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Effects of dietary lycopene supplementation on serum biochemistry, meat quality and oxidative stability of breast muscle in broilers

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ABSTRACT. Poultry meat is prone to oxidation and spoilage due to its high content of unsaturated fatty acids. Lycopene is a natural antioxidant belonging to the carotenoid family with strong free radical quenching properties. The present study evaluated the effects of varying dietary levels of lycopene supplementation on serum biochemistry, meat quality and oxidative stability of broiler breast muscles. A total of 256 male one-day-old Arbor Acres broilers were randomly allocated to four groups (eight replicates per group with eight birds per replicate) and fed a basal diet supplemented with 0 (control), 100, 200, and 400 mg/kg lycopene, respectively. The final growth performance of broilers supplemented with lycopene was not significantly different compared to the control group. The inclusion of dietary lycopene improved meat quality of broiler breast muscle by increasing the *a** value and decreasing the *L** value, as well as improving the pH value 24 h after slaughter, decreasing cooking loss and shearing force. Additionally, lycopene supplementation increased the activity of glutathione peroxidase and hydroxyl radical scavenging capacity in the breast muscle. Broilers fed a diet with lycopene showed reduced hydrogen peroxide and malonaldehyde content in the breast muscle 45 min and 24 h post-mortem. Furthermore, lycopene increased mRNA expression of nuclear factor erythroid 2-related factor 2 (*NRF2*) and its downstream antioxidant factor in the breast muscle. In conclusion, dietary lycopene supplementation enhanced the antioxidant capacity of broilers, thereby improving meat quality. These regulatory effects were likely mediated through the *NRF2* signalling pathway.

Introduction

With increasing concerns for the safety of animal products, there is a growing trend to prohibit the use of antibiotics and certain drug feed additives. This shift has sparked global interest in the beneficial effects of botanical additives on animal health and production. Over the past two decades, phytochemicals have been widely reported to promote growth, modulate metabolism, and exhibit anti-inflammatory, anti-microbial, and particularly antioxidative properties in animal production (Kikusato, 2021). Poultry, especially broilers, is also an important source of animal-derived food; however, its meat is susceptible to oxidation and spoilage due to its high unsaturated fatty acid content (Grashorn, 2007). Feed manipulation using phytogenic bioactive substances has been demonstrated to support growth and health of broilers (Wan et al., 2017; Ahmadipour et al., 2021; Kikusato, 2021).

Lycopene, a natural antioxidant belonging to the carotenoid family found in many red-coloured vegetables and fruits, is recognized for its excellent antioxidant capacity and associated health benefits (Khalaf and Awad, 2023). Research has demonstrated that dietary lycopene supplementation can improve meat quality and oxidative stability in pigs and lambs (An et al., 2019; Xu et al., 2019; Wen et al., 2022), as well as enhance meat nutritive value in rabbits (Czauderna et al., 2021). However, studies investigating the effects of lycopene in broilers are limited and provide inconsistent results. Lycopene has been shown to improve the production and reproductive performance, increase immunity and antioxidant capacity, as well as alleviate heat stress and mycotoxin damage in poultry (Sun et al., 2015; Sahin et al., 2016; Sarker et al., 2021; Wan et al., 2021). However, Pozzo et al. (2013) reported that dietary lycopene supplementation in broilers led to a decrease in the weight of the spleen and bursa of Fabricius, the development of degenerative lesions, reduced serum protein, albumin, α and γ globulin concentrations, and had no effect on growth, slaughter and antioxidant performance. These discrepancies might be related to the source, dosage, and purity of lycopene. In addition, the effect of dietary lycopene supplementation on meat quality of broilers remains unclear. Therefore, to provide a reference for the application of lycopene and lycopene-containing plant material in poultry production, the present study investigated the effects of different levels of lycopene dietary supplementation on growth performance, serum biochemistry, meat quality and oxidative stability of breast muscle in broilers.

Material and methods

Experimental design and animal treatment

All animal research procedures in the present study were approved by the Animal Care and Welfare Committee of the Yangzhou University (Permission No. SYXK (Su) 2016-0020) (Yangzhou, China), and complied with the Regulations for the Administration of Affairs Concerning Experimental Animals of the People's Republic of China.

A total of 256 male one-day-old Arbor Acres broilers were randomly assigned to four groups (eight replicates per group, eight birds per replicate). The birds were fed basal diet supplemented with 0 (control), 100, 200, or 400 mg/kg lycopene, respectively. All birds were reared in an environmentally controlled room with free access to feed and water from 1 to 42 days of age. The temperature was maintained at 32–34 °C for the first 3 days, and gradually reduced by 2–3 °C per week until 22 ± 1 °C, with a light cycle of 23 h light/1 h dark. The basal diet was formulated as in our previous study to meet the nutritional requirements of Arbor Acres broilers (Wan et al., 2021). Lycopene (purity ≥80%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Dietary supplementation with lycopene had no significant effect on the final growth performance of broilers.

Sample collection

At the end of the experiment, one bird with an average body weight from each replicate (eight birds per group) was selected for sampling. Blood samples were collected from the wing vein and centrifuged at 3 000 × *g* for 10 min at 4 ℃ to obtain serum, which was subsequently stored at −20 ℃ for further analysis. After blood collection, the broilers were slaughtered, and the left breast muscle samples were collected. Part of the breast muscle samples were snap frozen in liquid nitrogen, and then stored at −80 ℃ for further analysis. The remaining breast muscle tissue was stored at 4 ℃ for meat quality assessment.

Serum biochemical parameters

Serum concentrations of total protein (TP), albumin (ALB), globulin (GLO), uric acid (UA), and glucose (GLU) were determined using a UniCel Synchron DxC-800 fully automatic biochemical analysis system (Beckman Coulter, Los Angeles, CA, USA).

Meat quality

Meat colour was determined using a colorimeter (Konica Minolta CR-400, Osaka, Japan) 45 min and 24 h post-mortem, based on the CIELab classification system: L^* (lightness), a^* (redness), and b^* (yellowness). A portable pH meter (pH-STAR, Matthaus, Berlin, Germany) was used to measure the pH value of the breast muscle 45 min and 24 h after slaughter. Cooking loss was assessed according to Wan et al. (2018) and expressed as the percentage of weight loss relative to the initial weight. Shearing force was measured using a C-LM3B digital tenderness meter (Tenovo, Beijing, China).

Oxidative stability

The commercial kits of the Nanjing Jiancheng Bioengineering Institute (Nanjing, China) were used to measure protein concentration, antioxidant

enzyme activity, free radical scavenging capacity and oxidative damage in breast muscles. Protein concentration was determined using the bicinchoninic acid (BCA) assay.

The activity of antioxidant enzymes, including total superoxide dismutase (T-SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), were measured using colorimetric assays following the procedures provided by the manufacturers of the commercial kits. The results were calculated based on the protein concentration in the breast muscle and expressed as units per mg of protein (U/mg protein). The free radical scavenging capacity of hydroxyl radical ($OH^{\bullet}$) and superoxide radical ($O_2^{-\bullet}$) was measured based on the protein content in the breast muscle, and expressed as units per milligram of protein for $OH^{\bullet}$ (U/mg protein) and units per gram of protein for $O_2^{-\bullet}$ (U/g protein).

The concentration of hydrogen peroxide (H_2O_2) was measured spectrophotometrically to determine the level of reactive oxygen metabolites in the breast muscle 45 min and 24 h post-mortem (storage at 4 °C). Results were expressed as nanogram per gram of protein (ng/g protein). Malondialdehyde (MDA) levels were evaluated using thiobarbituric acid reactive substances, with measurements taken spectrophotometrically at 45 min and 24 h post-mortem (storage at 4 °C), and the result was expressed as nanomoles per milligram of protein (nmol/mg protein).

Relative mRNA expression levels

Total RNA was isolated from breast muscle samples using the Uni-10 Column Trizol Total RNA Extraction Kit (Sangon Biotech Co. Ltd., Shanghai, China) according to the manufacturer's protocol. RNA quality and quantity were assessed by agarose gel electrophoresis and absorbance measurements using a NanoDrop 2000c microspectrophotometer (Thermo Scientific, Waltham, MA, USA). Total RNA was then reverse-transcribed into cDNA using the Prime Script™ RT Master Mix kit (TaKaRa Biotechnology Co. Ltd., Dalian, China). The primer sequences are listed in Table 1. Real-time PCR analysis was conducted using a 7500 Real-Time PCR System (Applied Biosystems, USA), and the TB Green™ *Premix Ex* Taq™ II Kit (TaKaRa), following the manufacturer's guidelines. Relative mRNA levels of target genes were calculated using the $2^{-\Delta\Delta Ct}$, with beta-actin (ACTB) as a housekeeping gene. Results were reported as fold changes relative to the control group.

Table 1. Primer sequences for real-time PCR

Accession ID	Gene name	Primer sequence 5'-3'	Product length, bp
NM_205518	*ACTB*	F: TGATATTGCTGCGCTCGTTG R: ATACCTCTTTTGCTCTGGGCTT	183
NM_205117	*NRF2*	F: CACCAAAGAAAGACCCTCCTG R: CACTGAACTGCTCCTTCGACAT	201
XM_025145847	*KEAP1*	F: CATCGGCATCGCCAACTT R: GCAGTGGGACAGGTTGAAGAA	127
NM_001277621	*NQO1*	F: AACCCCGAGTGCTTTGTCTA R: TCAGGTCAGCCGCTTCAA	109
NM_205344	*HMOX1*	F: ACGAGCAGGCGGAGAACA R: CATCGGAAAATAAACAGGAGCA	170
NM_205064	*SOD1*	F: AAGGGAGGAGTGGCAGAAGT R: TTTCAGGTACAACGGTTAG-CACT	210
NM_204211.1	*SOD2*	F: CACTCTTCCTGACCTGCCTTAC R: CACCTGAGCTGTAACAT-CACCTT	169
NM_001031215	*CAT*	F: TCTTGAGTCTTCGCCCTGAG R: TGATCGGTCTTAACGTGGAAC	166
NM_001277854	*GPX2*	F: GTTCCAGAAGTGCCAGGTGA R: CTGTAGCGGCGGAAAGGT	207

ACTB – beta-actin; *NRF2* – nuclear factor erythroid 2-related factor 2; *KEAP1* – kelch-like ECH-associated protein 1; *NQO1* – NAD(P)H quinone dehydrogenase 1; *HMOX1* – heme oxygenase 1; *SOD1* – superoxide dismutase 1; *SOD2* – superoxide dismutase 2; *CAT* – catalase; *GPX2* – glutathione peroxidase 2

Statistical analysis

Data analysis was conducted using SPSS statistical software (version 22.0, SPSS Inc, Chicago, IL). One-way ANOVA was employed to assess the differences among treatment groups, and post-hoc comparisons were performed using Tukey's test. Statistical significance was determined at $P < 0.05$.

Results

Serum biochemistry

The effects of different levels of lycopene inclusion on the serum biochemistry of broilers are presented in Table 2. Lycopene supplementation did not significantly alter broiler serum of TP, ALB, GLO, UA, and GLU levels in broilers ($P > 0.05$).

Meat quality

The effects of various dietary lycopene supplementation levels on broiler meat quality are presented in Table 3. Dietary lycopene supplementation at 400 mg/kg increased the a^* value 45 min

Table 2. Effects of different levels of dietary lycopene supplementation on serum biochemistry of broilers

Items	Control	Lycopene addition level, mg/kg			SEM	*P*-value
		100	200	400		
TP, g/l	34.06	34.35	33.66	35.38	0.521	0.707
ALB, g/l	10.09	9.94	10.44	10.70	0.158	0.323
GLO, g/l	23.98	24.41	23.23	24.68	0.448	0.702
UA, µmol/l	128.88	131.88	134.25	127.13	3.963	0.931
GLU, mmol/l	12.40	11.38	12.53	11.99	0.316	0.595

TP – total protein, ALB – albumin, GLO – globulin, UA – uric acid, GLU – glucose; SEM – standard error of the mean; $P > 0.05$ indicates that the data are not significantly different; n = 8

Table 3. Effects of different levels of dietary lycopene supplementation on the quality of breast muscle meat in broilers

Items	Control	Lycopene addition level, mg/kg			SEM	*P*-value
		100	200	400		
L^*_{45min}	45.71	46.26	44.68	47.33	0.615	0.509
a^*_{45min}	1.80^b	1.87^b	2.03^{ab}	2.47^a	0.082	0.010
b^*_{45min}	6.36	6.69	6.06	6.34	0.114	0.286
L^*_{24h}	57.38^a	55.14^{ab}	56.72^{ab}	53.83^b	0.462	0.021
a^*_{24h}	1.40^b	1.60^{ab}	1.72^a	1.65^{ab}	0.042	0.045
b^*_{24h}	8.24	8.69	8.88	8.58	0.250	0.848
pH_{45min}	6.41	6.32	6.49	6.38	0.033	0.334
pH_{24h}	5.73^b	5.81^{ab}	5.88^a	5.91^a	0.019	0.002
Cooking loss, %	22.47^a	20.26^{ab}	19.44^b	19.73^{ab}	0.429	0.044
Shearing force, N	53.41^a	46.00^b	45.79^b	45.18^b	1.013	0.006

L^* – lightness, a^* – redness, b^* – yellowness; SEM – standard error of the mean; ^{ab} means within a row with different superscripts are significantly different at $P < 0.05$; n = 8

Table 4. Effects of different levels of dietary lycopene supplementation on the antioxidant capacity of breast muscle in broilers

Items	Control	Lycopene addition level, mg/kg			SEM	*P*-value
		100	200	400		
T-SOD, U/mg protein	26.87	25.77	25.37	25.04	0.533	0.660
CAT, U/mg protein	13.72	12.31	12.58	12.03	0.447	0.583
GSH-Px, U/mg protein	113.04^b	129.73^a	131.98^a	126.31^{ab}	2.436	0.020
$OH^{\cdot}$, U/g protein	39.57^b	38.83^b	44.18^{ab}	47.94^a	1.197	0.015
$O_2^{-\cdot}$, U/g protein	6.10	6.13	6.50	6.46	0.186	0.830

T-SOD – total superoxide dismutase, CAT – catalase, GSH-Px – glutathione peroxidase, $OH^{\cdot}$ – scavenging capacity of hydroxyl radical, $O_2^{-\cdot}$ – scavenging capacity of superoxide radical; SEM – standard error of the mean; ^{ab} means within a row with different superscripts are significantly different at $P < 0.05$; n = 8

Table 5. Effects of different levels of dietary lycopene supplementation on oxidative damage to breast muscle in broilers

Items	Control	Lycopene addition level, mg/kg			SEM	*P*-value
		100	200	400		
$H_2O_{2\ 45min}$, ng/g protein	13.25^a	10.12^b	10.78^b	11.48^b	0.300	<0.001
$H_2O_{2\ 24h}$, ng/g protein	15.91^a	11.62^b	12.22^b	12.53^b	0.399	<0.001
MDA_{45min}, nmol/mg protein	4.44^a	3.91^{ab}	3.84^b	3.96^{ab}	0.083	0.032
MDA_{24h}, nmol/mg protein	5.32^a	4.39^b	4.06^b	4.36^b	0.116	<0.001

H_2O_2 – hydrogen peroxide, MDA – malonaldehyde; SEM – standard error of the mean; ^{ab} means within a row with different superscripts are significantly different at $P < 0.05$; n = 8

after slaughter and decreased the L^* value 24 h after slaughter. Additionally, a dose of 200 mg/kg lycopene inclusion significantly increased the a^* value 24 h post-mortem ($P < 0.05$). Broilers receiving 200 and 400 mg/kg lycopene had significantly higher pH values in breast muscle 24 h after slaughter compared to the control group ($P < 0.05$). In addition, a 200 mg/kg lycopene supplementation to the diet reduced cooking loss, and doses of 100, 200 and 400 mg/kg lowered the shearing force of breast muscle ($P < 0.05$).

Oxidative stability

Tables 4 and 5 show the oxidative stability of breast muscle, including antioxidant capacity and oxidative damage. Compared to the control group, supplementation with 100 and 200 mg/kg of lycopene significantly increased GSH-Px activity, while 400 mg/kg lycopene enhanced the scavenging capacity of $OH^{\cdot}$ ($P < 0.05$). Additionally, H_2O_2 concentrations both 45 min and 24 h post-mortem, as well as MDA concentrations 24 h post-mortem, were significantly lower in the lycopene-supplemented groups compared to the control group ($P < 0.05$). MDA levels 45 min post-mortem were significantly reduced in the 200 mg/kg lycopene group compared to the control group ($P < 0.05$).

Relative mRNA expression levels

As illustrated in Figure 1, supplementing 100–400 mg/kg of lycopene to the broiler diet upregulated the relative mRNA expression level of nuclear factor erythroid 2-related factor 2 (*NRF2*) ($P < 0.05$). Additionally, broilers fed a diet with 200 and 400 mg/kg lycopene showed a significant decrease in mRNA levels of kelch-like ECH-associated protein 1 (*KEAP1*) and an increased mRNA levels of NAD(P)H quinone dehydrogenase 1 (*NQO1*) and glutathione peroxidase 2 (*GPX2*) ($P < 0.05$). Moreover, the relative mRNA expression level of superoxide dismutase 2 (*SOD2*) was significantly higher in the 200 mg/kg lycopene group compared to the control group ($P < 0.05$).

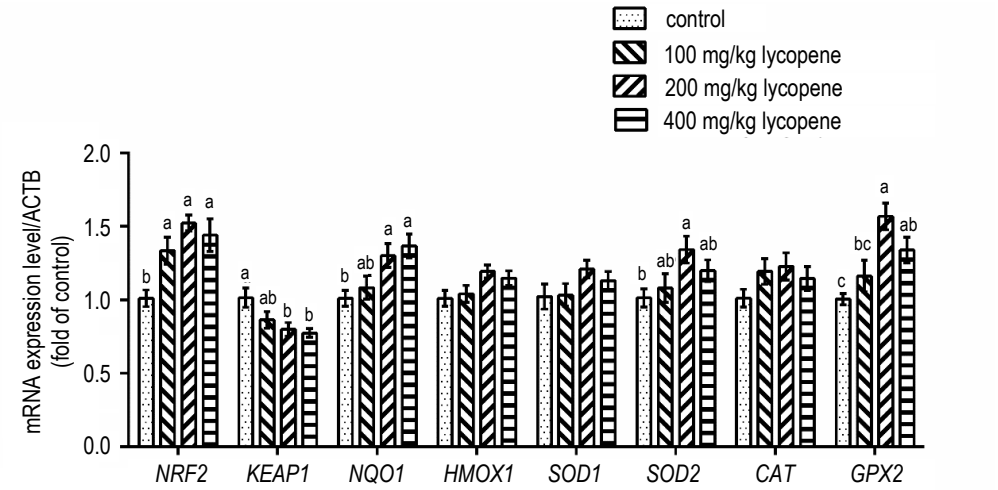

Figure 1. Effect of different levels of dietary lycopene supplementation on relative mRNA expression levels of antioxidant-related genes in the breast muscle of broilers

ACTB – beta-actin, *NRF2* – nuclear factor erythroid 2-related factor 2, *KEAP1* – kelch-like ECH-associated protein 1, *NQO1* – NAD(P)H quinone dehydrogenase 1, *HMOX1* – heme oxygenase 1, *SOD1* – superoxide dismutase 1, *SOD2* – superoxide dismutase 2, *CAT* – catalase, *GPX2* – glutathione peroxidase 2. Data are presented as mean ± standard error of the mean (n = 8). Bars with different letters differ significantly at $P < 0.05$

Discussion

Meat quality indicators influence consumers' purchasing decisions. A post-slaughter stress causes a shift from aerobic to anaerobic respiration in muscle tissue, leading to the continuous generation of free radicals. These free radicals oxidise bright red myoglobin into dark brown metmyoglobin, causing a decrease in the *a** value (Faustman et al., 2010). The oxidation of myoglobin typically results in an increase in the *L** value and a decrease in the *a** value. Antioxidants play a crucial role in enhancing the activity of antioxidant enzymes, which scavenge free radicals in muscle, thereby inhibiting myoglobin oxidation, reducing the *L** value and increasing the *a** value of muscle. Glycolysis leads to the accumulation of lactic acid in animal muscles after slaughter, causing a decrease in pH. A low pH value weakens the binding force between heme and globin in myoglobin, thus accelerating myoglobin oxidation (Ylä-Ajos and Puolanne, 2007). Antioxidant can mitigate the leakage of Ca^{2+} into the sarcoplasm from mitochondria and the sarcoplasmic reticulum by stabilising the cell membrane, which in turn inhibits glycolysis and slows the post-mortem pH decline (den Hertog-Meischke et al., 1997). This stabilisation likely explains the higher pH observed 24 h post-mortem in the lycopene supplementation groups compared to the control group in the present study. The elevated pH value helps to prevent myoglobin oxidation, resulting in improved meat colour. Additionally, meat pH is also an essential factor for water-holding capacity and tenderness (Maribo et al., 1998). Broilers in the lycopene supplementation groups showed reduced cooking loss and shearing force in the breast muscle, suggesting an increase in water-holding capacity and meat tenderness. Therefore, the current results indicate that dietary lycopene supplementation can enhance meat quality in broilers.

Antioxidant enzymes are an important part of the antioxidant defence system. *NRF2* is a key transcription factor within this system, playing a pivotal role in mitigating stress by regulating the expression of genes such as antioxidant and detoxification enzymes or anti-inflammatory factors. Under normal, non-induced conditions, *NRF2* is bound to *KEAP1*, forming an inactive complex. KEAP1 has 5 active cysteine residues that can react with inducers. When an inducer interacts with any of the cysteine sulfhydryl groups, *NRF2* is released from KEAP1. Subsequently, *NRF2* translocates into the nucleus, where it binds to the antioxidant response element, initiating the expression of target genes coding for detoxification and antioxidant enzymes (Niture et al., 2010; Pall and Levine, 2015). Free radicals produced in the body can lead to oxidative stress when their levels become imbalanced, potentially causing harm to overall health. Among these, the hydroxyl radical ($OH^{\bullet}$) is particularly reactive and abundant. Lycopene can effectively scavenge $OH^{\bullet}$ through an addition reaction (Prasad and Mishra, 2014). H_2O_2

is a major redox metabolite, and high concentrations of H_2O_2, another significant redox metabolite, can induce oxidative damage to biomolecules when present in high concentrations (Sies, 2017). MDA is a marker of lipid peroxidation, also indicating oxidative stress in tissues. In the present study, dietary lycopene addition increased GSH-Px activity and $OH^{\bullet}$ scavenging capacity, decreased H_2O_2 and MDA levels in the breast muscle 45 min and 24 h after slaughter. Furthermore, lycopene supplementation upregulated mRNA expression levels of *NRF2* and antioxidant enzymes, and downregulated *KEAP1* transcription. These effects align with findings from previous studies. For instance, Xu et al. (2019) demonstrated that lycopene reduced the oxidation of meat proteins and lipids, thereby improving the quality and shelf life of lamb meat. Similarly, An et al. (2019) and Wen et al. (2022) reported that lycopene enhanced oxidative stability in meat and positively influenced the *NRF2*-regulated antioxidant signalling pathway in pigs. Taken together, our findings indicate that dietary lycopene supplementation significantly improves the antioxidant capacity in broilers, which is beneficial for the quality and shelf life of broiler meat.

Conclusions

This study demonstrated that the inclusion of lycopene in the diet could improve meat quality and oxidative stability in broilers, with the regulatory effect likely linked to the nuclear factor erythroid 2-related factor 2 (*NRF2*) signalling pathway. These findings provide a reference for the application of lycopene and lycopene-containing plant materials in poultry production. Based on the results, a supplemental level of 100–200 mg/kg of lycopene in broiler diets is recommended.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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