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PHYTOSTEROL DIETARY SUPPLEMENTATION EFFECTS ON THE MEAT QUALITY AND ANTIOXIDANT ACTIVITY IN BROILERS

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ABSTRACT

Phytostrols are a group of naturally occurring plant compounds located in cell membranes. It is used as a dietary for lowering cholesterol intake. We assessed effect of different phytosterol supplementation level feed rations on broiler chicken. We randomly divided 112 One-day old broiler chickens as into 3 main groups. The control group was fed on normal feed; the second group was sub divide into 3 sub-groups fed with 15, 20 and 25 g/kg polyhydroxy phytosterol (Castastesrone). The third groups was also subdivided and fed with 25, 50 and 75 g/kg hydroxyl phytosterol (β-sitosterol)and all fed 45 days. We showed phytosterol did not have a significant ($p<0.05$) effect on the feed efficiency and feed conversion ratio (FCR), however there was a body weight increase in the phytosterol fed group which clearly corresponded with the diameter, area, and density of myofibers. We noted larger diameter fibers in Fast-growing chickens than slow-growing lines. Moreover, the antioxidant capability of broilers was significantly ($p<0.05$) improved when fed with phytosterol supplemented diet with no effect on the broiler meat texture properties, area, density and fiber diameter. Consequently, a different amount of phytosterol in feed had no negative effect on broiler meat and produced more suitable meat for human health needs.

Key words: Polyhydroxyphytosterol, Hydroxyphytosterol, Supplementation, Antioxidant status, Meat quality, Broiler chicken.

INTRODUCTION

Poultry meat consumption has become very popular due to its nutritional characteristics. In fact, chicken meat contains high protein (around and low fat levels of approximately 20 g/100g and 5 g/100 g raw meat without skin respectively, and supplies essential vitamins and minerals, while it is among the most affordable meat sources [1].It is also referred to as white meat, characterized by low fat content, different amino acid glucose and creatine pattern compared to red meat, like, beef and pork [2]. Currently there is increased demand for functional poultry products enrichment with phytosterol, iodine and low unsaturated fatty acids. The higher the polyunsaturated fatty acids (PUFAs) meat content, the higher the lipid oxidation susceptibility and the more human health benefits [3]. Food research and production are aimed at increasing the food nutritional value without lowering the sensory quality or consumer's acceptability. Human health can be improved by increasing the intake of biologically valuable ingredients.

Currently there is an exponential increase in poultry meat consumption and this mainly due to the meat nutrition quality which is determined by the poultry feed composition [4]. The basic broiler diet mainly consists of cereals like wheat and corn, but its nutritional value can be enrichment by addition biological active additives and this mostly applied in commercial broiler feed production. Phytosterol is regarded as an essential dietary supplement which is vital in improving the bird health and performance as well as improving the poultry meat quality for human consumption by enhancing the immune function and reducing the risk of developing cancer [5]. More than 200 different types of phytosterols are reported to be present in plant species, but the most abundant are the -sitosterol (24-ethylcholesterol), campesterol (24-methylcholesterol) and stigmasterol (22, 24-ethylcholesterol). Vegetable oils and oil derived products

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are regarded as the richest natural sources of sterols, followed by cereal grains, nuts and vegetables. Plant sterols Metabolism has been demonstrated in higher organisms [6]. The cholesterol lowering physiological benefit of Phytosterols has sparked off its increased application in food products and diets as a nutritional supplement. Since the bovine spongiform encephalopathy (BSE) and dioxin outbreak, animal feed supplementation with animal fat has been banned from, since then vegetable materials are widely used as alternative nutritional supplements. This implied that phytosterols are the main source of sterols in animal feed, instead of cholesterol [7].

The food model studies provide a simplified multilevel insight into oxidative behavior of phytosterols in common food lipids. In fact, the phytosterols and non-saturated fatty acid fed combination has been shown to not only have complementary but also synergistic effects on lipid circulation levels, without adverse effects [5,6]. Therefore the combination of phytosterols and non-saturated fatty acids may offer great cardiovascular benefits than either of the supplements alone [8]. This out has positively impacted the development of "functional foods with more human health benefits and is the basis for" the generation new of food products enriched with bioactive components that display a therapeutic effect. Works in the field of functional food production has reverted to the use of Phytosterols hypocholesterolemic properties [6,8]. Despite abundant evidence on the therapeutic benefits of phytosterols, most previous studies have neither eliminated nor quantified phytosterols in the background diet, leaving many questions unanswered regarding the effective dose of phytosterols required to obtain health benefits and the mechanisms by which such benefits occur. With the view of increasing the nutritional quality of poultry meat without altering the sensory quality, we assessed feeding regimens based on several levels of phytosterol in the rations of broiler chickens.

MATERIALS AND METHODS

Experimental animals

A total of one hundred and twelve one -day old broiler chickens (Ross308 strain) purchased from a commercial hatchery were used in this experiment. The birds were housed in individual 40 x 45 x 50 cm cages in temperature controlled environment (24^{o}C). All chicken housed in pens were fed with chicken mash and water adds labium for 24 h under light. We assigned the experimental birds different 7 treatment groups using a randomized design. Chicken were fed at 16 per diet for a total 45 days of which 5 days were for acclimatization and 40 days were used for active intervention. The experimental birds were weighed, in a range of ±20 g mean, and allotted to 4 birds in 4 replicates each. Diets were formulated to meet or exceed nutrient requirements of broiler chicken consuming 120 g/d (Table 1)[9].The experimental diet was prepared by the addition of phytosterol [(polyhydroxy phytosterol (Castastesrone) L1, L2, L3 (15, 20, 25 g/kg) respectively, hydroxyphytosterol (β-sitosterol) H1, H2, H3 (25, 50, 75 g/kg) respectively] to the control diet according to their initial body weights, to groups. Mortalities and feed consumption per pen were recorded daily during the 6-wk experiment. Feed intake (FI), body weights (BW) and feed conversion ratios (FCR) of each group were determined weekly. The 21, 45 day old experimental, birds were deprived of feeds for 12 h and weighed just prior to slaughter., we slaughtered 6 and 10 chicken at 21 and 45 days per experimental treatment per pen (4 birds per replicate) using cervical dislocation for meat analyses. Birds were slaughtered and dissected by a trained team. Muscle were collected and stored at −70^{o}C prior to analysis. All the experiments complied with ethics approved by the international animal guidelines.

Choline chloride, 800 mg; cobalamin, 15 g; cholecalciferol, 18.5 g; vitamin E (DL-α-tocopherol acetate), 20 IU; vitamin A (trans-retinyl acetate), 10,000 IU; biotin, 0.1 mg; folic acid, 0.75 mg; $FeSO_4$ $7H_2O$, 300 mg; MnO, 100 mg; $CuSO_4$ $5H_2O$, 20 mg; ZnSO4 H_2O, 150 mg; $NaSeO_3$, 0.15 mg; KI, 0.5 mg; ethoxyquin, 100 mg; and avoparcin, 15 mg. The Carrier was zeolite.

Determination of lipid peroxidation

The lipid peroxidation was expressed as malondialdehyde (MDA) in nano moles per milligram protein. This method was conducted as previously described [10]. MDA formed as an end product of lipid peroxidation was treated with thiobarbituric acid to generate a coloured product that was measured at 532 nm (MDA detecting kit purchased from Jiancheng Bioengineering Institute, Nanjing, China).

Measurements of antioxidant status

Total antioxidant capacity (TAC), glutathione (GSH), oxidized glutathione (GSSG) and catalase (CAT) in tissue were assayed using the appropriate commercial kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, P.R. China).

Water-holding Capacity (WHC)

We extracted Pectoralis major muscles of broilers were from the slaughtered chicken, trimmed it to 5 × 2 ×1 cm, blotted it to remove the surface water, and then determined the initial breast muscle weight. We then placed the samples in plastic bags filled with air, fastened to avoid evaporation and vertically hanged them in the refrigerator at 4^{o}C. The final breast muscle weight was determined after 24 and 48 h post slaughter. The drip loss percentage was calculated using the following formula:

(Initial breast muscle weight-final muscle fillet weight)/initial weight×100.

Mechanical properties measurement

We analyzed the Mechanical properties using the TA.XT2i texture analyzer (Stable Microsystems, Godalming UK) and continuously recorded the force (N) during compression presented in a texture profile curve (Texture Profile Analysis (TPA60)) with the trigger force 5 g. The samples were compressed twice at a deformation rate of 1.0 mm/s to 60% of their original height (TPA60). The holding time between the compressions was 5 s. The

maximum force of compression in the force–time curve (hardness), adhesiveness, cohesiveness, resilience, springiness, gumminess and chewiness were all recorded and calculated. Measurements (n = 6) were run on each broiler breast samples.

Histological studies

Chicken broiler muscles, with an approximate volume of 0.5 to 0.7 cm^3, samples were cut from the middle and fixed for 24 h with 0.1 g/mL paraformaldehyde solution, then embedded in paraffin and sectioned at 6–8 μm. Sections were stained with hematoxylin-eosin (HE) and evaluated under a microscope.

Statistical analysis

The statistical analyses were computed using the GLM procedures of SAS software, using Duncan's multiple range tests to compare treatment means. Differences at $p<0.05$ were considered to be significant. Experiments were analyzed as Replicates to determine the performance.

Fig 1. Effects of phytosterol on texture profile analysis: Water-holding capacity, hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience properties of broiler semitendinosus muscle

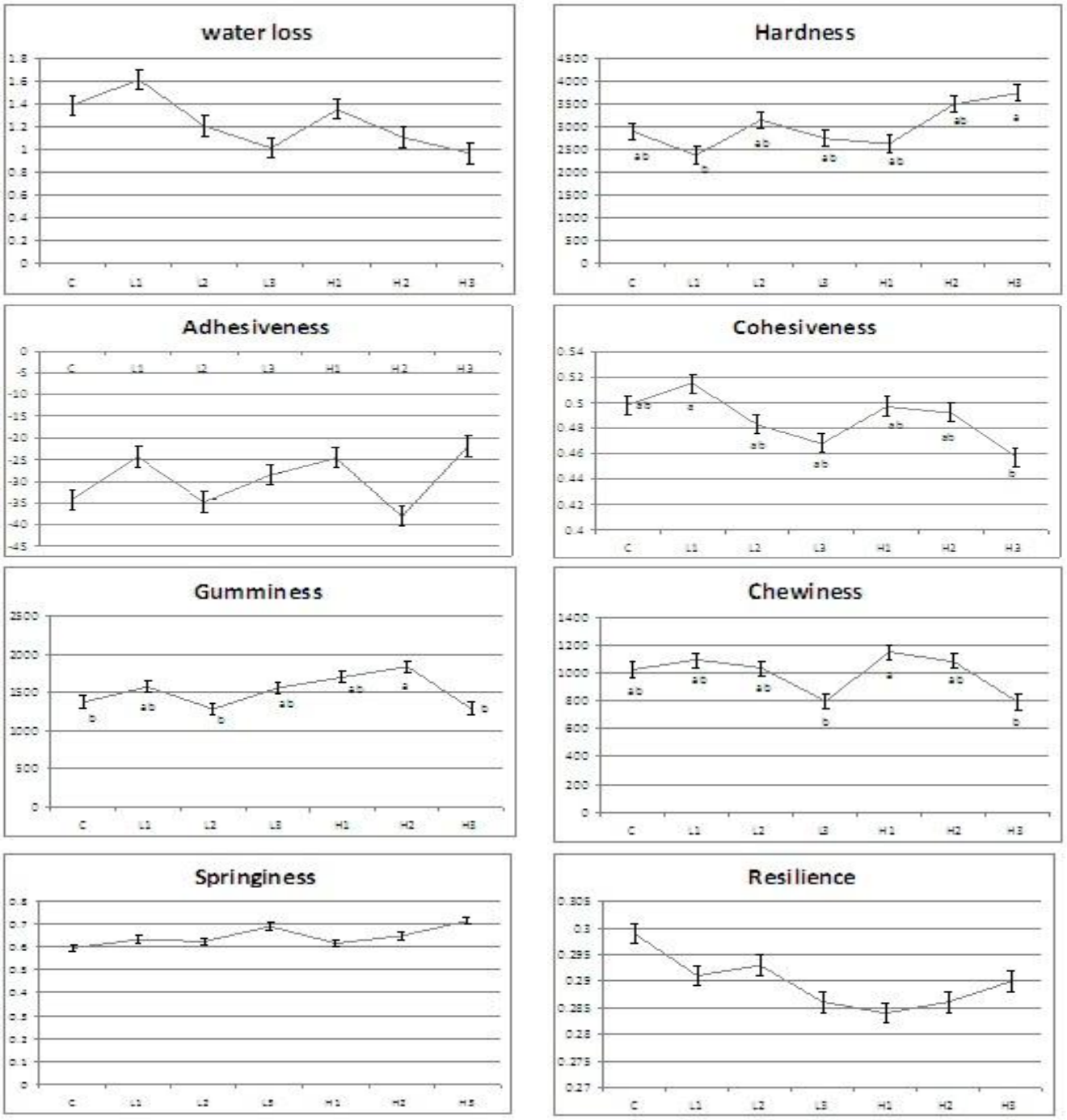

Table 1. Composition of the experimental diet (g/100 g of diet)

Ingredient (%)	Starter (1-15)	Grower (16-35)	Finisher (36-45)
corn	45.00	40.00	45.00
Wheat	8.00	8.30	8.50
Soybean meal	38.00	36.00	31.00
Calcium phosphate	1.90	1.50	1.20
Limestone	1.00	1.10	1.10
Salt	0.34	0.34	0.38
Vitamin-mineral premix*	0.30	0.30	0.30
Santoquin	0.04	0.04	0.04
Sobean oil	5.00	12.00	12.00
Polyhydroxy phytosterol	0,1.5,2.0,2.5	0,1.5,2.0,2.5	0,1.5,2.0,2.5
Hydroxy phytosterol	0,2.5,5.0,7.5	0,2.5,5.0,7.5	0,2.5,5.0,7.5

* Supplied per kilogram of diet: riboflavin, 8.0 mg; niacin, 48 mg; pantothenic acid, 16 mg; 50%

Table 2. Effect of phytosterol levels on feed consume by broiler chicken, growth performance and feed efficiency on broilers

		Week2 (g)	Week4 (g)	Week6 (g)
Effect of phytosterol levels on growth performance of broiler chicken				
Control		634±9.77	1972±20.84b	2881±23.02d
Polyhydroxy Phytosterol	**L1**	631.73±26.50	2145.3±60.52ab	2970.83±11.89c
	L2	642.00±3.33	2408.3±34.09a	3163.67±47.23ab
	L3	640.27±2.75	2388±28.50a	3182.63±23.15ab
Hydroxy phytosterol	**H1**	624.27±12.01	2202.1±13.33ab	3104±12.76bc
	H2	656.07±12.52	2362±11.76a	3119.67±65.78bc
	H3	647.13±7.45	2321.7±14.99a	3286.57±55.16a
Effect of phytosterol levels on Feed consume of broiler chicken				
Control		133.33±10.53ab	162.033±4.32	106.93±8.01b
Polyhydroxy Phytosterol	**L1**	149.17±1.03a	166.400±6.67	136.30±5.77a
	L2	147.63±2.67a	163.867±3.34	129.67±9.24ab
	L3	124.70±2.59b	157.967±1.56	108.20±9.19b
Hydroxy phytosterol	**H1**	124.50±5.25b	159.100±6.45	108.50±8.56b
	H2	138.01±6.54ab	158.700±2.22	128.03±8.47ab
	H3	129.17±2.60b	156.200±4.42	116.13±2.03ab
Effect of phytosterol levels on Feed efficiency of broiler chicken				
Control		1.79±0.18c	1.65±0.14ab	1.46±0.13
Polyhydroxy phytosterol	**L1**	1.67±0.07c	1.89±0.15b	1.39±0.16
	L2	1.61±0.12bc	1.59±0.17ab	1.50±0.18
	L3	1.46±0.08a	1.41±0.07a	1.51±0.48
Hydroxyl phytosterol	**H1**	1.50±0.09bc	1.90±0.54b	1.43±0.29
	H2	1.58±0.01bc	1.64±0.07ab	1.22±0.17
	H3	1.53±0.17bc	1.81±0.19b	1.45±0.32

Data are means ± SD (mmol/L). *a,b,c* Values with different superscripts are significantly different (P<0.05) from each other.

Table 3. Effect of phytosterol levels on relative weight of organs of broilers

(g)		Leg Muscle	Breast Meat	Liver	Kidney	Bursa	Spleen	Heart	Gastrocnemius	Net weight
Control		171.49±13.48ab	269.48±23.96b	69.78±3.26bc	11.11±1.13ab	3.22±0.25ab	3.84±0.31a	7.85±0.47	13.68±0.83	2436.72±71.82d
Polyhydroxy Phytosterol	L1	195.72±10.13ab	276.20±19.60ab	64.02±4.17c	10.01±0.65b	2.93±0.24b	3.40±0.44a	8.05±0.49	11.87±1.09	2510.53±22.36c
	L2	194.46±8.10ab	295.66±12.99ab	83.22±4.42a	11.94±1.20ab	3.97±0.46a	3.39±0.52a	8.04±0.46	14.18±0.85	2653.22±63.09ab
	L3	203.76±17.21ab	323.93±13.73a	72.10±3.87abc	12.57±0.86ab	3.12±0.23ab	4.05±0.81a	8.25±0.22	13.45±1.29	2682.10±84.64ab
Hydroxy phytosterol	H1	213.03±17.42a	282.04±14.49ab	77.58±5.13ab	12.16±1.27ab	3.54±0.31ab	3.67±0.63a	8.35±0.19	14.19±0.91	2614.43±18.11bc
	H2	174.31±11.31b	300.41±15.80ab	71.86±4.12abc	12.15±1.37ab	3.41±0.21ab	3.86±0.73a	8.20±0.57	14.39±1.02	2605.28±39.27bc
	H3	197.04±12.95ab	303.28±17.24ab	76.18±4.48ab	13.40±0.66a	3.77±0.33ab	5.10±0.62a	7.76±0.17	14.39±0.95	2768.09±71.93a

Values shown are means ±SD for 16 birds. a,b,c Values with different superscripts are significantly different (P<0.05) from each other

Table 4. Effect phytosterol levels on oxidative stability of muscle in broiler chicken at 21 days

(U/mg protein)		MDA	CAT	TAC	GSH	GSSG	GSH/GSSG
Control		4.71±0.64a	2.89±0.46	0.21±0.04b	1.62±0.58c	14.77±2.10a	0.12±0.04d
Polyhydroxy Phytosterol	**L1**	1.957±0.31b	5.03±1.03	0.63±0.06ab	1.78±0.38bc	12.70±2.12ab	0.14±0.03cd
	L2	1.56±0.18b	5.40±0.91	0.56±0.08ab	2.55±1.09abc	10.66±0.59bc	0.21±0.10bcd
	L3	1.68±0.22b	4.54±1.01	0.97±0.31a	3.24±1.01ab	9.89±2.13bc	0.29±0.13ab
Hydroxy phytosterol	**H1**	2.65±0.42b	4.39±1.01	0.44±0.13ab	2.33±1.04abc	10.23±1.2bc	0.18±0.01bcd
	H2	2.50±0.24b	4.61±1.23	0.63±0.25ab	3.04±0.32abc	9.75±2.16bc	0.25±0.03abc
	H3	1.93±0.33b	6.05±1.70	0.92±0.30a	3.59±0.84a	9.11±0.87c	0.372±0.03a

Values shown are means ±SD for 16 birds. a,b,c,d Values with different superscripts are significantly different (P<0.05) from each other

Table 5.Effect of phytosterol levels on oxidative stability of muscle in broiler chicken at 45 days

(U/mg protein)		MDA	CAT	TAC	GSH	GSSG	GSH/GSSG
Control		4.03±0.36a	1.54±0.30c	0.39±0.07c	3.03±0.49b	30.66±1.05a	0.12±0.01b
Polyhydroxy Phytosterol	**L1**	4.09±0.36a	2.60±0.47c	0.35±0.10c	4.88±0.43a	16.66±2.22d	0.24±0.03a
	L2	3.40±0.14ab	3.54±0.70bc	0.70±0.16bc	5.25±0.32a	27.38±1.64ab	0.19±0.01a
	L3	2.52±0.26c	3.82±0.74bc	0.93±0.37cb	4.91±0.19a	25.63±0.97abc	0.23±0.03a
Hydroxy phytosterol	**H1**	3.25±0.23abc	3.01±0.75bc	0.81±0.12bc	5.43±0.56a	23.85±1.59bc	0.23±0.03a
	H2	4.14±0.24a	5.13±1.05ab	1.18±0.19b	5.56±0.25a	27.31±0.72ab	0.19±4.39a
	H3	3.01±0.29bc	6.79±1.15a	2.08±0.44a	4.70±0.38a	21.94±2.69c	0.20±0.01a

Values shown are means ±SD for 16 birds. a,b,c,d Values with different superscripts are significantly different (P<0.05) from each other.

Table 6. Effect phytosterol levels on area, fiber density and diameter of muscle in broilers

		Area (mm^2)	Density (num/mm^2)	Diameter(mm)
Control		7099.7±2699.48a	3318.01±17.36b	40.61±9.07b
Polyhydroxy Phytosterol	**L1**	3792.2±849.18b	3529.37±62.79ab	37.11±6.53b
	L2	3177.9±1643.48b	3472.58±41.53b	44.95±10.88b
	L3	3043.3±921.97b	3533.49±75.19ab	38.90±12.61b
Hydroxy phytosterol	**H1**	3367.0±2216.76b	3485.59±28.41b	65.29±13.20a
	H2	3185.0±606.61b	3561.66±33.72ab	35.75±7.74b
	H3	2986.8±959.42b	4172.19±59.50a	33.58±15.43b

Values shown are means ±SD for 16 birds. a,b,c Values with different superscripts are significantly different (P<0.05) from each other

RESULTS

Performance parameters

The final body weight of the 6 week old birds significantly (p<0.05) increased when supplemented with of H3, L3and H2 Table 2. There was no distinct difference in Feed consumption (p<0.05). L3 treatment induced the highest feed efficiency value compared to the other treatments and control (Table 2). However the leg muscle, Breast meat, bursa, Spleen, Liver, heart, did not differ across all treatment groups, but the net weight increased in phytosterol group (Table 3).

Reactive oxygen species and malondialdehyde levels

The effects of phytosterol supplementations on malondialdehyde MDA concentrations evaluated (Table 4, 5). Phytosterol treatment induced a significant response (p<0.05) however it reduced with increase in MDA concentrations where phytosterol were added to the diet of broiler chickens while comparing to the broiler control group.

Antioxidant Enzyme Activity

The antioxidant status in muscle of broilers was assessed (Tables 4 and 5). The GSH, CAT, and TAC activities in broilers increased on phytosterol addition. There was a clear significantly (p<0.05) improvement at the 75 level on hydroxyl phytosterol supplementation. However, hydroxyl phytosterol diet supplementation at the 75 level clearly showed a reduction in glutathione (GSSG) oxidation in broilers.

Waterless and TPA investigations

One of the methods of texture measurement applied most frequently is texture profile analysis; (TPA) carried out by means of the TA.XT2i apparatus. There was no significant effect (p<0.05) on water loss (a) hardness (b), adhesiveness (c), cohesiveness (d), gumminess (e), chewiness (f), resilience (g), springiness (c), (Figure 1).

Histological tests

As it can be seen from Table 6 the phytosterol addition showed a no clear effects on meat quality properties like area, density, and the muscle fiber diameter.

DISCUSSION

Phytosterols and non-saturated fatty acids feed combination offers greater cardiovascular benefits than either of the supplements alone [5,7]. Vegetable fat is used as a substitute for animal fat in meat products. It contains phytosterols such as stigmasterol and β-sitosterol, which are absent in animal fat. When the fatty acids are saturated, the oxygen molecules present will change sterols without fatty acid inhibition. When fatty acids are unsaturated, the oxygen molecules will first oxidize the fatty acid, followed by oxidation of the sterol [11,12].This indicates that highly unsaturated oils use more oxygen before the total destruction of sterols through the oxidative pathway. This attack causes lipid peroxidation. Furthermore, the decomposition of peroxidized lipids yields a wide variety of end-products, including malondialdehyde (MDA), it is a major degradation product of lipid hydroperoxides that has attracted much attention as a marker for assessing the extent of lipid peroxidation [13]. This compound is of particular concern since it has been shown to be mutagenic, carcinogenic and implicated in other pathological process that is widely used in practice as an indicator of free radical damages [13,14].

Antioxidant system has a cellular protective property against oxidative stress. Dietary trace elements/antioxidants help to maintain appropriate antioxidant balance in during several infections [3,15,16]. Thus, when unsaturated oil such as soybean is subjected to the oxidative stresses, it is possible to recover more intact sterols and more sterol oxides. We demonstrated dietary phytosterol supplementation greatly improves the antioxidant capability in broilers. Increase in the total antioxidant capacity (TAC), concentration of a non-enzymatic substance (GSH) and catalase (CAT), along with the metabolic product of lipid peroxides of malonaldehyde MDA and oxidized glutathione (GSSG)

levels indicates that lipid and nucleic acids oxidative modifications and decreased proteins content. These findings are in agreement with findings reported by Gruneet al. [17]; Wang et al. [18].

Usually, it is assumed that good quality food should not only be faultless but it should also possess sensory features required by the consumers. A very important property for determining the food quality is texture. Texture is one of the principal factors in determining acceptability of foods. Texture profile analysis (TPA) is an objective test commonly used in other industries for texture assessment of foods[19].Texture profile analysis uses a double compression cycle to simulate the first and second bites, similar to a human subject. Texture parameters such as: hardness, cohesiveness, springiness, gumminess, and chewiness are assessed using TPA. Utilization of these texture parameters has been proven to be a useful method in determining textural properties of food borne, it was found that water holding capacity values proportional with early content of products, where water holding capacity values were low in products high in fat. The water holding capacity functionality nature was influenced by how effective the protein matrix binds to the scattered excess fat and water within the products[7]. While most of the reports by high value broilers producing companies refers to some technological and economical features of their products (microclimate, nutrition requirements, weight gains, FCR, slaughtering efficiency), We report some partial results from a study onto the textural quality of the broiler meat which showed that the increase in amount of phytosterol had minor effects on the chicken meat properties. Diet with the different amount of phytosterol had no pronounced effect on the water holding capacity, hardness, adhesiveness, springiness, cohesiveness, gumminess, resilience, and chewiness. However, during the 45 days, the meat of broilers groups L3, H3 had higher parameters compared to broilers from the other feeding and control groups ($p<0.05$) whereas, broiler muscles fed phytosterol groups exhibited muscular fibers with the average diameter and had lower average value than the control group. There is a clear positive correlation between fiber diameter and body weight [20]. In terms of area (mm^2) the situation is reversed, with density of muscle fibers in broiler fed phytosterol compared to broiler control.

We demonstrated that large body weight of chicken is dependent on the larger myofiber diameter and area, less myofiber density. Fast-growing chickens showed larger diameter fibers than slow-growing lines. We suggest that this is associated with increase in giant fibers quantity, which indicated three to five times' cross-sectional areas larger than normal, although these may result from severe contraction (hypercontracted fibers). Smaller fiber diameters may allow a higher packing density and increase toughness of the meat [19-22].

CONCLUSION

The antioxidative status of chickens determined as antioxidative enzymes and the oxidative stability of lipids, increased after phytosterol diet supplementation. Phytosterol enriched diet improved the antioxidant status and growth performance of broiler chicken. Consequently a different amount of phytosterol in feed had no negative effect on broiler meat and produced more suitable and healthy meat for human consumption.

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