effects on broiler and suppresses the efficiency of production. During this period, broiler has to make major thermo-regulatory adaptations against the death, because they have a greater challenge in maintaining homoeothermic body temperature rather than the other animals. Regarding the fact that poultry lack sweat glands and have relatively high body temperature (41.5°C),they rely on evaporation cooling (through panting) to keep themselves cool (Ensminger et al. , 1990). Several methods to alleviate the negative effects of high environmental temperature on performance of poultry have been recommended, but nutritional manipulation with its low cost is a common approach in poultry production (Shane, 1988). The role of dietary supplements such as, vitamins for alleviating the effect of heat stress in poultry has been reviewed extensively by Sahin and Kucuk (2003).

Several studies have shown a positive effect of n-3 on bone mineral density (BMD) and bone mineral content (BMC) in animals. Liu et al. (2003) found a significantly higher BMC in quail, fed a fish oil-supplemented diet (high in n-3) compared with a soybean oil diet group (high in n-6). Similarly, fish oil-supplemented rats had a significantly higher BMD in the distal femur and proximal tibia than a corn oil-supplemented group (high n-6; Sun et al., 2003). However, other studies have shown no significant effect of a high n-3 diet on BMD or BMC (Mollard et al., 2005; Johnston et al., 2006). At the other

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hand, a diet with a high saturated fat level adversely affected bone mineralization and consequently compromised structural and mechanical properties of bones, in growing rats (Hou et al., 1990; Li et al., 1990; Salem et al., 1992; Zernicke et al., 1995) and chicks (Atteh et al., 1983; Wohl et al., 1998).

Zinc is used in poultry diets because of its antistress effects. Moreover, its requirement increases and its retention decreases during stress (Bartlett and Smith, 2003; Sahin and Kucuk, 2003b).The result of Sandoval et al.(1998) who supplemented basal ration with zinc amount between 1,000 and 1,500 mg/kg , showed that dietary zinc supplementation leads to increased tibia zinc concentration. Zinc deficiency also causes shortness and thickness in long bones of legs and wings. There is also some evidence suggesting supplemental zinc can alleviate negative effects of heat stress in broiler chickens (Klasing, 1984).

Bone breaking strength is measured by evaluating the reaction of the bone to stress and force. An increase in bone mineralization is accompanied by an increase in bone stress and bending moment values. Bone mechanical strength is affected by nutrition, genes, age, sex, environment etc., for the expression of collagen and proteins, quantity and quality of the organic and inorganic material, content and size of the mineral material and design and structure of the bone (Boskey et al., 1999). Velleman (2000) explained the structural complexity and composition of bone associated with strength, which varied according to the age and nutritional status of the bird, femur compression strength = 11.9 + 0.0141 (bird BW). However, contradictory data regarding the effect of vitamin E on collagen synthesis exists and few studies have reported its influence on cartilage development. In fact, increase in thickness of the growth plate was proportional to DL-α-tocopheryl acetate dose. The effect of vitamin E on chondrocytes was observed by Xu et al. (1995) in chicks, fed by two levels of DL-α-tocopheryl acetate and two dietary lipids. In their study, the thickness of the entire growth plate cartilage and of the lower hypertrophic chondrocyte (mineralized) zone was significantly wider in animals fed the greater level of vitamin E. α-tocopherol protects PUFA from lipid oxidation. However, the effect of α-tocopherol and zinc supplementation on bone strength in poultry has rarely been studied and is rather controversial.

In this study, the relation between dietary fat, zinc and vitamin E with heat stress on femur breaking strength, has been studied separately. Therefore, the objective of this experiment was to determine the effect of the four aforementioned objects in performance parameters and TBS too.

MATERIALS AND METHODS

Experimental design, animals, housing and diets

Two hundred and forty female Arbor Acres broiler chicks were used for evaluating effects of diet and temperature, on the immune response and blood parameters. Two factors of temperature and feed (2×3) were considered in a complete random design to form 6 separate treatments. Each treatment had 4 replications and 10 birds and it lasted for 6 weeks. Diets included: 1) basal diet supplemented with 5% saturated fatty acid, 2) basal diet supplemented with 5% unsaturated fatty acids (2% of canola oil plus 3% of fish oil); 3) basal diet supplemented with 5% unsaturated fatty acids (2% of canola oil plus 3% of fish oil) and antioxidant (100 IU vitamin E and 50 mg/kg zinc); that birds received these 3 diets in two temperatures (22 and 32°C). Table 1 shows the ingredients and chemical analyses of the starter and grower diets fed to experimental broiler chickens. The chicks were placed in floor pens equipped with stainless steel feeders and automatic water drinkers. Lighting was continuous and water and feed were available *ad libitum* for 56 days. The weights of birds were recorded at the end of the experiment and also at 21st day of age. Feed intakes per pen were recorded. Birds were always deprived of feed 12 h before being weighed. The experimental chickens were slaughtered at the 56th day of age.

Bone sampling

At the end of the experiment (56th day), birds were killed by cervical dislocation and weighed, afterward the right tibia bones were removed. Much of the adhering tissues were removed from each bone at the time of dissection. Each bone was subsequently placed in boiling water for 15 min to complete tissue removal. The length of each bone was determined with a ruler and the width was measured using a micrometer. Tibia bones from 10 birds of each group were evaluated. Tibia breaking strength was determined with instron set. A compression test determined the behavior of materials under crushing loads. The biomechanical strength of each bone was measured using a material testing machine. The bones were held in vertical position and the compression load was applied in the cross-sectional area up to bone failure; the total crushing load at failure was determined in Newton (N/mm^2). The bone ash was determined after ashing in a muffle furnace at 600°C for 24 h.

Fatty acid content of diets

Fatty acid content of feeds was determined following the methodology described by Metcalf et al. (1996). The fatty acid (FA) content was determined using a gas chromatograph (HP68908, Unicam 4600, USA) equipped with a flame ionization detector and a HP 19091 to 136 capillary column9 (30 m, 0.22 mm internal diameter) with a film thickness (0.25 µm) of stationary phase. Helium was used as gas carrier. Oven temperature was programmed as follows: from 140 to 160°C at 1.50°C/min; from 160 to 180°C at 20°C/min; and from 180 to 190°C at 20°C/min. The other chromatographic conditions were injector and detector temperatures, 280°C; sample volume injected, 1 µl. Fatty acids were identified by matching their retention times with those of their relative standards, as well as by mass spectrometry (HP5973) with 8 at each peak. Fatty acid composition of diets is shown in Table 3.

Statistical analysis

As mentioned before, the experimental design consisted of a 2 × 3 factorial arrangement with 6 treatment groups, 4 replications of 10 birds each. Data for chickens were analyzed with analysis of variance (ANOVA). Performance parameters were analyzed for each pen as a replication. The general linear model (GLM) of the Statistical Analysis System (SAS Institute, 1999) was conducted for the statistical processing of data. Differences were considered

Table 1. Ingredients and chemical analyses of the starter and grower diets.

Treatment	Starter percentage of DM			Grower percentage of DM		
	O	A	T	O	A	T
Ingredient						
Ground corn	54.75	54.75	54	61.73	61.73	61
Soybean meal	37.28	37.28	37.28	30.25	30.25	30.25
Fish oil	4.51	4.51	-	4.65	4.65	-
Tallow	-	-	5.26	-	-	5.38
Dicalcium phosphate	1.37	1.37	1.37	1.33	1.33	1.33
Sodium chloride	0.1	0.1	0.1	0.2	0.2	0.02
Limestone ground	0.53	0.53	0.53	0.53	0.53	0.53
Calcium carbonate	0.1	0.1	0.1	0.16	0.16	0.16
DL-methionine	0.24	0.24	0.24	0.25	0.25	0.25
Lysine	-	-	-	0.12	0.12	0.12
Cavilamycin	0.1	0.1	0.1	0.1	0.1	0.1
Ca- propionate	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin E IU	-	100	-	-	100	-
Zinc mg/Kg	-	50	-	-	50	-
Clinao	0.1	0.1	0.1	0.1	0.1	0.1
Toxin	0.3	0.3	0.3	-	-	-
Vitamin premix*	0.27	0.27	0.27	0.27	0.27	0.27
Trace mineral premix**	0.16	0.16	0.16	0.16	0.16	0.16
DM%	89.65	89.65	89.63	89.68	89.68	89.68
Chemical analyses						
ME, kcal/kg	3100	3100	3100	3200	3200	3200
Cp	23	23	23	20.03	20.03	20.03
Crude fat	7.04	7.04	7.04	6.35	6.35	6.35

* Premix supplied for 2 kg: vitamin A, 15000 IU; cholecalciferol, 3 IU, vitamin E 15 IU; menadione, 2.5 mg; vitamin B1, 1 mg vitamin B2, 10 mg; niacin, 70 mg; d-pantothenic acid, 20 mg; vitamin B12, 4 mg; folic acid, 2 mg; biotin, 0.1 mg; ** oremix supplied for 2 kg; Mn, 80 mg: Mn, 80 mg; Fe, 25 mg; Zn, 50 mg; Cu, 7 mg; Iodine, 0.3 mg; Se, 0.15 mg; choline chloride, 350 mg.

significant at $P \leq 0.05$ and means were compared by Duncan test. Interactions were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Performance

The effects of dietary fat and temperature, on the performance of broilers have been shown in Table 2. Feed to gain ratio was not influenced by diet. However, high environmental temperature reduced the feed intake, body weight and increased ($P \leq 0.05$) feed to gain ratio in broiler (Table 2). This result showed that diet containing saturated fat, had a significant effect on feed intake and body weight; although, the same result on feed to gain was not convenient ($P \leq 0.05$). It might be due to high energy level of tallow in comparison with fish oil. Addition of zinc and vitamin E to the unsaturated oil ration, increased the body weight of broilers, though it was less than the body weight of tallow ration. Interaction of diet and temperature had a significant effect on broilers performance ($P < 0.05$). High environmental temperature had deleterious effects on performance including: feed intake and live weight. Not only did feed intake and live weight show reduction, also feed to gain ratio increased in broilers at high environmental temperature (Donkoh et al., 1989). This negative effect of heat stress on growth rate and production is speculated to be due to reduced feed intake (Hurwitz et al., 1980).

Bone strength

The effect of temperatures and diets are shown in Table 4. There are significant differences of bone parameters in different groups. The highest weight of dry bone, length, width, ash and maximum force for breaking bone were observed in low environmental temperature breeding groups ($P< 0.05$). High temperature causes excretion of some minerals, for example Ca, Fe, Zn and leads to decreased bone strength (Post et al., 2003). In chickens, bone mineralization as determined through dual-energy x-ray absorptiometry (DEXA) is positively correlated with

Table 2. Effect of temperature, oil type and supplemental antioxidant on feed intake, feed conversion and body weight.

Temperature	Feed intake	Feed conversion	Body weight
Heat stress(H)	4976 [b]	1.96 [a]	2539[b]
Normal(C)	6329 [a]	1.89 [b]	3349[a]
P	0.02	0.03	0.02
Feed			
O	5423 [b]	1.93	2810 [b]
T	5929 [a]	1.92	3088[a]
A	5662 [ab]	1.93	2934[ab]
P	0.03	n.s	0.027
Interaction (Temp × Feed)			
TH	5297[c]	1.95 [a]	2717 [b]
TC	6539[a]	1.89[b]	3460[a]
OH	4682[d]	1.97[a]	2377[c]
OC	6131[b]	1.89 [b]	3244[a]
AH	4947[cd]	1.96[a]	2524[bc]
AC	6301[ab]	1.89[b]	3334[a]
P	0.02	0.03	0.024
SEM	192	0.23	88

Table 3. Fatty acid composition in experimental diets (mg/g fat).

Fatty acids 2	Experimental diets1		
	O	T	A
16:00	26.6	26	36
16:01	6	6.4	6.22
18:00	6.6	5.3	6.5
18:01	38	42	37.9
18:02	15	11.6	12.9
18:03	1.57	0.58	1.4
EPA	1.53	0.09	1.52
DHA	1.16	0.047	1.09
SFA	33	32	33
UNFA	44.24	49.13	43.3
PUFA	19.73	17.2	13
UFA	63.97	62	61.5
omega-3	4.6	1.25	4
omega-6	15.2	11.81	13
n6/n3	3.27	9.61	3
UFA/SFA	1.95	1.95	1.84

[a b c d], values that do not have common superscripts are significantly different ($P < 0.05$). 1 O = basal diet + unsaturated fatty acid, A = basal diet + 100 IU/kg vitamin E and 50 mg/kg zinc T= basal diet + saturated fatty acid; 2SFA = Saturated fatty acids; UFA =. unsaturated fatty acids; MUFA = monounsaturated fatty acids; and PUFA = polyunsaturated fatty acids; n6/n3 = proportion of omega-6 to mega-3.

bone breaking force ($r = 0.58$ to 0.68; $P < 0.001$) and bone ash weight ($r = 0.73$ to 0.99; $P < 0.001$; Onyango et al., 2003; Mazzuco and Hester, 2005; Schreiweis et al., 2005). Higher increases in stress occur with smaller increases in ash content. Decrease in bone mineralization is accompanied by a decrease in bone breaking

Table 4. Tibia lengths, widths, ash, breaking strength in broiler chickens in 56 day of age.

Treatment	Ash (%)	Maximum force for breaking bones (N/mm^2)	Length of bones (ml)	Width of bones (mm)	Weight of dry bones (Gr)
Interaction					
Temperatures					
Heat stress (H)	41.91 b	79.88 b	93.91 b	6.89 b	7.22 b
Normal (c)	46.10 a	103.67 a	103.40 a	7.85 a	10.15 a
Diets					
O	42.7 b	86.92 b	96.75 b	7.22 b	8.51 b
T	41.15 b	88.37 c	93 c	6..9 b	8.71 c
A	48.2 a	110 a	106 a	8.3 a	9.48 a
Interaction (Temp × Feed)					
TH	40.16 d	66.25 e	96 bc	6.91 c	7.36 d
TC	42.77 c	90.50 c	100 b	7.46 b	10.22 b
OH	14.18 c	71.50 d	89.25 c	6.20 d	6.10 e
OC	44.32 b	102.35 b	98.75 b	7.09 c	9.20 c
AH	44.58 b	101.90 b	100.75b	7.06 c	7.95 d
AC	53.83 a	118.18 a	111.75 a	9	11.01 a
SEM	0.82	3.81	1.60	0.16	0.33

a b c d e , values that do not have common superscripts are significantly different ($P < 0.05$). O: Unsaturated fatty acid; A: basal diet + 100 IU/kg vitamin E and 50 mg/kg zinc; T: saturated fatty acid, OH: high temperature and O diet; OC: normal temperature and O diet; AH: high temperature and A diet; AC: normal temperature and A diet; TH: high temperature and T diet; TC: normal temperature and T diet.

force and bone ash weight (Crenshaw et al., 1981).

Our results showed that oil supplementation can affect the TBS and weight of dry and wet bone ($P < 0.05$). This result is in agreement with Liu et al. (2003) who showed that, high n-3 fatty acids increased the strength of bone in quail. In their experiment, quail fed soybean oil diets (high in n-6), had significantly lower values in shear force and stress than those fed fish oil or hydrogenated soybean oil. Sakaguchi et al. (1994) showed that dietary EPA and DHA led to increased bone strength. It is unknown whether the potential effects of n-3 fatty acids on bone are due to specific longer chain derivatives, such as DHA and EPA or not. Thus, the differences in specific n-3 sources on bone have to be clarified. The highest dry bone weight, width and length of bone, maximum force for breaking bone and ash value obtained in the group fed by diet supplemented with zinc and vitamin E. In fact, the increase in thickness of the growth plate was proportional to DL-α-tocopheryl acetate dose. The effect of vitamin E on chondrocytes was observed by Xu et al. (1995), in chicks fed two levels of DL-α-tocopheryl acetate and two dietary lipids.

In their study, the thickness of the entire growth plate cartilage and of the lower hypertrophic chondrocyte (mineralized) zone was significantly wider in animals fed the greater level of vitamin E. The authors maintained that the increased thickness of the mineralized zone may be due to decreased cartilage resorption and phagocytic activity on the metaphyseal side. The lowest bone strengthening was observed in the group fed with diet supplemented with tallow. It seems that, high level of arachidonic acid in supplemented diet with tallow, which is the precursor of PGE2 and may lead to decrease in bone formation, consequently, decrease in bone strength (Watkins et al., 1997). Our results are compatible with the results of Liu et al. (2003). The highest bone strength, dry matter and ash content were observed in broilers fed by supplemented diet with zinc and vitamin E in normal temperature.

Afterward, the highest dry bone weight was observed in broilers under heat stress but fed by diet supplemented with tallow. Higher growing rate in this group could be the reason for this fact. Feeding diet supplemented with zinc and vitamin E and diet supplemented with canola and fish oil in normal temperature, resulted to maximum bone strength and ash content in broiler, respectively. It seems this high bone strength is because of high level of EPA, DHA, omega-3 and Zn in the diet, which might increase Ca precipitation and collagen formation. The lowest ash value and force for breaking bone were observed in broiler under heat stress, which were fed with tallow. That would probably be due to high environmental temperatures and high omega-6 content in the diet. It might increase excretion of some minerals and PGE formation but decrease collagen formation.

Conclusion

In conclusion, the higher environmental temperature and

feeding chickens with tallow, lead to decrease in bone strength and on the contrary, supplementing diet with fish oil, zinc and vitamin E, leads to an increase in bone strength in broilers.

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