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Original Research Article

Effects of Phytosterol in Feed on Growth and Related Gene Expression in Muscles of Broiler Chickens

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Abstract

Purpose: *To investigate the effect of phytosterol in a feed on growth and gene expression of broiler chickens.*
Methods: *Seven isonitrogenous diets with graded levels of polyhydroxy phytosterol(Castastesrone) (15, 20, and 25 g/kg diet) and hydroxyphytosterol (β-sitosterol) (25, 50, and 75 g/kg diet) were used to feed broiler chickens for 45 d.*
Results: *The dietary phytosterol significantly improved ($p < 0.05$) the body weight and feed intake of broiler chickens, especially with the 75 g/kg diet. In contrast, phytosterol supplementation was associated with significant ($p < 0.05$) hypotriglyceridemic effects with concurrent modifications of depressed antioxidant defence systems in the broiler chickens. Myogen, eIF4E, and S6k1 gene expression levels in tissues were significantly ($p < 0.05$) improved by dietary phytosterol. mTOR gene expression levels in muscle tissues were increased significantly ($p < 0.05$), but myostatin (GDF-8) and ubiquitin levels were significantly decreased ($p < 0.05$).*
Conclusion: *These results suggest that giving immediate phytosterol feeding regimen to chicks is a good feeding program for appropriate morphological development of the pectoralis major muscle and the expression of genes necessary for muscle cell proliferation and differentiation. Furthermore, the data suggest that developmental decline in skeletal muscle protein synthesis, may be partly attributed to developmental regulation of the activation of growth factor and nutrient components.*

Keywords: *Broiler chicken, Polyhydroxy phytosterol; Hydroxyphytosterol, Feed, Antioxidant status, Gene expression.*

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INTRODUCTION

Currently, research required to align broiler products with optimum feeding programs can likely benefit by incorporating nutrigenomics [1]. Possible applications for nutrigenomic research may include "co-evolution" of nutrition and genetics in breeding programs, maximising response to diets used in breeding programs, helping safeguard consumer health, maximising feed efficiency in broilers and utilising epigenetics to improve broiler and breeder performance. The oxidative stability of meat was reported to be dependent on the balance of anti- and pro-oxidants as well as the composition of oxidation substrates, including cholesterol, polyunsaturated fatty acids, proteins and pigments [2,3]. Indeed, combined feed with phytosterols and unsaturated fatty acids has been demonstrated to have not only

complementary but also synergistic effects on circulating lipid levels without adverse effects [4]. Higher sterols are universally present in large amounts in eukaryotic plasma membranes. Cholesterol (cholest-5 α-en-3 β-ol) is an integral compound of animal and human cell membranes [5,6].

During the immediate posthatch period, muscle growth occurs through the process of hypertrophy. Hypertrophy entails the fusion of satellite cells with existing myofibres leading to an increase in muscle fibre size through increased protein synthesis [7]. Activating key genes in a sequence similar to that occurring in the normal organism is a reasonable approach to obtain differentiated muscle cells in vitro, but this approach depends on understanding the gene pathways leading to myogenic differentiation [8]. Mammalian target of rapamycin (mTOR) has emerged as a critical nutritional and cellular energy checkpoint sensor and regulator of cell growth in mammalian cells [7]. mTOR modulates the activity of two important translational regulators, namely ribosomal S6 kinases (S6K1 and S6K2) and the eukaryotic initiation factor 4E (eIF4E), following changes in amino acid levels and energy sufficiency as well as stimulation by hormones and mitogens. Myostatin-responsive genes in skeletal muscle have been recently analysed by microarray analysis [7]. Generally, mature muscle fibres exhibit a remarkable plasticity in response to external stimuli. Generaly broiler chickens are used as model systems to study and control the mechanisms of myogenesis rate and muscle cell size. Therefore, the objective of the current study was to evaluate the physiologic effects of phytosterol intake and to examine myogenin, myostatin, S6k1, eif4E, mTOR and ubiquitin expression levels by real-time PCR in developing broiler major muscles to determine the effects of phytosterol levels.

EXPERIMENTAL

Experimental animals

A total of 112 one-day old avian broiler chickens (Ross308 strain) obtained from a commercial hatchery were used in the present study. The birds were housed in individual 40 x 45 x 50 cm cages in an environmentally-controlled room (24 ^{o}C). All chickens kept in pens were given feed and water *ad libitum* with 24 h light and acquired immunity in accordance with the guidelines set by the Animal Experimental Ethical Committee (No. 13-BC-005, Jiangnan University, Wuxi, China)[9]. The birds were randomly assigned, and a completely randomised design with 7 treatments was used. Chickens (16 per diet) were systematically randomised to receive 7 treatment diets for 45 d (5 d of acclimatisation and 40 d of active intervention). The birds were weighed within ± 20 g of the mean and allotted to 4 replicates of 4 birds each. Diets were formulated to meet or exceed nutrient requirements of broiler chickens consuming 120 g/d (Table 1).

The experimental diet was prepared by the addition of phytosterol [(polyhydroxy phytosterol (Castastesrone) L1, L2, and L3 (15, 20, and 25 g/kg respectively) and hydroxy phytosterol (β-

Table1: Composition of the experimental diet (g/100g of diet)

Ingredient (%)	Starter (1-15)	Grower (16-35)	Finisher (36-45)
Corn	45.20	40.20	45.20
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
Soybean 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
hydroxyPhytosterol	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% cholinechloride, 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; $ZnSO_4$ H_2O, 150 mg; $NaSeO_3$, 0.15 mg; KI, 0.5 mg; ethoxyquin, 100 mg; and avoparcin, 15 mg. The carrier was zeolite.*

sitosterol) H1, H2, and H3 (25, 50, and 75 g/kg, respectively)] to the control diet according to their initial body weights and groups. Mortalities and feed consumption per pen were recorded daily during the 6-week experiment. Feed intake (FI), body weight (BW) and feed conversion ratio (FCR) were determined weekly for each group. At 45 d of age, the birds were deprived of feed for 12 h and weighed just prior to slaughter. Ten chickens per pen were killed by cervical dislocation for meat analyses. Birds were slaughtered and dissected by a trained team. Muscles were collected and stored at −70 °C prior to analysis. The divided parts were placed into cryopreservation tubes and were then quickly placed into liquid nitrogen (−196 °C) for preservation.

Determination of lipid peroxidation

Lipid peroxidation was expressed as malondialdehyde (MDA) in millimoles per milligram protein. The method was performed as previously described by James *et al.* [10]. MDA, which is formed as an end product of lipid peroxidation, was treated with thiobarbituric acid to generate a coloured product, and the coloured product was measured at 532 nm (MDA detection kit; Jiancheng Bioengineering Institute, Nanjing, China).

Measurements of antioxidant status

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

RNA extraction and real-time PCR

Real-time PCR was performed to quantify the mRNA expression levels of myogenin, myostatin, mTOR, S6k1, eIF4E and ubiquitin. Primer Premier 5 software was used (Table 2) to design the primers, and the Mx3000P Real-Time PCR System was used (Stratagene, USA). mRNA expression levels of target genes were quantified relative to β-actin. The nucleotide sequences of the primers and the PCR conditions for these genes are shown in Table 2. The 2-ΔΔCt method was used to analyse the real-time PCR data according to Eq 1 [11].

ΔΔCt = (Ct. Target — Ct. β-actin) sample — (Ct. Target — Ct. β-actin) …… (1)

Statistical analysis

Data were analysed using Statistical Package for Social Scientists for Windows 16 (SPSS, Inc. Chicago, USA). Data are presented as proportions, mean (standard error mean), odds ratio and the corresponding 95 % confidence interval. Tukey's HSD test was used to compare the means. Differences at *($p < 0.05$)* and *($p < 0.01$)* for gene expression levels were considered to be significant.

RESULTS

Growth performance

Table 3 shows the final body weight. At 6 weeks, the birds had significant (P<0.05) growth differences in terms of body weight increases due to the supplementation in the H3, L3 and H2 treatments. Feed consumption did not show any relevant differences (P<0.05) among the treatments (Table 3). Treatment L3 achieved the highest value of feed efficiency compared to the other treatments and control (Table 3).

Reactive oxygen species and malondialdehyde levels

The effects of phytosterol supplementations on

Table 2: Primer sequences used in real-time PCR

Myogenin	Sense	5'GCGGAGGCTGAAGAAGGT3'	NM_204184.1
	Antisense	5'AGGCGCTCGATGTACTGG3'	
Myostatin	Sense	5'GGGACGTTATTAAGCAGC3'	NM 001001461
	Antisense	5'ACTCCGTAGGCATTGTGA3'	
mTOR	Sense	5'GAAGTCCTGCGCTAGCATAAG3'	XM_426232.3
	Antisense	5'TTTGTGTCCATCAGCCTCCAGT3'	
S6K1	Sense	5'GGAGATGCTGGAGAAGTT3'	NM-001030721
	Antisense	5'GTTTGGTTGTCGGATTGG3'	
eIF4E	Sense	5'TCCAACCCTCAACCTTCAG3'	XM-420655
	Antisense	5'CAGCACCACATACATCATCGC3'	
Ubiquitin	Sense	5'CGCACCCTGTCTGACTACAA3'	X02650.1
	Antisense	5'GCCTTCACGTTCTCAATGGT3'	
β-Actin	Sense	5'TGCGTGACATCAAGGAGAAG3'	NM_205518.1
	Antisense	5'TGCCAGGGTACATTGTGGTA3'	

Table 3: Effect of phytosterol levels on growth performance, feed consumption and feed efficiency of broiler chickens

		Week 2 (g)	Week 4 (g)	Week 6 (g)
Control		634±9.77	1972±20.84^{b}	2881±23.02^{d}
Polyhydroxy	L1	631.73±26.50	2145.3±60.52^{ab}	2970.83±11.89^{c}
Phytosterol	L2	642.00±3.33	2408.3±34.09^{a}	3163.67±47.23^{ab}
	L3	640.27±2.75	2388±28.50^{a}	3182.63±23.15^{ab}
Hydroxyphytosterol	H1	624.27±12.01	2202.1±13.33^{ab}	3104±12.76b^{c}
	H2	656.07±12.52	2362±11.76^{a}	3119.67±65.78^{bc}
	H3	647.13±7.45	2321.7±14.99^{a}	3286.57±55.16^{a}
Effect of phytosterol level on feed consumption of broiler chickens				
		Week 2 (g)	Week 4 (g)	Week 6 (g)
Control		133.33±10.53^{ab}	162.033±4.32	106.93±8.01^{b}
Polyhydroxy	L1	149.17±1.03^{a}	166.400±6.67	136.30±5.77^{a}
Phytosterol	L2	147.63±2.67^{a}	163.867±3.34	129.67±9.24^{ab}
	L3	124.70±2.59^{b}	157.967±1.56	108.20±9.19^{b}
Hydroxyphytosterol	H1	124.50±5.25^{b}	159.100±6.45	108.50±8.56^{b}
	H2	138.01±6.54^{ab}	158.700±2.22	128.03±8.47^{ab}
	H3	129.17±2.60^{b}	156.200±4.42	116.13±2.03^{ab}
Effect of phytosterol level on feed efficiency of broiler chickens				
		Week 2 (g)	Week 4 (g)	Week 6 (g)
Control		1.79±0.18^{c}	1.65±0.14^{ab}	1.46±0.13
Polyhydroxy	L1	1.67±0.07^{c}	1.89±0.15^{b}	1.39±0.16
Phytosterol	L2	1.61±0.12^{bc}	1.59±0.17^{ab}	1.50±0.18
	L3	1.46±0.08^{a}	1.41±0.07^{a}	1.51±0.48
Hydroxyphytosterol	H1	1.50±0.09^{bc}	1.90±0.54^{b}	1.43±0.29
	H2	1.58±0.01^{bc}	1.64±0.07^{ab}	1.22±0.17
	H3	1.53±0.17^{bc}	1.81±0.19^{b}	1.45±0.32

Values shown are mean ± SEM (mmol/L); ^{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 boiler chickens at 45 days

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

Values shown are means ± SEM for 16 birds; ^{a,b,c,d} Values with different superscripts are significantly different

malo presented in Table 4. A significant (P<0.05) ndialdehyde (MDA) concentrations are decrease in MDA was found when phytosterol was added to the diets of broiler chickens as compared to the control treatment.

Antioxidant enzyme activity

Table 4 shows the antioxidant status in the muscle of broilers. Addition of phytosterol increased the activities of GSH, CAT, and TAC in the broilers. There was significant (P<0.05) improvements caused by the phytosterol supplement, particularly at the 75 g level, which was the highest supplemental level. These data indicated that the activities of GSH, CAT, and TAC increased with increasing levels of phytosterol. However, the addition of 75 g of hydroxy phytosterol to the diet significantly (P<0.05) decreased oxidised glutathione in broilers.

mRNA expression

As shown in Figure 1, myogen, mTOR, S6k1 and eIF4E mRNA levels were enhanced significantly (P<0.01) in the skeletal muscle of broilers by phytosterol supplementation. However, phytosterol in the diet significantly (P<0.01) decreased myostatin and ubiquitin mRNA levels in broilers (Figure 2).

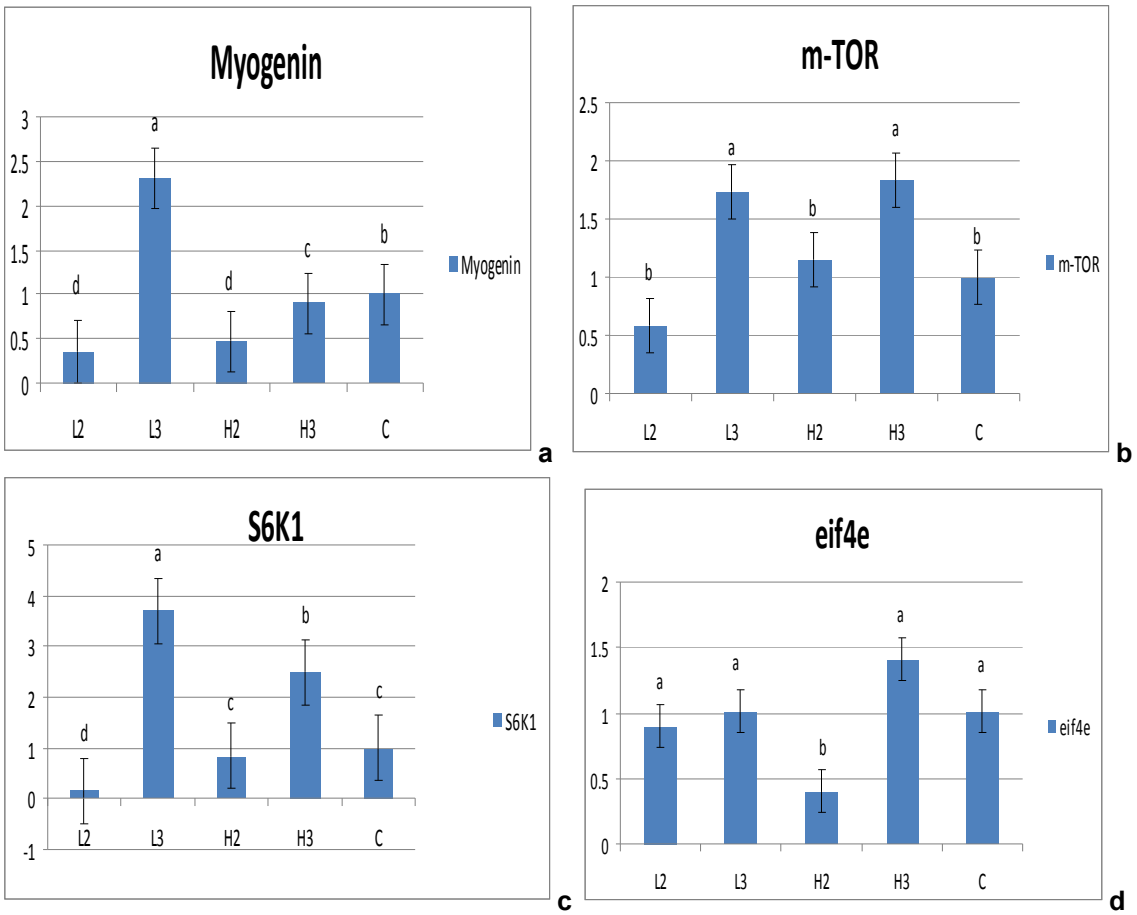

Figure 1: Effect of phytosterol on (a) myogenin, (b) mTOR, (c) S6K1 and (d) eif4E in broiler chicken skeletal muscle. Values are mean ± SEM (n = 10). Means with a different letter differ significantly (p <0.01).

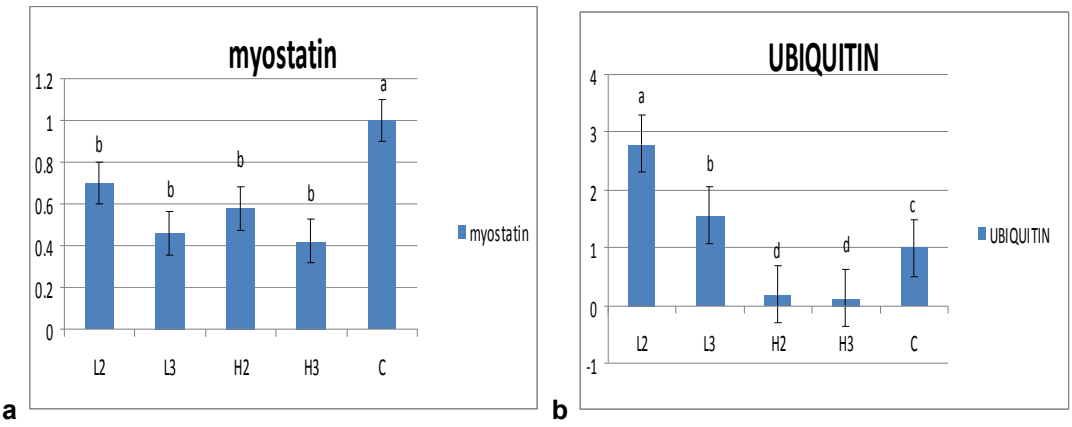

Figure 2: The effect of phytosterol on (a) myostatin and (b) ubiquitin in broiler chicken skeletal muscle. Values are mean ± SEM (n = 10). Means with a different letter differ significantly (p <0.01).

DISCUSSION

Phytosterols are known to be important to membrane permeability and for transport of nutrients from the blood into cells. [12]. Phytosterols play a crucial role when placed in water; it behaves as phospholipids and forms a bilayer where the hydrophobic tails line up together to avoid water, which forms a membrane with the hydrophilic heads extending into the water. This kind of behaviour favours membrane formation that can spontaneously form liposomes, which are small fat vesicles that are used to transport materials into living organisms [13-15]. However, this present study focused on different signalling pathways related to protein synthesis induced by phytosterol rather than the involvement of signalling pathways in translational regulation. Feeding induces high rate of protein synthesis. The divergent muscle

growth rates of broilers make them great models to study myogenesis. To discover the molecular mechanisms determining the divergent muscle growth rates and muscle mass control in different chickens, we systematically identified differentially expressed genes in broiler skeletal muscle cells during different developmental stages by real-time polymerase chain reaction.
The results from the present investigation revealed that both BW and muscle weight were significantly increased after 45 d. Myogenin scored lower than the control for all treatments with the exception of L3, which was high and significantly increased ($P<0.01$) compared to the control. However, total antioxidant capacity (TAC) and the concentrations of GSH (a non-enzymatic substance) and catalase (CAT) were increased. The metabolic product of lipid peroxidase, malondialdehyde (MDA), and oxidised glutathione (GSSG) can lead to oxidative stress, which may cause oxidative modifications to lipids, nucleic acids and proteins. Importantly, the MDA and GSSG contents decreased with the supplementations. The present data corroborated with the results of Kitts *et al* [16]. For myostatin, all treatments scored lower than the control, and the lowest scores were found for H3 and L3. Myogenin expression was increased by feed containing phytosterol suggesting an increment in muscle cell differentiation. Interestingly, myostatin expression was decreased when myogenin expression was increased. We observed higher overall levels of weight-related genes in phytosterol groups as compared to the control group because the expression changes of these different genes correlated with the body weight change rate of broilers. Thus, these genes might bring a new insight in regulating muscle cell growth indicating that more protein accumulation could be one reason for the larger body size of broilers.

As Figure 1b shows, the H3 and L3 treatments increased mTOR significantly ($P<0.01$), but the H2 treatment did not cause a significant increase in mTOR. Moreover, S6K1 was increased the most with the addition of 25 g/kg (L3) and 75 g/kg (H3) compared to the control and other treatments. The addition of phytosterol did not negatively affect the eif4e gene in broiler chicken skeletal muscle. mTOR is known to regulate the function of eIF4E by regulating the phosphorylation of the eIF4E inhibitory proteins, 4E binding proteins (4E-BPs); and phosphorylation of 4E-BP1 promotes its dissociation from eIF4E bound to the mRNA 7-methylguanosine cap structure [7]. mTOR regulates mRNA translation by phosphorylating two of its effectors, ribosomal protein S6 kinase 1 (S6K1) and eIF4E-binding protein-1 (4E-BP1). mTOR has been demonstrated to integrate nutrient and growth factor signals via multiple mechanisms, including phosphorylation of the protein kinase, S6K1, and the translation factor, 4E-BP1, resulting in modulation of protein synthesis and cell growth [7,17]. The activation of mTOR, a major protein kinase that modulates translation initiation components, is regulated by nutrients [7,17-19]. Phosphorylation of mTOR, which activates the kinase, is stimulated by both insulin and/or amino acids [18]. Furthermore, the association of raptor with mTOR is essential for TOR signalling and the binding of mTOR with 4E-BP1 and S6K1 [18,19]. mTOR is considered a master protein kinase that is regulated independently by insulin, amino acids, and energy sufficiency, and mTOR participates in the control of protein synthesis components that are responsible for cell growth [7,20]. The activation of S6K1 is initiated by mTOR/raptor-mediated phosphorylation, which requires the TOS motif located at the N terminus of S6K1. In response to stimulation by certain nutrients and growth factors, mTOR phosphorylates eIF4E binding protein-1 (4E-BP1) and ribosomal protein S6 kinase-1 (S6K1), thereby promoting the initiation of polypeptide formation. Furthermore, enhanced translation is induced by increased initiation through the supply of eukaryotic translation initiation factor 4E (eIF4E) and phosphorylation of ribosomal protein S6. If compensatory gain does occur in a manner that does not affect the biochemistry or morphological structure of the muscle, the following events must take place: muscle growth by satellite cell-induced hypertrophy must remain the same with growth restriction; growth restriction must not alter cellular fate; and inhibited muscle hypertrophy must not change muscle fibre organisation, muscle mass, or meat quality.

In this study, the effect of phytosterol feeding and development on the activation of translation initiation factors led to the stimulation of protein synthesis. The postprandial rise in skeletal muscle protein synthesis in the broiler is regulated by the activation of translation initiation factors, including mTOR, S6K1, 4E-BP1, and eIF4F. However, ubiquitin is thought to degrade myofibrillar proteins and most soluble proteins [7,17,18]. Ubiquitin mediates cachexia muscle wasting associated with diseases and physiological muscle atrophy due to starvation, denervation, or disuse [15]. The resulting overload of muscles may lead to ongoing production of damaged myofibrillar protein, which would be reflected in the activity of muscle extracts. The fact that this increase was not accompanied by increased polyubiquitin mRNA

expression confirms the view that ubiquitin concentrations were not limiting for conjugation in broiler muscle. Differential growth rates of chickens and ubiquitin expression or conjugation were not related to the extent of high phytosterol feed. Therefore, ubiquitin is unlikely to be useful as a biochemical selection marker, but it may be related to muscle growth. In this study, the H3 and H2 treatments had the best positive effect on ubiquitin by significantly decreasing ubiquitin ($P<0.01$).

In conclusion, the addition of phytosterol improved the antioxidant system in broiler chickens, and the different amounts of phytosterol in feed did not have negative effects on broiler meat production. mTOR played a critical role in the regulation of cell growth, proliferation, survival and motility by phosphorylation of the two best-characterised downstream effector molecules, S6K1 and eif4e, which promote mRNA translation and ribosome biogenesis. The H3 and L3 treatments increased mTOR significantly, but the H2 treatment did not significantly increase mTOR. The addition of phytosterol in the L3 treatment substantially increased myogenin. For myostatin, all treatments scored lower than the control, and the lowest scores were detected for the H3 and L3 treatments. Researchers and poultry producers need to collaborate to nutritionally improve products and to understand fatty acid metabolism and phytosterol in broiler chickens. Moreover, this collaboration is important to efficiently optimise the use of phytosterol in broiler chicken diets.

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REFERENCES

1. *Gabriel I, Mallet S, Leconte M, Travel A, Lalles JP. Effects of whole wheat feeding on the development of the digestive tract of broiler chickens. Anim Feed Sci Technol 2008; 142: 144-162.*
2. *Fondjo FA, Kamgang R, Oyono JLE, Yonkeu JN. Anti-dyslipidemic and antioxidant potentials of methanol extract of kalanchoe crenata whole plant in streptozotocin-induced diabetic nephropathy in rats. Trop J Pharm Res 2012; 11: 767-775.*
3. *Yasin M, Asghar A, Anjum FM, Butt MS, Khan MI, Arshad MS, Shahid M, El-Ghorab AH, Shibamoto T..Oxidative stability enhancement of broiler bird meats with α-lipoic acid and α-tocopherol acetate supplemented feed. Food Chem 2012; 131: 768-773.*
4. *Demonty I, Ras R, Knaap HM, Meijer L, Zock P, Geleijnse J, Trautwein E. The effect of plant sterols on serum triglyceride concentrations is dependent on baseline concentrations: a pooled analysis of 12 randomised controlled trials. Eur J Nutr 2013; 52: 153-160.*
5. *Moreau RA, Whitaker BD, Hicks KB. Phytosterols, phytostanols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. Progress Lipid Res 2002; 41: 457-500.*
6. *Syed A Hassan, Lata Chauhan, Ritu Barthwal, Aparna Dixit A. Qualitative and Quantitative Assay to Study DNA/Drug Interaction Based on Sequence Selective Inhibition of Restriction Endonucleases. Trop J Pharm Res 2012; 11: 721-727.*
7. *Yang X, Yang C, Farberman A, Rideout TC, de Lange CF, France J, Fan MZ.The mammalian target of rapamycin-signaling pathway in regulating metabolism and growth. J Anim Sci 2008; 86: 12.*
8. *Pinheiro CHdJ, Silveira LR, Nachbar RT, Vitzel KF, Curi R. Regulation of glycolysis and expression of glucose metabolism-related genes by reactive oxygen species in contracting skeletal muscle cells. Free Rad Biol Med 2010; 48: 953-960.*
9. *Standing Committee on Agriculture and Resource Management. Model code of practice for the Welfare of Animals Domestic Poultry. In: CSIRO, 1995; Editor: 3rd Edition.*
10. *James BW, Goodband RD, Unruh JA, Tokach MD, Nelssen JL, Dritz SS, O'Quinn PR, Andrews BS. Effect of creatine monohydrate on finishing pig growth performance, carcass characteristics and meat quality. An Feed Sci Technol 2002; 96: 135-145.*
11. *Rath NC, Richards MP, Huff WE, Huff GR, Balog JM. Changes in the Tibial Growth Plates of Chickens with Thiram-induced Dyschondroplasia. J Comp Pathol 2005; 133: 41-52.*
12. *Ottestad I, Ose L, Wennersberg MH, Granlund L, Kirkhus B, Retterstøl K. Phytosterol capsules and serum cholesterol in hypercholesterolemia: a randomized controlled trial. Atherosclerosis 2013: http://dx.doi.org/10.1016/j.atherosclerosis.2013.100 3.1001.*
13. *Amadou I, Le GW, Amza T, Sun J, Shi YH. Purification and characterization of foxtail millet-derived peptides with antioxidant and antimicrobial activities. Food Res Inter 2013; 51: 422-428.*
14. *Yang X, Zhong L, An X, Zhang N, Zhang L, Han J, Yao J, Cote C, Sun Y. Effects of diets supplemented with zinc and manganese on performance and related parameters in laying hens. Ani Sci J 2012; 83: 474-481.*
15. *Lecker SH, Goldberg AL, Mitch WE. Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. J Am Soc Nephrol 2006; 17: 1807-1819.*
16. *Kitts DD, Kopec A, Zawistowski J, Popovich DG. Effects of high molecular weight alcohols from sugar cane fed alone or in combination with plant sterols on lipid profile and antioxidant status of Wistar rats. Appl Physiol Nutr Metab 2012; 37: 938-946.*
17. *van Blitterswijk WJ, Verheij M. Anticancer mechanisms and clinical application of alkylphospholipids. Biochim Bioph Acta (BBA) - Mol Cell Biol Lipids 2013; 1831: 663-674.*
18. *Han B, Tong J, Zhu MJ, Ma C, Du M. Insulin-like growth factor-1 (IGF-1) and leucine activate pig myogenic satellite cells through mammalian target*

of rapamycin (mTOR) pathway. Mol Reprod Dev 2008; 75: 810-817.

19. *Zou D, Coudron TA, Liu C, Zhang L, Wang M, Chen H. Nutrigenomics in Arma chinensis: Transcriptome Analysis of Arma chinensis Fed on Artificial Diet and Chinese Oak Silk Moth Antheraea pernyi Pupae. PLoS One 2013; 8.*
20. *Richardson CJ, Schalm SS, Blenis J. PI3-kinase and TOR: PIKTORing cell growth. Semi Cell Develop Biol 2004; 15: 147-159.*