

Effects of graded levels of dietary squalene supplementation on the growth performance, plasma biochemical parameters, antioxidant capacity, and meat quality in broiler chickens

Yueping Chen,^{*,†} Yunfeng Gu,^{*} Haoran Zhao,^{*} Hao Zhang,^{*} and Yanmin Zhou^{*,1}

**College of Animal Science and Technology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China; and[†]Postdoctoral Research Station of Food Science and Engineering, College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, People's Republic of China*

ABSTRACT This study was conducted to evaluate the effects of dietary squalene supplementation on the growth performance, plasma biochemical indices, antioxidant status, and meat quality in broilers. Two hundred and forty 0-day-old male chicks were allocated into 5 groups of 6 replicates and were fed a basal diet supplemented with 0 (Control group), 250, 500, 1,000, or 2,000 mg/kg squalene for 42 d. Dietary squalene supplementation linearly increased weight gain and feed efficiency of broilers during the grower and overall periods ($P < 0.05$). Squalene linearly decreased 21-d malondialdehyde (MDA) level and 42-d glutathione peroxidase (GSH-Px) activity, and both linearly and quadratically decreased 42-d MDA level in plasma ($P < 0.05$). In contrast, squalene linearly increased plasma reduced form of glutathione (GSH) level on 21 and 42 d and superoxide dismutase activity on 42 d ($P < 0.05$). Squalene supplementation linearly decreased 21-d MDA accumulation but linearly increased GSH level on 21 d and 42 d and both linearly and quadratically

increased 21-d GSH-Px activity in liver ($P < 0.05$). Supplementing squalene linearly increased pH value at 48 h and linearly decreased lightness at 48 h and 24-h drip loss of breast muscle ($P < 0.05$). The lightness at 24 h and cooking loss of breast muscle were both linearly and quadratically reduced by squalene ($P < 0.05$). Dietary squalene administration linearly decreased MDA accumulation but linearly increased GSH level and GSH-Px activity of breast muscle ($P < 0.05$). Compared with the control group, aforementioned growth performance, antioxidant-related parameters (except 42-d GSH-Px in plasma and breast and hepatic GSH), and meat quality were improved by squalene when its level was 1,000 and 2,000 mg/kg ($P < 0.05$), with their results being similar between these 2 groups ($P > 0.05$). It was concluded that squalene administration especially at a level of 1,000 mg/kg can improve growth performance, antioxidant status, and meat quality in broilers, providing insights into its application as a potential feed additive in broiler production.

Key words: squalene, growth performance, antioxidant capacity, meat quality, broilers

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INTRODUCTION

The squalene is a naturally occurring polyunsaturated triterpene and is an important precursor for the biosynthesis of cholesterol, phytosterol, and other sterols and hopanoids in various types of cells, ranging from microbes to mammals (Reddy and Couvreur, 2009; Spanova and Daum, 2011). It is widely present in nature, and substantial amounts are available in shark liver oil, olives, wheat germ, rice bran, amaranth, and palm (Spanova and

Daum, 2011; Lou-Bonafonte et al., 2018). Squalene can therefore be consumed from food sources in humans in addition to being synthesized in the liver and skin (Reddy and Couvreur, 2009). In mammals, around 60 to 85% of an orally administered squalene, similar to cholesterol, is absorbed through lymphatic vessels of the gastrointestinal tract and then transported in the blood by the very low-density lipoproteins and low-density lipoproteins to the various target organs and tissues where it exerts in vivo biological function (Strandberg et al., 1990; Gylling and Miettinen, 1994; Miettinen and Vanhanen, 1994). Only a small amount of the consumed squalene as a nutrient is actually converted to cholesterol by enzymes in animals, and an even higher intake of squalene does not appear to change intracellular cholesterol pool (Strandberg et al., 1990). The antioxidative, antiaging, immune-regulatory, anti-inflammatory, cytoprotective,

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¹Corresponding author: zhouym6308@163.com

anticancer, and cardioprotective activities of squalene have been already reported in animal models and/or in vitro studies (Lou-Bonafonte et al., 2018). Among them, its antioxidant properties have drawn particularly increasing attention because squalene has been demonstrated as a singlet oxygen scavenger because of its special triterpene structure (Kim and Karadeniz, 2012). An in vitro study has reported that the rate constant of quenching of singlet oxygen by squalene, the first target lipid in human skin surface by various oxidative damage, is much higher than other skin surface lipids in humans and is even comparable to that of 3,5-di-*t*-butyl-4-hydroxytoluene, an effective lipophilic antioxidant (Kohno et al., 1995). In a cell culture experiment, squalene-induced protection against cisplatin and carboplatin-induced toxicity in mesenchymal stem cells have been shown to be directly associated with the decreased reactive oxygen species production and improved cellular glutathione homeostasis (Das et al., 2008). Squalene is well tolerated and active in both humans and rodents, and its clearance from the circulation is slower than that of triglycerides and plant sterols (Relas et al., 2001; Tegenge et al., 2016). The oral administration of squalene has been reported to attenuate cyclophosphamide-induced oxidative damage to the heart, red blood cells, and reproductive tissues in rats by normalizing antioxidant enzymes activities, maintaining nonenzymatic antioxidants levels and inhibiting lipid peroxidation (Senthilkumar et al., 2006; Motawi et al., 2010). Likewise, squalene can protect male rats against isoproterenol-induced myocardial infarction by maintaining redox homeostasis during cellular oxidative stress (Sabeena Farvin et al., 2004, 2007).

It has been reported that squalene administration can prevent alcohol-induced damage in chick embryo retina (Aguilera et al., 2005). In addition to this finding, dietary administration with squalene can improve reproductive performance in male meat-type broiler chickens (Li et al., 2010). Moreover, the growth-promoting effects of squalene-rich feed supplements, such as olive oil (Zhang et al., 2013), wheat germ oil (Arshad et al., 2013), and palm oil (Long et al., 2019), have been reported previously in broiler chickens. Little, however, is known about whether dietary squalene supplementation could bring beneficial effects on the growth performance and especially antioxidant redox system in broiler chickens. Actually, modern broilers are particularly susceptible to oxidative stress when compared with other animal species because of the genetic selection toward lean and large breast muscles and fast growth rate (Sihvo et al., 2013), and the occurrence of oxidative damage in broiler chickens could result in impaired growth performance, disrupted antioxidant defence, and inferior meat quality such as pale, soft, and exudative meat, which makes the meat unattractive and watery (Estévez, 2015). It is, therefore, imperative to develop effective interventions to prevent and counteract oxidative damage in broilers, which are inevitably and continuously exposed to oxidative stimuli from environment and diet, although they are always reared in

normal conditions that are free from oxidative stress. According to its favorable antioxidant characteristics, we hypothesized that dietary supplementation with squalene may exert beneficial consequences on the growth and antioxidant status of broilers. The current study was hence conducted to investigate effects of graded levels of dietary squalene supplementation on growth performance, plasma biochemical indices, antioxidant status, and meat quality of breast muscle in broiler chickens under normal physiological conditions, and the results of which would provide guidance and reference for its further application as a promising feed additive in broiler feed.

MATERIALS AND METHODS

Animals, Diets, and Treatments

All experiments in this study involving animals were carried out following an ethics committee-approved protocol (SYXK (SU) 2,017-0007) in compliance with Nanjing Agricultural University.

A total of two hundred and forty 0-day-old male Ross 308 broiler chicks with similar hatching weight (47.40 ± 0.38 g) were allocated into a completely randomized design with 5 treatments for a 42-d feeding trial after separate weighing, and each treatment consisted of 6 replicates (cages) with 8 birds each. Birds were fed a basal diet supplemented with 0 (Control group), 250, 500, 1,000, and 2,000 mg/kg squalene, respectively. The dosage of squalene was selected according to previous studies (Sabeena Farvin et al., 2004, 2007; Sánchez-Fidalgo et al., 2015). The squalene used in this study was extracted from deodorizer distillate generated during rice bran oil production using methanol as solvent and then purified by molecular distillation and was kindly provided by Yichun Dahaigui Life Science Co., Ltd. (Yichun City, Jiangxi Province, P.R. China), with a purity of approximately 92%. The squalene was added on top of the original diet formulation (basal diet). In detail, the liquid squalene was mixed thoroughly with soybean oil used in broiler feed formulation before feed preparation. Ingredient composition of the basal diet and its nutrient levels are presented in Table 1. The basal diet was formulated according to NRC (1994) recommendations. Broiler chickens were reared in cages (120 cm × 60 cm × 50 cm) in a temperature-controlled house under a light schedule of 23 h of light and 1 h of dark and were provided with mash feed and water *ad libitum*. The temperature of facility was kept between 32°C and 34°C during the first week of age, and it was reduced by 2°C to 3°C at 1 wk interval until a final temperature of 24°C was reached. The relative humidity was maintained at around 70% during the initial 3 d and was then set at approximately 60 to 65% thereafter. Birds were vaccinated against Newcastle disease virus, infectious bronchitis virus, and infectious bursal disease virus through neck injection on 11 d of age, using commercially available vaccines.

Table 1. Composition and nutrient level of the basal diet (g/kg, as-fed basis unless otherwise stated).

Items ¹	0 to 21 d	22 to 42 d
Ingredients		
Corn	570.10	615.20
Soybean meal	315.00	250.00
Corn gluten meal	34.00	46.00
Soybean oil	31.00	41.00
Limestone	12.00	17.00
Dicalcium phosphate	20.00	14.00
L-Lysine	3.40	3.00
DL-Methionine	1.50	0.80
Sodium chloride	3.00	3.00
Premix ²	10.00	10.00
Calculated nutrient levels		
Apparent metabolizable energy (MJ/kg)	12.55	13.06
Crude protein	213.32	194.87
Calcium	9.99	9.80
Available phosphorus	4.58	4.04
Lysine	12.09	10.41
Methionine	5.01	4.15
Methionine + cystine	8.56	7.47
Analyzed composition		
Crude protein	209.41	188.74
Calcium	9.59	9.44
Total phosphorus	6.91	6.19
Methionine	4.79	3.94
Lysine	11.61	10.62

¹The squalene was added on top of the basal diet; The liquid squalene was mixed thoroughly with soybean oil used in broiler feed formulation before feed preparation at a level of 0 (Control group), 250, 500, 1,000, and 2,000 mg/kg, respectively.

²Premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D₃ (cholecalciferol), 3,000 IU; vitamin E (all-rac- α -tocopherol), 30 IU; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 600 mg; calcium pantothenate, 10 mg; pyridoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B₁₂ (cobalamin), 0.013 mg; Fe (from ferrous sulfate), 80 mg; Cu (from copper sulphate), 8.0 mg; Mn (from manganese sulphate), 110 mg; Zn (from zinc oxide), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.

Sample Collection

On 21 and 42 d of age, 1 bird from each replicate (cage) of groups (6 birds from each treatment in total) was randomly selected for sampling after a 12-h feed withdrawal period. Blood samples were collected from wing vein, using heparin as an anticoagulant. Plasma was then harvested after centrifugation at $2,000 \times g$ for 15 min at 4°C and immediately stored at -20°C until further analysis. Birds were euthanized by cervical dislocation after blood sampling. After necropsy, liver samples were dissected free from connective tissues, washed with phosphate-buffered saline (pH = 7.4), and dried with filter paper before placing on a chilled stainless-steel tray. The right lobe of liver sample (around 5 g) was excised from the remainder of the fresh liver tissue, minced, snapped frozen in liquid nitrogen, and stored at -80°C for subsequent analysis. The left pectoralis major muscle sample was then collected and stored at 4°C environment for the measurement of meat color, pH value, and drip loss, and 2 portions of the right pectoralis major muscle sample were collected to determine cooking loss (stored at 4°C before analysis) and antioxidant-related parameters (stored at -80°C before analysis), respectively.

Growth Performance Measurement

All birds in each replicate (cage) were weighed individually after a 12-h feed deprivation period on 21 and 42 d of age, and the consumption of feed by birds was recorded on cage (replicate) basis to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed to gain ratio (F/G) during the starter (0–21 d), grower (22–42 d), and overall (0–42 d) periods.

Determination of Plasma Biochemical Parameters

The total protein (cat. No.A045-4-1), albumin (cat. No.A028-2-1), glucose (cat. No.F006-1-1), triglyceride (cat. No.A110-2-1), and total cholesterol (cat. No.A111-1-1) levels and aspartate aminotransferase (cat. No.C010-1) and alanine aminotransferase (cat. No.C009-2) activities were quantified colorimetrically with commercial assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China), strictly following manufacture's manuals.

Assay of Antioxidant-Related Parameters

Approximately 0.5 g of fresh liver and pectoralis major muscle samples were homogenized with a cold physiological saline solution (4°C) in an ice-cold bath at a ratio of 1:9 (wt/vol) and 1:4 (wt/vol), respectively, using a homogenizer (PRO-PK-02200D, Pro Scientific, Inc., Monroe, CT) until no tissue particles were visible in the homogenate solution (around 60 s). The prepared homogenized solution was then centrifuged at $4450 \times g$ at 4°C for 20 min to remove debris, and the acquired supernatant was immediately stored at -20°C until assay.

The assay kits of superoxide dismutase (SOD, cat. No.A001-1-1) activity, glutathione peroxidase (GSH-Px, cat. No.A005-1-2) activity, reduced form of glutathione (GSH, cat. No.A006-1) level, and malondialdehyde (MDA, cat. No.A003-1) concentration in plasma and tissues (liver and pectoralis major muscle) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, P.R. China). A hydroxylamine method (Kono, 1978) was employed to determine SOD activity. One unit of SOD activity was defined as the amount of enzyme per milliliter of plasma or per milligram protein of tissues that was required to produce 50% inhibition of the rate of nitrite generation at 37°C. The activity of GSH-Px and GSH level was measured by 5, 5'-dithiobis (2-nitrobenzoic acid) method (Owens and Belcher, 1965), and one unit of GSH-Px was calculated as the amount of enzyme depleting 1.0 μ mol of GSH per 5 min at 37°C in 0.1 mL of plasma or 1.0 mg of tissue protein. The MDA concentration was measured using the thiobarbituric acid method (Placer et al., 1966). The total protein concentration of tissues was quantified with Bradford method (Kruger, 1994), using crystalline bovine serum albumin as a standard protein. All

Table 2. Effects of graded levels of dietary squalene supplementation on the growth performance of broiler chickens from 0 to 42 d of age.

Items	Squalene level (mg/kg)					SEM	P-values		
	0	250	500	1,000	2,000		ANOVA	Linear	Quadratic
0 to 21 d									
ADFI (g/d/bird)	39.01	40.99	39.92	40.91	39.00	0.404	0.342	0.977	0.101
ADG (g/d/bird)	28.36	28.29	28.62	29.26	27.89	0.313	0.749	0.985	0.409
F/G (g:g)	1.38	1.45	1.40	1.40	1.40	0.012	0.472	0.969	0.363
22 to 42 d									
ADFI (g/d/bird)	152.34	150.51	147.97	158.94	156.96	1.722	0.238	0.146	0.353
ADG (g/d/bird)	79.26 ^c	81.08 ^c	81.80 ^{b,c}	91.35 ^a	88.49 ^{a,b}	1.304	0.004	0.001	0.952
F/G (g:g)	1.94 ^a	1.86 ^{a,b}	1.81 ^{a,b}	1.74 ^b	1.78 ^b	0.022	0.042	0.005	0.234
0 to 42 d									
ADFI (g/d/bird)	94.88	93.71	93.33	98.67	97.26	0.854	0.208	0.107	0.452
ADG (g/d/bird)	53.81 ^c	54.69 ^{b,c}	55.21 ^{b,c}	60.30 ^a	58.19 ^{a,b}	0.687	0.006	0.001	0.765
F/G (g:g)	1.77 ^a	1.72 ^{a,b}	1.69 ^{a,b}	1.64 ^b	1.67 ^b	0.014	0.037	0.006	0.154

^{a-c}Means within a row with different superscripts are different at $P < 0.05$.

Abbreviations: ADFI, average daily feed intake; ADG, average daily gain; F/G, feed to gain ratio.

acquired results from tissue samples were normalized against the total protein level of each sample for inter-sample comparisons.

Meat Quality Determination

The measurement of meat quality was performed on 6 birds selected from each group (1 bird per replicate). The determination of meat color and pH value in pectoralis major muscle was performed as previously reported in our lab (Chen et al., 2018). The meat color was measured at 45 min, 24 h, and 48 h postmortem by a handheld colorimeter (Minolta CR-400, Konica Minolta, Tokyo, Japan) based on the CIELAB system (L^* = lightness; a^* = redness; b^* = yellowness). The meat color measurement was performed on a tray that was placed in a cabinet at 4°C under a light-emitting diode light source with an intensity of approximately 700 lx. The pH value of meat at 45 min, 24 h, and 48 h postmortem was determined by directly inserting a pH probe (HI9125, HANNA

Instruments, Padova, Italy) into the cranial aspect of the breast muscle. The measurement of meat color and pH value was performed in triplicate at 3 different places of meat samples, and the average values were used for calculation. The cooking loss of pectoralis major muscle was measured after a 24-h storage period postmortem at 4°C according to previous studies (Christensen, 2003; Cheng et al., 2017). In brief, a piece of regular-shaped muscle sample from the same location in the pectoralis major muscle was weighed, placed into a zip-sealed plastic bag, and then heated in a water bath to an internal temperature of 75°C for 20 min. The core temperature of the meat was monitored using a thermometer. After that, muscle sample was taken out, cooled to room temperature, dried with filter papers, and weighed again to determine cooking loss, which was calculated as gram of weight loss per kilogram of weight before cooking. The 24-h and 48-h drip loss of pectoralis major muscle were determined as previously described (Wang et al., 2013; Chen et al., 2018). In detail, muscle samples were

Table 3. Effects of graded levels of dietary squalene supplementation on the plasma biochemical parameters of broiler chickens.

Items	Squalene level (mg/kg)					SEM	P-values		
	0	250	500	1,000	2,000		ANOVA	Linear	Quadratic
21 d									
Total protein (g/L)	26.67	29.46	29.73	30.34	28.17	0.665	0.444	0.418	0.098
Albumin (g/L)	18.51	19.90	22.94	21.79	21.27	0.621	0.190	0.091	0.120
Glucose (mmol/L)	15.89	16.19	16.43	16.98	15.73	0.332	0.806	0.851	0.345
Triglyceride (mmol/L)	0.758	0.750	0.768	0.753	0.695	0.019	0.805	0.398	0.440
Total cholesterol (mmol/L)	5.04	4.79	5.12	5.48	5.16	0.155	0.756	0.422	0.929
Alanine aminotransferase (U/L)	6.97	6.87	6.92	7.37	7.18	0.153	0.685	0.201	0.902
Aspartate aminotransferase (U/L)	20.00	21.02	19.80	18.68	18.47	0.620	0.717	0.246	0.663
42 d									
Total protein (g/L)	34.65	37.86	35.55	36.67	33.73	0.622	0.245	0.483	0.091
Albumin (g/L)	17.78	18.93	17.46	17.20	19.12	0.382	0.402	0.725	0.398
Glucose (mmol/L)	15.29	17.40	16.75	16.89	16.43	0.318	0.305	0.426	0.107
Triglyceride (mmol/L)	0.697	0.655	0.717	0.785	0.782	0.027	0.516	0.134	0.720
Total cholesterol (mmol/L)	4.22	4.51	4.12	4.79	4.72	0.136	0.468	0.202	0.766
Alanine aminotransferase (U/L)	8.13	8.92	9.12	9.26	9.07	0.197	0.403	0.120	0.230
Aspartate aminotransferase (U/L)	29.54	30.65	34.83	30.50	32.72	1.024	0.512	0.403	0.474

Table 4. Effects of graded levels of dietary squalene supplementation on the plasma antioxidant status of broiler chickens.

Items	Squalene level (mg/kg)					SEM	P-values		
	0	250	500	1,000	2,000		ANOVA	Linear	Quadratic
21 d									
MDA (nmol/mL)	1.94 ^a	1.60 ^b	1.43 ^{b,c}	1.49 ^{b,c}	1.25 ^c	0.062	0.002	<0.001	0.294
GSH (mg/L)	9.28 ^b	9.59 ^b	9.48 ^b	13.68 ^a	13.58 ^a	0.550	0.002	<0.001	0.340
SOD (U/mL)	165.65	175.15	166.42	154.43	167.70	4.478	0.727	0.617	0.913
GSH-Px (U/mL)	683.92	691.75	700.30	694.36	685.10	10.590	0.990	0.951	0.613
42 d									
MDA (nmol/mL)	2.05 ^a	1.82 ^{a,b}	1.33 ^c	1.56 ^{b,c}	1.40 ^c	0.065	<0.001	<0.001	0.032
GSH (mg/L)	8.99 ^b	8.79 ^b	9.42 ^b	9.77 ^{a,b}	10.89 ^a	0.239	0.032	0.004	0.189
SOD (U/mL)	180.54 ^b	178.33 ^b	175.67 ^b	205.83 ^a	203.18 ^a	3.630	0.004	0.002	0.203
GSH-Px (U/mL)	726.90 ^a	743.91 ^a	720.46 ^a	672.64 ^{a,b}	616.78 ^b	13.139	0.006	0.001	0.069

^{a-c}Means within a row with different superscripts are different at $P < 0.05$.

Abbreviations: GSH, reduced form of glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

trimmed of adjacent fat and connective tissues, taken along the fiber direction, weighed, hung in a sealed plastic bag, and stored at 4°C for 24 h or 48 h. After removing surface moisture, the meat was weighed again to determine 24-h and 48-h drip loss, respectively.

Statistical Analysis

Data were analyzed by one-way analysis of variance using SPSS (2008) statistical software (Ver.16.0 for windows, SPSS Inc., Chicago, IL). The statistical model for data analysis was $Y_{ij} = \mu + T_i + \epsilon_{ij}$, where Y_{ij} = dependent observation; μ = overall mean; T_i = effect of diet (level of squalene); ϵ_{ij} = the random error. A cage (replicate) was the experimental unit for the growth performance data, whereas an individual bird from each cage was the experimental unit for other measured parameters (plasma biochemical parameters, antioxidant-related parameters, and meat quality). Orthogonal polynomial contrasts were employed to test the linear and quadratic effects of the increasing levels of squalene. Differences among groups were examined using Duncan’s multiple range test. The differences were considered as statistically significant when P value was less than 0.05. Results were presented as means with their pooled standard errors.

RESULTS

Growth Performance

The growth performance (Table 2, ADFI, ADG, and F/G) of broilers was similar among groups during the starter period ($P > 0.05$). Dietary supplementation with squalene linearly increased ADG (grower and overall periods, $P = 0.001$) but linearly decreased F/G (grower period, $P = 0.005$; overall period, $P = 0.006$) of broilers during the grower and overall periods. Compared with the control group, supplementing 1,000 and 2,000 mg/kg squalene increased ADG (grower period, $P = 0.004$; overall period, $P = 0.006$) but decreased F/G (grower period, $P = 0.042$; overall period, $P = 0.037$) of broilers during the late and whole experimental periods, with their values being similar between the 2 squalene-supplemented groups ($P > 0.05$). However, the ADFI of broilers during the grower and overall periods did not differ among treatments ($P > 0.05$).

Plasma Biochemical Parameters

As shown in Table 3, dietary squalene administration did not affect plasma total protein, albumin, glucose, triglyceride, or total cholesterol level of broilers on 21 and 42 d of age ($P > 0.05$). The similar result was also found

Table 5. Effects of graded levels of dietary squalene supplementation on the hepatic antioxidant status of broiler chickens.

Items	Squalene level (mg/kg)					SEM	P-values		
	0	250	500	1,000	2,000		ANOVA	Linear	Quadratic
21 d									
MDA (nmol/mg protein)	0.903 ^a	0.784 ^{a,b}	0.724 ^{a,b}	0.610 ^b	0.586 ^b	0.036	0.024	0.001	0.610
GSH (mg/g protein)	9.58 ^b	13.15 ^a	12.46 ^a	12.93 ^a	13.48 ^a	0.414	0.010	0.005	0.105
SOD (U/mg protein)	224.36	213.72	203.37	209.13	202.49	3.725	0.344	0.075	0.441
GSH-Px (U/mg protein)	37.92 ^b	43.79 ^{a,b}	47.65 ^a	47.58 ^a	46.58 ^a	1.091	0.012	0.003	0.030
42 d									
MDA (nmol/mg protein)	0.863	0.767	0.709	0.721	0.720	0.033	0.593	0.179	0.365
GSH (mg/g protein)	11.15 ^b	13.05 ^{a,b}	13.54 ^{a,b}	12.96 ^{a,b}	15.51 ^a	0.459	0.042	0.006	0.946
SOD (U/mg protein)	140.95	148.60	164.49	166.61	154.21	3.911	0.192	0.105	0.098
GSH-Px (U/mg protein)	45.94	43.60	46.94	42.95	42.98	0.956	0.591	0.350	0.753

^{a-c}Means within a row with different superscripts are different at $P < 0.05$.

Abbreviations: GSH, reduced form of glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

Table 6. Effects of graded levels of dietary squalene supplementation on the meat quality of breast muscle in broiler chickens.

Items	Squalene level (mg/kg)					SEM	P-values		
	0	250	500	1,000	2,000		ANOVA	Linear	Quadratic
pH _{45 min}	6.42	6.48	6.43	6.44	6.50	0.015	0.426	0.259	0.620
pH _{24 h}	5.79	5.90	5.87	5.91	5.87	0.015	0.098	0.099	0.076
pH _{48 h}	5.76 ^c	5.83 ^{a,b,c}	5.81 ^{b,c}	5.89 ^{a,b}	5.91 ^a	0.016	0.007	0.001	0.902
L* _{45 min}	41.76	42.24	42.70	42.50	42.21	0.236	0.796	0.511	0.295
L* _{24 h}	46.73 ^a	47.48 ^{a,b}	46.06 ^{a,b}	45.47 ^b	43.45 ^c	0.334	<0.001	<0.001	0.023
L* _{48 h}	48.88 ^a	48.27 ^{a,b}	46.81 ^{a,b,c}	46.30 ^{b,c}	45.39 ^c	0.410	0.031	0.002	0.908
a* _{45 min}	4.42	5.09	4.09	4.71	5.33	0.194	0.272	0.296	0.351
a* _{24 h}	6.05	5.83	5.75	6.04	7.08	0.192	0.180	0.092	0.072
a* _{48 h}	7.03	7.04	6.42	6.57	7.66	0.227	0.481	0.637	0.139
b* _{45 min}	21.99	22.32	22.16	22.52	22.88	0.285	0.899	0.360	0.817
b* _{24 h}	26.75	25.36	25.62	26.14	24.01	0.429	0.352	0.131	0.735
b* _{48 h}	28.06	25.86	26.48	27.35	26.92	0.439	0.602	0.805	0.323
Cooking loss (g/kg)	213.01 ^a	192.35 ^{a,b}	182.39 ^b	172.40 ^b	183.96 ^b	4.201	0.019	0.005	0.043
Drip loss _{24 h} (g/kg)	34.38 ^a	30.81 ^{a,b}	28.77 ^{a,b}	24.76 ^b	25.36 ^b	1.091	0.018	0.001	0.425
Drip loss _{48 h} (g/kg)	48.27	43.82	45.97	45.55	45.75	0.906	0.680	0.619	0.398

^{a-c}Means within a row with different superscripts are different at $P < 0.05$.

Abbreviations: a*, redness; b*, yellowness; L*, lightness.

in plasma transaminase activity, as evident by the insignificant aspartate aminotransferase and alanine aminotransferase activities on 21 and 42 d among treatments ($P > 0.05$).

Plasma Antioxidant Status

On 21 d of age, dietary squalene supplementation linearly decreased MDA concentration (Table 4, $P < 0.001$) but linearly increased GSH level ($P < 0.001$) in plasma of broilers. Compared with the control group, the administration of squalene, regardless of its level, decreased circulating MDA concentration ($P = 0.002$), and its lowest value was found in the 2,000 mg/kg squalene-supplemented group, which was near to that of 500 and 1,000 mg/kg groups ($P > 0.05$). The plasma GSH concentration ($P = 0.002$) was increased by both 1,000 and 2,000 mg/kg squalene in comparison with the control group, and there was no significant difference between these 2 groups ($P > 0.05$). However, the activities of SOD and GSH-Px in plasma were similar among the 5 groups ($P > 0.05$).

On 42 d of age, feeding squalene linearly ($P < 0.001$) and quadratically ($P = 0.032$) decreased MDA accumulation and linearly decreased GSH-Px activity ($P = 0.001$) but linearly increased GSH level ($P = 0.004$) and SOD activity ($P = 0.002$) in plasma

of broilers. Compared with the control group, the administration of squalene from 500 to 2,000 mg/kg all decreased plasma MDA level ($P < 0.001$), and the difference was insignificant among the 3 squalene-supplemented groups ($P > 0.05$). The administration of 1,000 and 2,000 mg/kg squalene both increased plasma SOD activity ($P = 0.004$) to a similar extent ($P > 0.05$). Only the highest level of dietary squalene supplementation (2,000 mg/kg) increased GSH level ($P = 0.032$) and decreased GSH-Px activity ($P = 0.006$) in plasma of broilers.

Hepatic Antioxidant Status

Dietary squalene supplementation linearly decreased hepatic MDA accumulation (Table 5, $P = 0.001$) but linearly increased GSH level ($P = 0.005$) as well as linearly ($P = 0.003$) and quadratically ($P = 0.030$) increased GSH-Px activity in liver of broilers on 21 d of age. Compared with the control group, the 21-d hepatic MDA concentration of broilers was decreased by 1,000 and 2,000 mg/kg squalene supplementation ($P = 0.024$) to a similar level ($P > 0.05$). The administration of squalene, irrespective of its dosage, increased hepatic GSH level on 21 d of age ($P = 0.010$), and its concentration was similar among the squalene-supplemented treatments ($P > 0.05$). The 21-d hepatic GSH-Px activity was increased by squalene when its

Table 7. Effects of graded levels of dietary squalene supplementation on the antioxidant status of breast muscle in broiler chickens.

Items	Squalene level (mg/kg)					SEM	P-values		
	0	250	500	1,000	2,000		ANOVA	Linear	Quadratic
MDA (nmol/mg protein)	0.475 ^a	0.420 ^a	0.290 ^b	0.259 ^b	0.273 ^b	0.024	0.004	<0.001	0.159
GSH (mg/g protein)	4.29 ^c	4.72 ^{b,c}	5.32 ^{a,b,c}	5.95 ^a	5.68 ^{a,b}	0.194	0.029	0.003	0.344
SOD (U/mg protein)	98.28	83.17	88.22	94.78	99.64	3.350	0.509	0.555	0.156
GSH-Px (U/mg protein)	19.29 ^b	18.95 ^b	19.91 ^b	23.43 ^{a,b}	25.34 ^a	0.810	0.029	0.003	0.246

^{a-c}Means within a row with different superscripts are different at $P < 0.05$.

Abbreviations: GSH, reduced form of glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

supplemental level ranged from 500 to 2,000 mg/kg ($P = 0.012$), and the value of this parameter did not differ among the 3 squalene-supplemented groups ($P > 0.05$). On 42 d of age, dietary squalene administration linearly increased hepatic GSH level ($P = 0.006$), and its level in the 2,000 mg/kg squalene group was higher than that of the control group ($P = 0.042$). However, treatment did not affect hepatic MDA concentration on 42 d, SOD activity on both 21 d and 42 d, or GSH-Px activity on 42 d ($P > 0.05$).

Meat Quality and Its Antioxidant Capacity

Dietary supplementation with squalene linearly increased pH value at 48 h (Table 6, $P = 0.001$) and linearly decreased lightness at 48 h ($P = 0.002$) and 24-h drip loss ($P = 0.001$) of breast muscle. In addition, the lightness at 24 h (linear, $P < 0.001$; quadratic, $P = 0.023$) and cooking loss (linear, $P = 0.005$; quadratic, $P = 0.043$) of breast muscle were both linearly and quadratically reduced after supplementation with squalene. Compared with the control group, the administration of squalene at levels of 1,000 and 2,000 mg/kg increased pH value at 48 h ($P = 0.007$) and decreased lightness at 48 h ($P = 0.031$) and 24-h drip loss ($P = 0.018$) of breast muscle, with the values of these parameters being similar between these 2 supplemental groups ($P > 0.05$). The 24-h lightness was higher in broilers fed a basal diet supplemented with 1,000 and 2,000 mg/kg squalene when compared with their counterparts receiving a basal diet ($P < 0.001$), but its level was still lower in 1,000 mg/kg group than that of 2,000 mg/kg group ($P > 0.05$). The cooking loss of breast muscle was decreased by squalene when its dosage was from 500 to 2,000 mg/kg ($P = 0.019$); however, no significant difference was observed in these 3 groups treated with squalene ($P > 0.05$). Dietary treatment did not alter pH values at both 45 min and 24 h, redness, yellowness, or 48-h drip loss of breast muscle in broiler chickens ($P > 0.05$).

Dietary squalene administration linearly decreased MDA accumulation (Table 7, $P < 0.001$) but linearly increased GSH level ($P = 0.003$) and GSH-Px activity ($P = 0.003$) of breast muscle on 42 d of age. The administration of squalene from 500 to 2,000 mg/kg decreased muscular MDA accumulation ($P = 0.004$) when compared with the control group, but no significant difference was found among the 3 squalene-supplemented groups ($P > 0.05$). Dietary supplementation with 1,000 and 2,000 mg/kg squalene increased GSH level ($P = 0.029$) of breast muscle to a similar extent ($P > 0.05$). And only 2,000 mg/kg squalene supplementation increased GSH-Px activity ($P = 0.029$) of breast muscle. However, the SOD activity of breast muscle did not differ among dietary treatments ($P > 0.05$).

DISCUSSION

Up to date, the effects of dietary supplementation with squalene on the growth performance have not

actually been investigated in broiler chickens and other domestic animal species. An in vivo study has shown that oral squalene administration can significantly improve body weight gain in male Wistar rats challenged with cyclophosphamide (Motawi et al., 2010). In this study, we firstly found that supplementing squalene from 250 to 2,000 mg/kg linearly improved weight gain and feed efficiency of broilers during the grower and overall periods, and the most pronounced results were observed, when its dosage was 1,000 mg/kg. The improved weight gain noticed in the current study was most likely related to an increase in feed efficiency, because the feed intake of broilers did not differ among treatments. The beneficial effects of squalene on the growth performance of broilers may be possibly explained by its anti-inflammatory, antioxidative, immune-promoting, detoxifying, and gut protective effects, as reported previously (Reddy and Couvreur, 2009; Spanova and Daum, 2011; Kim and Karadeniz, 2012; Sánchez-Fidalgo et al., 2015; Lou-Bonafonte et al., 2018). Available studies conducted in both humans (18.1 mg through intravenous administration) and rodents (27.5 μ L through intramuscular administration) have demonstrated that squalene is safe and tolerant in the body (Relas et al., 2001; Tegenge et al., 2016), and squalene has been already used as emulsions for vaccine and drug delivery because of its high surface tension, stability, and biocompatibility characteristics (Fox, 2009). In this study, squalene administration, irrespective of its level, did not alter plasma metabolites levels and transaminase activity, the sensitive indices of health status, suggesting that squalene supplementation from 250 to 2,000 mg/kg is safe for broiler chickens, which can be further supported by the simultaneously improved growth performance of broiler chickens found in this study. Usually, squalene is generalized as an important precursor for cellular cholesterol synthesis (Reddy and Couvreur, 2009; Spanova and Daum, 2011). However, the plasma cholesterol concentration was similar among treatments in this study. In agreement with our finding, Strandberg et al. (1990) have reported that squalene feeding (900 mg/d for 7–30 d) does not alter serum cholesterol and triglyceride levels in humans, although the administration of squalene increases fecal excretions of cholesterol and its nonpolar derivatives as well as bile acids. Likewise, Tilvis and Miettinen (1983) found that feeding 1% squalene markedly increased squalene accumulation in the intestinal mucosa, liver, and adipose tissues, and hepatic cholesterol concentration, but the serum cholesterol level remained unchanged. The similar plasma cholesterol level observed currently may indicate that blood cholesterol concentration is not sensitive to squalene administration, and further investigation should be conducted to explore the underlying metabolism.

Reactive oxygen species produced inside body during normal cellular activities are essential in abundant biochemical pathways and play an important role in various physiological processes, but this potential double-edged sword would damage cellular DNA,

proteins, and lipids when present in a high enough level, resulting in harmful consequences on the growth performance and product quality of animals (Seifried et al., 2007; Estévez, 2015). To combat this, living cells have evolved antioxidant defences to protect against free radicals, including antioxidant enzymes (e.g., SOD and GSH-Px) and the small antioxidants (e.g., GSH) (Pisoschi and Pop, 2015). In the current study, dietary squalene administration improved antioxidant capacity of broilers by increasing plasma SOD and hepatic GSH-Px activities, elevating GSH levels in liver and plasma, and reducing the accumulations of plasma and hepatic MDA, an end product of lipid peroxidation. This result indicates that squalene can exert beneficial consequences on antioxidant capacity of broilers. In agreement with our finding, an oral administration of squalene for 7 d can improve antioxidant status of rats subjected to the cyclophosphamide-induced oxidative stress by increasing GSH-Px activity and GSH concentration and by reducing MDA accumulation in tissues (myocardium, testicles, and urinary bladder) (Motawi et al., 2010). Likewise, squalene treatment can help to maintain cellular redox homeostasis in cyclophosphamide-challenged rats by increasing SOD and GSH-Px activities as well as GSH level and by inhibiting the generation of thiobarbituric acid reactive substances in heart and hemolysate of red blood cells (Senthilkumar et al., 2006). The protective effects of squalene against isoproterenol-induced myocardial infarction in rats have also been reported to be closely correlated with its regulation on antioxidant defence system, such as the increased antioxidant enzyme activities, improved cellular GSH homeostasis, and reduced lipid peroxidation (Sabeena Farvin et al., 2004, 2007). Although belonging to a class of triterpene derivatives, squalene is actually not susceptible to lipid peroxidation and is stable for attacks by peroxide radicals, and its ability to scavenge free radicals is even comparable to that of 3,5-di-*t*-butyl-4-hydroxytoluene (Kohno et al., 1995). A cell culture study has shown that squalene can directly reduce cellular reactive oxygen species and alleviate the hydrogen peroxide-induced oxidative damage of human mammary epithelial cells in a dose-dependent manner (Warleta et al., 2010). Therefore, the improved antioxidant status in the plasma and liver of broilers in this study can be at least partially explained by its antioxidant characteristics. Moreover, it has also been reported that squalene can improve liver mitochondrial function of rats by maintaining activities of tricarboxylic acid cycle enzymes and respiratory marker enzymes to improve energy status and by increasing mitochondrial antioxidant capacity (Buddhan et al., 2007), which may also contribute to the improved redox status of broilers observed in this research, because mitochondria are the major intracellular source and target of reactive oxygen species (James et al., 2016). It is necessarily to mention that dietary squalene supplementation actually linearly reduced plasma GSH-Px activity on 42 d of age in this study, which is opposite to the elevated plasma GSH

level. This result may be because of the negative feedback effect of the simultaneously increased GSH concentration (Wang et al., 1997; Chen et al., 2013).

The ability to retain moisture is considered as the most critical quality characteristic of fresh meat (Huff-Lonergan and Lonergan, 2005). After slaughter, the muscular antioxidant defence will gradually collapse because of cellular biochemical changes that are taken place during the conversion of muscle to meat (Min and Ahn, 2005). The meat oxidation especially lipid peroxidation will subsequently impair hydrolysis sensitivity, weaken protein degradation, reduce water retention in myofibrils, and destroy membrane integrity of muscle cells, ultimately leading to the increased meat juice loss (Huff-Lonergan and Lonergan, 2005; Cheng and Sun, 2008; Min et al., 2008). The α -tocopherol, aromatic herbs, essential oils, and other plant-derived antioxidants have been widely used as a feed additive strategy to improve water holding capacity of meat (Jiang and Xiong, 2016). In our study, dietary squalene administration linearly reduced cooking loss and drip loss at 24 h of breast muscle, 2 important parameters reflecting water holding capacity. The increased water holding capacity of breast muscle after squalene supplementation is in parallel with the improved antioxidant status, including the increased GSH level and GSH-Px activity and inhibited lipid peroxidation, as evident by a decrease in MDA accumulation. This result, in turn, suggests that squalene administration may improve water holding capacity of breast muscle by improving its antioxidant capacity (Kim and Karadeniz, 2012; Lou-Bonafonte et al., 2018). The pH decline rate and ultimate pH value at 24 h postmortem are highly dependent on amounts of adenosine triphosphate and glycogen stored in muscle, which will be gradually depleted after slaughter and are highly correlated with genetic background (Ali et al., 2008; Le Bihan-Duval et al., 2008; Kim et al., 2014). The normal broiler breast ultimate pH value (24 h) is usually around 5.8 to 5.9 (Duclos et al., 2007; Alnahhas et al., 2014). In this study, the pH value of breast muscle at 45 min postmortem did not differ among treatments, and the similar 24-h pH value among groups also ranged around 5.8 to 5.9. The redox status is crucial for meat quality maintenance during cold storage. It has been reported that garlic juice or onion juice can increase pH value of meat stored at 4°C from 1 to 7 d postmortem by inhibiting lipid peroxidation (Kim et al., 2010). In consistent with this finding, squalene linearly increased pH value of breast muscle at 48 h postmortem, which may be associated with the improved antioxidant capacity of breast muscle. Many factors, such as concentration of heme pigments, ligand formation of heme pigments, oxidative status, and physical characteristics of meat (pH value and storage time) can contribute to meat color variation (Qiao et al., 2001; Xiao et al., 2011). The administration of squalene reduced lightness of breast muscle at both 24 and 48 h postmortem in this study. The improved oxidative status, elevated water holding capacity, and increased pH value all account for the reduction in lightness of meat

as summarized previously (Hughes et al., 2014; Carvalho et al., 2017). In the current research, the improved antioxidant capacity of breast muscle is most likely to account for the simultaneous decrease in lightness value, because the lipid peroxidation and protein oxidation occurred during storage can damage cell membrane integrity and myofibril structure and then induce juice loss of meat, leading to increased water availability on the meat surface and light reflection, and ultimately an increased lightness value of meat, as reported previously (Wang et al., 2009; Zhang et al., 2017).

This study suggests dietary supplementation with squalene can improve growth performance, antioxidant status of liver and plasma, and antioxidant capacity and meat quality of breast muscle in broiler chickens, and the recommended level of squalene is 1,000 mg/kg in broiler feed.

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REFERENCES

- Aguilera, Y., M. E. Dorado, F. A. Prada, J. J. Martínez, A. Quesada, and V. Ruiz-Gutiérrez. 2005. The protective role of squalene in alcohol damage in the chick embryo retina. *Exp. Eye Res.* 80:535–543.
- Ali, M., G. H. Kang, and S. T. Joo. 2008. A review: Influences of pre-slaughter stress on poultry meat quality. *Asian-Australas. J. Anim. Sci.* 21:912–916.
- Alnahhas, N., C. Berri, M. Boulay, E. Baéza, Y. Jégo, Y. Baumard, M. Chabault, and E. Le Bihan-Duval. 2014. Selecting broiler chickens for ultimate pH of breast muscle: analysis of divergent selection experiment and phenotypic consequences on meat quality, growth, and body composition traits. *J. Anim. Sci.* 92:3816–3824.
- Arshad, M. S., F. M. Anjum, M. I. Khan, M. Shahid, S. Akhtar, and M. Sohaib. 2013. Wheat germ oil enrichment in broiler feed with α -lipoic acid to enhance the antioxidant potential and lipid stability of meat. *Lipids Health Dis.* 12:164.
- Buddhan, S., R. Sivakumar, N. Dhandapani, B. Ganesan, and R. Anandan. 2007. Protective effect of dietary squalene supplementation on mitochondrial function in liver of aged rats. *Prostaglandins Leukot. Essent. Fatty Acids* 76:349–355.
- Carvalho, R. H., E. I. Ida, M. S. Madruga, S. L. Martínez, M. Shimokomaki, and M. Estévez. 2017. Underlying connections between the redox system imbalance, protein oxidation and impaired quality traits in pale, soft and exudative (PSE) poultry meat. *Food Chem.* 215:129–237.
- Chen, Y., Y. Cheng, C. Wen, Y. Kang, A. Wang, and Y. Zhou. 2018. Effects of dietary synbiotic supplementation as an alternative to antibiotic on the growth performance, carcass characteristics, meat quality, immunity, and oxidative status of Cherry Valley ducks. *J. Poult. Sci.* 55:182–189.
- Chen, Y. P., X. Chen, H. Zhang, and Y. M. Zhou. 2013. Effects of dietary concentrations of methionine on growth performance and oxidative status of broiler chickens with different hatching weight. *Br. Poult. Sci.* 54:531–537.
- Cheng, Y., Y. Chen, X. Li, W. Yang, C. Wen, Y. Kang, A. Wang, and Y. Zhou. 2017. Effects of synbiotic supplementation on growth performance, carcass characteristics, meat quality and muscular antioxidant capacity and mineral contents in broilers. *J. Sci. Food Agric.* 97:3699–3705.
- Cheng, Q., and D. W. Sun. 2008. Factors affecting the water holding capacity of red meat products: a review of recent research advances. *Crit. Rev. Food Sci. Nutr.* 48:137–159.
- Christensen, L. B. 2003. Drip loss sampling in porcine *m. longissimus dorsi*. *Meat Sci.* 63:469–477.
- Das, B., R. Antoon, R. Tsuchida, S. Lotfi, O. Morozova, W. Farhat, D. Malkin, G. Koren, H. Yeager, and S. Baruchel. 2008. Squalene selectively protects mouse bone marrow progenitors against cisplatin and carboplatin-induced cytotoxicity *in vivo* without protecting tumor growth. *Neoplasia* 10:1105–1119.
- Duclos, M. J., C. Berri, and E. L. Bihan-Duval. 2007. Muscle growth and meat quality. *J. Appl. Poult. Res.* 16:107–112.
- Estévez, E. 2015. Oxidative damage to poultry: from farm to fork. *Poult. Sci.* 94:1368–1378.
- Fox, C. B. 2009. Squalene emulsions for parenteral vaccine and drug delivery. *Molecules* 14:3286–3312.
- Gylling, H., and T. A. Miettinen. 1994. Post absorptive metabolism of dietary squalene. *Atherosclerosis* 106:169–178.
- Huff-Lonergan, E., and S. M. Lonergan. 2005. Mechanisms of water-holding capacity of meat: the role of postmortem biochemical and structural changes. *Meat Sci.* 71:194–204.
- Hughes, J. M., S. K. Oiseth, P. P. Purslow, and R. D. Warner. 2014. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. *Meat Sci.* 98:520–532.
- James, J. E., G. Piganeau, and A. Eyre-Walker. 2016. The rate of adaptive evolution in animal mitochondria. *Mol. Ecol.* 25:67–78.
- Jiang, J., and Y. L. Xiong. 2016. Natural antioxidants as food and feed additives to promote health benefits and quality of meat products: a review. *Meat Sci.* 120:107–117.
- Kim, Y. J., S. K. Jin, W. Y. Park, B. W. Kim, S. T. Joo, and H. S. Yang. 2010. The effect of garlic or onion marinade on the lipid oxidation and meat quality of pork during cold storage. *J. Food Qual.* 33:171–185.
- Kim, S. K., and F. Karadeniz. 2012. Biological importance and applications of squalene and squalane. *Adv. Food Nutr. Res.* 65:223–233.
- Kim, Y. H. B., R. D. Warner, and K. Rosenvold. 2014. Influence of high pre-rigor temperature and fast pH fall on muscle proteins and meat quality: a review. *Anim. Prod. Sci.* 54:375–395.
- Kohno, Y., Y. Egawa, S. Itoh, S. Nagaoka, M. Takahashi, and K. Mukai. 1995. Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in *n*-butanol. *Biochim. Biophys. Acta* 1256:52–56.
- Kono, Y. 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch. Biochem. Biophys.* 186:189–195.
- Kruger, N. J. 1994. The Bradford method for protein quantitation. *Methods Mol. Biol.* 32:9–15.
- Le Bihan-Duval, E., M. Debut, C. M. Berri, N. Sellier, V. Santé-Lhoutellier, Y. Jégo, and C. Beaumont. 2008. Chicken meat quality: genetic variability and relationship with growth and muscle characteristics. *BMC Genet.* 9:53.
- Li, S., Z. Liang, C. Wang, Y. Feng, X. Peng, and G. Yong. 2010. Improvement of reproduction performance in AA⁺ meat type male chicken by feeding with squalene. *J. Anim. Vet. Adv.* 9:486–490.
- Long, G. L., W. X. Hao, L. F. Bao, J. H. Li, Y. Zhang, and G. H. Li. 2019. Effects of dietary inclusion levels of palm oil on growth performance, antioxidative status and serum cytokines of broiler chickens. *J. Anim. Physiol. Anim. Nutr.* 103:1116–1124.
- Lou-Bonafonte, J. M., R. Martínez-Beamonte, T. Sanclemente, J. C. Surra, L. V. Herrera-Marcos, J. Sanchez-Marco, C. Arnal, and J. Osada. 2018. Current insights into the biological action of squalene. *Mol. Nutr. Food Res.* 62:1800136.
- Miettinen, T. A., and H. Vanhanen. 1994. Serum concentration and metabolism of cholesterol during rapeseed oil and squalene feeding. *Am. J. Clin. Nutr.* 59:356–363.
- Min, B., and D. U. Ahn. 2005. Mechanism of lipid peroxidation in meat and meat products—A review. *Food Sci. Biotechnol.* 14:152–163.
- Min, B., K. C. Nam, J. Cordray, and D. U. Ahn. 2008. Endogenous factors affecting oxidative stability of beef loin, pork loin, and chicken breast and thigh meats. *J. Food Sci.* 73:C439–C466.

- Motawi, T. M., N. A. Sadik, and A. Refaat. 2010. Cytoprotective effects of DL-alpha-lipoic acid or squalene on cyclophosphamide-induced oxidative injury: an experimental study on rat myocardium, testicles and urinary bladder. *Food Chem. Toxicol.* 48:2326–2336.
- NRC. 1994. *Nutrient Requirements of Poultry*. 9th rev. ed. National Academy Press, Washington, DC.
- Owens, C. W. I., and R. V. Belcher. 1965. A colorimetric micro-method for the determination of glutathione. *Biochem. J.* 94:705–711.
- Pisoschi, A. M., and A. Pop. 2015. The role of antioxidants in the chemistry of oxidative stress: a review. *Eur. J. Med. Chem.* 97:55–74.
- Placer, Z. A., L. L. Cushman, and B. C. Johnson. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. *Anal. Biochem.* 16:359–364.
- Qiao, M., D. L. Fletcher, D. P. Smith, and J. K. Northcutt. 2001. The effect of broiler breast meat color on pH, moisture, water-holding capacity, and emulsification capacity. *Poult. Sci.* 80:676–680.
- Reddy, L. H., and P. Couvreur. 2009. Squalene: a natural triterpene for use in disease management and therapy. *Adv. Drug Deliver. Rev.* 61:1412–1426.
- Relas, H., H. Gylling, and T. A. Miettinen. 2001. Fate of intravenously administered squalene and plant sterols in human subjects. *J. Lipid Res.* 42:988–994.
- Sabeena Farvin, K. H., R. Anandan, S. Hari Senthil Kumar, K. S. Shiny, T. V. Sankar, and T. K. Thankappan. 2004. Effect of squalene on tissue defense system in isoproterenol-induced myocardial infarction in rats. *Pharmacol. Res.* 50:231–236.
- Sabeena Farvin, K. H., S. Hari Senthil Kumar, R. Anandan, S. Mathew, T. V. Sankar, and P. G. Viswanathan Nair. 2007. Supplementation of squalene attenuates experimentally induced myocardial infarction in rats. *Food Chem.* 105:1390–1395.
- Sánchez-Fidalgo, S., I. Villegas, M. Á. Rosillo, M. Aparicio-Soto, and C. A. de la Lastra. 2015. Dietary squalene supplementation improves DSS-induced acute colitis by downregulating p38 MAPK and NFκB signaling pathways. *Mol. Nutr. Food Res.* 59:284–292.
- Seifried, H. E., D. E. Anderson, E. I. Fisher, and J. A. Milner. 2007. A review of the interaction among dietary antioxidants and reactive oxygen species. *J. Nutr. Biochem.* 18:567–579.
- Senthilkumar, S., S. K. Yogeeeta, R. Subashini, and T. Devaki. 2006. Attenuation of cyclophosphamide induced toxicity by squalene in experimental rats. *Chem. Biol. Interact.* 160:252–260.
- Sihvo, H. K., K. Immonen, and E. Puolanne. 2013. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle of broilers. *Vet. Pathol.* 51:619–623.
- Spanova, M., and G. Daum. 2011. Squalene—biochemistry, molecular biology, process biotechnology, and applications. *Eur. J. Lipid Sci. Technol.* 113:1299–1320.
- SPSS. 2008. SPSS 16. 0 for Windows. SPSS Inc., Chicago, IL.
- Strandberg, T. E., R. S. Tilvis, and T. A. Miettinen. 1990. Metabolic variables of cholesterol during squalene feeding in humans: comparison with cholestyramine treatment. *J. Lipid Res.* 21:1637–1643.
- Tegenge, M. A., L. S. Von Tungeln, R. J. Mitkus, S. A. Anderson, M. M. Vanlandingham, R. A. Forshee, and F. A. Beland. 2016. Pharmacokinetics and biodistribution of squalene-containing emulsion adjuvant following intramuscular injection of H5N1 influenza vaccine in mice. *Regul. Toxicol. Pharmacol.* 81:113–119.
- Tilvis, R. S., and T. A. Miettinen. 1983. Dietary squalene increases tissue sterols and fecal bile acids in the rat. *Lipids* 18:32–36.
- Wang, S. T., H. W. Chen, L. Y. Sheen, and C. K. Lii. 1997. Methionine and cysteine affect glutathione level, glutathione-related enzyme activities and the expression of glutathione-S-transferase iso-enzymes in rat hepatocytes. *J. Nutr.* 127:2135–2141.
- Wang, X. Q., X. Chen, H. Z. Tan, D. X. Zhang, H. J. Zhang, S. Wei, and H. C. Yan. 2013. Nutrient density and slaughter age have differential effects on carcass performance, muscle and meat quality in fast and slow growing broiler genotypes. *Br. Poult. Sci.* 54:50–61.
- Wang, Z. G., X. J. Pan, Z. Q. Peng, R. Q. Zhao, and G. H. Zhou. 2009. Methionine and selenium yeast supplementation of the maternal diets affects color, water-holding capacity, and oxidative stability of their male offspring meat at the early stage. *Poult. Sci.* 88:1096–1101.
- Warleta, F., M. Campos, Y. Allouche, C. Sánchez-Quesada, J. Ruiz-Mora, G. Beltrán, and J. J. Gaforio. 2010. Squalene protects against oxidative DNA damage in MCF10A human mammary epithelial cells but not in MCF7 and MDA-MB-231 human breast cancer cells. *Food Chem. Toxicol.* 48:1092–1100.
- Xiao, S., W. G. Zhang, E. J. Lee, C. W. Ma, and D. U. Ahn. 2011. Effects of diet, packaging, and irradiation on protein oxidation, lipid oxidation, and color of raw broiler thigh meat during refrigerated storage. *Poult. Sci.* 90:1348–1357.
- Zhang, C., L. Wang, X. H. Zhao, X. Y. Chen, L. Yang, and Z. Y. Geng. 2017. Dietary resveratrol supplementation prevents transport-stress-impaired meat quality of broilers through maintaining muscle energy metabolism and antioxidant status. *Poult. Sci.* 96:2219–2225.
- Zhang, Z. F., T. X. Zhou, and I. H. Kim. 2013. Effects of dietary olive oil on growth performance, carcass parameters, serum characteristics, and fatty acid composition of breast and drumstick meat in broilers. *Asian-Australas. J. Anim. Sci.* 26:416–422.