Veterinary and Animal Science 26 (2024) 100398

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

The influence of Quercetin on behavior, performance and splenic immunity in broiler chickens

Sara Elsayed El-Kazaz [a], Mona Hafez Hafez [b,*], Ghadeer M. Albadrani [c], Muath Q. Al-Ghadi [d], Mohamed M. Abdel-Daim [e,f], Yasser Said El-Sayed [g]

[a] Animal Husbandry and Animal Wealth Development Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria, 22758, Egypt
[b] Physiology Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria, 22758, Egypt
[c] Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, 84428, Riyadh 11671, Saudi Arabia
[d] Department of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[e] Department of Pharmaceutical Sciences, Pharmacy Program, Batterjee Medical College, P.O. Box 6231 Jeddah 21442, Saudi Arabia
[f] Pharmacology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt
[g] Forensic Medicine and Toxicology Department, Faculty of Veterinary Medicine, Damanhour University, Damanhour, Egypt

ARTICLE INFO

Keywords:
Behavior and productive performance
Broiler chicken
Gene expression
Immune function
Oxidative stress
Quercetin

ABSTRACT

Quercetin (QRT), a potent flavonoid, holds immense mechanistic potential in enhancing various aspects of broiler chicken physiological status. This comprehensive study explores the profound effects of QRT on behavior, productive performance, and immune functions, unraveling the underlying mechanisms behind its efficacy. Four hundred, one-day-old Cobb 500 chicks were placed randomly into 4 supplementation groups (100 birds /group and five replicates) and provided diets enriched with varying concentrations of QRT (0, 200, 400, and 600 ppm) for six weeks. Visual scanning revealed significant ($P > 0.05$) improvements in feeding and body care behaviors, accompanied by reduced instances of idleness and walking in the QRT-supplemented groups. Moreover, QRT supplementation exerted a substantial ($P > 0.05$) positive influence on weight gain, feed intake and the final body weight of the broilers. In-depth evaluation of immune parameters, QRT supplementation elicited significant ($P > 0.05$) enhancements in immune functions, including improved spleen, thymus, and bursa indices, enhanced secretion of immunoglobulin M (IgM) and immunoglobulin A (IgA), and stimulated humoral immunity against sheep red blood cells (SRBCs). Furthermore, QRT displays potent antioxidant properties, as showed by diminished splenic malondialdehyde levels and augmented activity of antioxidative stress enzymes. Remarkably, QRT supplementation elicited dose-dependent upregulation ($P < 0.001$) of key immune-related genes, such as interleukin-4 (IL-4), interferon-γ (INF-γ), Toll-like receptor 2 (TLR2), and tumor necrosis factor-α (TNF-α) in the splenic tissue. Collectively, these mechanistic insights underscore the profound impact of QRT as a functional feed additive, fostering enhanced behavior, performance, and immune function in broiler chickens, while delivering robust antioxidant fortification.

1. Introduction

In broiler production systems, chickens experience a wide range of stresses that negatively affect their physiological status, behavioral patterns, welfare conditions, and adaptability to the environment. These stresses also lead to immunological changes, decreasing performance, disrupting immune homeostasis, and suppressing immune-related gene expression (Sierzant, Korzeniowska, Polbrat, Rybarczyk, & Smolinski, 2022; Yang et al., 2020), thereby impacting the economic benefits of commercial chicken production (Broom & Kogut, 2019; Zahoor, Ghayas & Basheer, 2018). The complex immune system, comprising immune organs, cells, and molecules, plays a vital role in defending against harmful substances (Kishawy, Amer, Abd El-Hack, Saadeldin & Swelum, 2019; Mchunu, Mthana & Mthiyane, 2024). Immunosuppressive disorders, caused by factors like nutrient deficiencies, viruses, parasites, bacteria, and toxins, compromise both cellular and humoral immune responses, damaging immune organs plus reducing normal immune functions (Sarrigeorgiou et al., 2023; Selim, Abdel-Megeid, Abou-Elnaga

* Corresponding author at: Physiology Department, Faculty of Veterinary Medicine, Alexandria University, Alexandria, 22758, Egypt.
E-mail addresses: saraelkazaz@alexu.edu.eg (S.E. El-Kazaz), Mona.hafez@alexu.edu.eg (M.H. Hafez), gmalbadrani@pnu.edu.sa (G.M. Albadrani), malghadi@ksu.edu.sa (M.Q. Al-Ghadi), abdeldaim.m@vet.suez.edu.eg (M.M. Abdel-Daim), elsayed-ys@vetmed.dmu.edu.eg (Y.S. El-Sayed).

https://doi.org/10.1016/j.vas.2024.100398

Available online 18 September 2024

& Mahmoud, 2021; Yang et al., 2020). Environmental stressors in poultry production induce oxidative stress, negatively affecting bird welfare and performance (Mchunu et al., 2024).

Flavanols, including naturally occurring polyphenols such as flavonoids, possess a range of beneficial properties in monogastric animals, including regulating feed intake and exhibition of antimicrobial, immunomodulatory, antioxidant, and anti-inflammatory effects (Wenk, 2003). These flavonoids serve as attractive candidates for antioxidative feed additives due to their natural origin and safer profile compared to synthetic alternatives (Serra, Salvatori & Pastorelli, 2021). Quercetin (QRT; 3,30,40,5,7-pentahydroxyflavone), a powerful bioflavonoid found in fruits (grapes, apples, berries), vegetables (onions, broccoli), and herbs, possesses diverse beneficial characteristics, which are anti-inflammatory, antioxidant, anti-obesity, antimicrobial, anti--hypercholesterolemic, anticancer and antiaging effects (Anand David, Arulmoli & Parasuraman, 2016). It is recognized for its stable chemical structure, water solubility, and multiple beneficial impacts, counting antioxidative, antibacterial, anti-inflammation properties, free radical scavenging, and immunity functions enhancement (Dang et al., 2022; Fernández-Quintela et al., 2019). Its primary effect is attributed to its antioxidative action, making it a commonly used dietary supplement for metabolic and inflammatory diseases (Lakhanpal & Rai, 2007). Additionally, QRT modulates gut microbiota by improving the beneficial bacteria like *Lactobacilli* while decreasing harmful bacteria such as *C. perfringens* and total coliform counts, thus positively impacting the performance and the general health status of poultry (Abdel-Latif et al., 2021; Liu et al., 2014). Former experiments have revealed that QRT administration improves performance in laying hens and has been related with the regulation of the intestinal functions and the superoxide dismutase concentration in the liver (Liu et al., 2014). While the exact immune modulatory impact of dietary QRT in chickens remains unclear, animal and in vitro researches have established the ability of flavonoids, including QRT, to relieve inflammation by regulating the secretion of anti-inflammatory and pro-inflammatory cytokines in the cells of adaptive and innate immune system, such as T cells and macrophages, leading to a reduction in the inflammatory immune function to protect against the stimuli that triggered the inflammatory processes (Dang et al., 2022; Kishawy et al., 2019; Sierzant et al., 2022; Yang et al., 2020).

The physiological status, environmental adaptation, welfare and behavioural patterns of broiler chickens are affected by various stressors. These stressors lead to immunological changes, decreased performance, disrupted oxidative homeostasis, and suppressed immune-related gene expression. There is a need for natural, safe feed additives that can mitigate these negative effects and improve broiler health, performance, and immune function. This study aims to investigate the potential of quercetin, a natural flavonoid, as a functional feed additive to address these challenges in broiler production on the birds immune organs, growth performance, behaviors, oxidative-antioxidant balance parameters, and the mRNA expression of immune-related gene to gain insights into the immunomodulatory properties and mechanisms of action of dietary QRT.

2. Materials and methods

2.1. Experimental setup and diet formulation for quercetin supplementation

In the current experiment, a total of 400 unsexed one-day-old broiler chicks (Cobb 500) were used, with 100 birds allocated to each group and five replicates. The chicks were obtained from a commercial hatchery and were distributed to different experimental groups based on their weight to reduce variations in mean body weight. The flooring consisted of cement covered with a 7 cm depth of a fresh straw litter, supplying a suitable environment for broilers (8 birds/m^2 throughout the whole experiment). They were given ad libitum access to water and feed (Manual systems). The diets, prepared to accommodate the nutrient requirements for poultry outlined by National Research Council (NRC, 1994), were provided in different forms throughout the study period. The chicks were initially fed starter crumbs for two weeks, then a grower pellets for a week, and lastly finisher pellets till end of the research. Temperature in pens was carefully controlled, starting at 32 °C for the first 7 days and gradually reduced by 3 °C every week till reaching 24 °C at end of the experiment. Relative humidity for chicken was set between 60 and 65 %. Artificial light was used for 23 h daily throughout the entire experiment period with an Automated-Tunnel ventilation system. Regular health inspections were conducted daily to check the well-being of the chickens. Throughout the study, every effort was made to ensure that the broilers experienced minimal distress or pain. The broilers were housed in crushed litter pens with a complete organized environmental system.

For experimental groups, the birds were divided into 4 categories (100 birds /group and five replicates): control group 1, group 2 (QRT200), group 3 (QRT400), and group 4 (QRT600) (Yang et al., 2020). Control birds was given a commercial basal diet, whilst the remaining groups were fed the same basal diet administered with varying concentrations of QRT: 200 ppm, 400 ppm, and 600 ppm, respectively. Quercetin dihydrate powder with clarity of 97 % was obtained from Sigma-Aldrich Company (St. Louis, MO) and mixed with the basal diet. The basal diets, composed of a corn-soybean-based formulation, were prepared in accordance with nutrient recommendations specified for broilers (Cobb 500) by the NRC (1994). Nutrient content of ingredients was according to the guidelines provided by the Association of Official Analytical Chemists (AOAC, 2005). Detailed information on the percentage composition and evaluated nutrient analysis of basal diet can be found in Table 1.

Table 1
Composition of experimental starter, grower and finisher diets (g/kg diet) and calculated chemical analysis of the basal diet.

Ingredients	Diet		
	Starter	Grower	Finisher
Yellow corn	542	558.8	606
Soybean meal (44 %)	319	281	253.3
Corn gluten meal (60 %)	71	81	48.1
Vegetable oil	29.8	41	54.4
Limestone[1]	15	15	15
Monocalcium phosphate[2]	14	14	14
Common salt	3	3	3
Mineral Premix[3]	1.5	1.5	1.5
Vitamin Premix[3]	1.5	1.5	1.5
Methionine[4]	1	1	1
Lysine[5]	1	1	1
Anti Coccidial[6]	0.2	0.2	0.2
Antimold[7]	1	1	1
Calculated Analysis			
Crude protein (CP)%	23.1	22.18	19.39
Metabolizable Energy (ME) Kcal/kg diet[8]	3053	3160.7	3252.6
Calorie/protein ratio (ME Kcal/CP%)	132.16	142.5	167.7

[1] Limestone (holds 36 % calcium).
[2] Monocalcium phosphate: hold 22 % phosphorus and 16 % calcium.
[3] Mineral and Vitamin premix produced by Heropharm and composed (per 3 kg) of vitamin A 12,000,000 IU, vitamin D3 2,500,000 IU, vitamin E 15,000 IU, vitamin K3 1000 mg, vitamin B1 1000 mg, vitamin B2 3000 mg, vitamin B6 1500 mg, vitamin B12 13.3 mg, niacin 30,000 mg, biotin 50 mg, folic acid 600 mg, pantothenic acid 10,000 mg, Mn: 60,000 mg, Zn: 50,000 mg, Fe: 30,000 mg, Cu: 4000 mg, I: 300 mg, Se: 100 mg and Co:100 mg.
[4] DL-Methionine (Produced by Evonic Co and contains 99 % methionine).
[5] Lysine = lysine hydrochloride (contain 98 % lysine).
[6] Kill Cox, Produced by the Arabian Company for Pharmaceutical Industries.
[7] Produced by EL TOBA CO. For Premixes & Feed El-Sadat city Egypt.
[8] ME calculated according to NRC (1994).

2.2. Behavioral observations

Continuous visual screening was conducted to observe the behavior of the flock using a digital camera (Teledyne Flir LCC, OR, USA, and EOS Rebel T7 DSLR Camera, Canon, Tokyo, Japan). All birds were checked at predetermined time intervals from 2 weeks old till end of 5-week study. The observations took place during daytime, spanning 12 h from 6:00 a.m. to 6:00 p.m. Every day was divided into 2 periods: morning (6:00 a.m. - 12:00 p.m.) and afternoon (12:00 p.m. - 6:00 p.m.). For each period, a 2-hour observation period was alternated between the morning and afternoon. Specific observation times were as follows: on day 1, observations were conducted from 6:00–8:00 a.m. and 12:00–2:00 p.m.; on day 2, from 8:00–10:00 a.m. and 2:00–4:00 p.m.; and same schedule was followed for day 3 (Reiter & Bessei, 1999). Each hour of observation was separated into 5-minute intermissions, during which the behavior of the broilers was scanned. The observed behavioral configurations included drinking, feeding, standing idle, walking, crouching, comfort behaviors (leg stretch, wing stretch, and wing and leg stretch), and body care behaviors (ruffling, shaking, and preening). These behaviors and their frequencies are revealed in Table 2. Data was expressed as percentages of broilers engaging in each categorized behavior out of the total number of broilers manually scanned (Reiter & Bessei, 1999).

2.3. Performance assessment: weight and feed

Parameters of the broilers performance, containing body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were evaluated. Individual weighing of broilers using electronic digital scale (TESA3301 Electronic Scale, China) was weekly conducted throughout the study to track the gaining of weight and record feed intake. FCR, a measure of efficiency, was determined by dividing the total intake of food (in grams) by the total gaining of weight (in grams) of birds (El-Kazaz & Hafez, 2020) from start of the study till the end of the experiment (from week 2 till 35 days of age).

2.4. Immunization with SRBCs and antibody titration

Preparation of sheep red blood cells (SRBCs) was conducted by collecting whole blood from a healthy sheep using anticoagulant (EDTA). The blood was centrifuged (Eppendorf 5810 R, Hanau, Germany) and washed with phosphate-buffered saline (PBS) (E105, Econ Labs, China), and then mixed with Alsevier's solution (A3551, Sigma-Aldrich, Germany) to achieve a final hematocrit (Hct) of 10 %. The SRBCs were kept in refrigerator and diluted with PBS to a 1 % Hct for immunizing broilers (Olsson & Oldenborg, 2008). At the third week of age, ten birds per treatment were intravenously injected with 100 μL of 1 % SRBC in PBS through the brachial vein. Six days after the SRBCs injection, samples of blood were gathered from the immunized broilers to examine the plasma titer of anti-SRBCs antibody, using hemagglutination test (Hager-Theodorides, Goliomytis, Delis & Deligeorgis, 2014). The blood samples were centrifuged, and the heat-inactivated complement was obtained from the supernatant. For total antibody titration, 25 μL of plasma were combined with 25 μL of PBS in the 1st well of a 96-well U-bottom microtiter plate. The plasma was then serially diluted through a row, and 25 μl of 1 % SRBCs was added to every well. The plate was incubated at 37 °C for 1 hour, and the titer of anti-SRBCs total antibody was calculated as the logarithm of highest dilution displaying hemagglutination. To evaluate the IgY antibody titer (mammalian IgG equivalent) that is resistant to β-mercaptoethanol (2-ME), 25 μl of plasma were mixed with 25 μl of 0.2 M 2-ME in the 1st column of a 96-well U-bottom microtiter plate at 37 °C for one hour. The hemagglutination test was then conducted as for the total antibody titer. The titer of IgM antibody was calculated by subtracting IgY titer from total antibody titer. All samples of plasma were examined in duplicates.

Table 2
Ethogram of the recorded behaviors.

Behavior	Description
Feeding	Pecking at feed-on-feed troughs.
Drinking	Obtaining water from the cup.
Standing idle	The bird remains standing and does not perform any activity.
Crouching	The bird remains sitting and lying on the litter, looking about or with closed eyes, with no other behavior.
Walking	Taking at least two successive steps.
Wing stretch	The bird stretches one wing or both.
Leg stretch	The bird stretches one leg.
Wing and leg stretch	The bird stretches one leg and one wing of the same body hemisphere.
Preening	The bird cleans and aligns its feathers using the beak.
Ruffling	Action of ruffling or shacking all body feathers.
Shaking	Action of shaking body.

2.5. Serum sampling and organ weights

On day 42 of age, following a 12-hour period of feed deprivation; ten birds from each group with similar body weights were randomly chosen. These broilers were weighed, blood samples (10 mL) were gathered from jugular vein and kept on ice, then the birds were euthanized through rapid decapitation. Following the blood samples centrifugation using cooling centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) at 3000 r.p.m at 4 °C for 15 min, the serum was obtained and kept at −20 °C for later immunoglobulin determination.

Immune organs, including the spleen, bursa of Fabricius, and thymus, were carefully dissected and individually weighed (Analytical Electronic Scale Chinese Laboratory Accurate Digital Scale 0.001 g - 1 kg). Final recorded weight of the live body at the end of the study was used to determine the immune organs relative weights. Spleen was kept at −80 °C in sterile tubes for the analysis of oxidative stress markers and mRNA extraction.

2.6. Serum immunoglobulin analysis

The values of immunoglobulins (IgA, IgG, and IgM) in the serum were determined (Bomski, 1995) through ELISA kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, P.R. China). Measurements were conducted with an enzyme-linked immunosorbent assay (ELISA) reader (Bio-Rad, Hercules, CA, USA).

2.7. Splenic oxidative stress and antioxidant activity

The concentration of malondialdehyde (MDA) (the product of lipid peroxidation), was determined by spectrophotometry following Buege and Aust (1987) in the spleen and superoxide dismutase (SOD) activity was determined following Sun, Oberley and Li (1988)) by kits provided by Ransod Diagnostics (London, UK). Additionally, the glutathione peroxidase (GPx) and catalase (CAT) enzymatic activities were examined according to Aebi (1984) using test kits from Bio-Diagnostic Co. (Cairo, Egypt) according to the manufacturer's instructions.

2.8. Splenic gene expression

Expression of genes related to immunity in spleen, including interferon gamma (IFN-γ), interleukin-4 (IL-4), Toll-like receptor 2 (TLR2), and tumor necrosis factor α (TNF-α), was quantitatively measured. Total mRNA was extracted and examined following the method described by Selim et al. (2021), and real-time polymerase chain reaction (PCR) cycling conditions were followed. Primers used for amplification were presented in Table 3. Amplification curves and cycle threshold (CT) levels were analyzed through Strata gene MX3005P software (Agilent Technologies, Inc., Santa Clara, CA, USA). The housekeeping gene reference consumed 28S rRNA. Relative mRNA expression was determined by the $2^{(-\Delta\Delta Ct)}$ method, and the results were reported as fold changes (Rao et al., 2013).

Table 3
Oligonucleotide sequence of splenic gene primers.

Gene	Sequence (5′−3′)	Annealing Temperature (°C)	Product Size (bp)	Accession	Number Reference
Interleukin- 4 (IL-4)	F-AATGACATCCAGGGAGAGGTTTC R-GCTAGTTGGTGGAAGAAGGTACG	55	219	JN639847	(Hager-Theodorides et al., 2014)
Tumor necrosis factor alpha (TNF-α)	F-AGACCAGATGGGAAGGGAATGAA R-GAAGAGGCCACCACACGACAG	55	219	JN942589	(Selim et al., 2021)
Interferon-γ (INF-γ)	F-AGCTGACGGTGGACCTATTATTGT R-CGGCTTTGCGCTGGATTC	58	260	JN942588	(Hager-Theodorides et al., 2014)
Toll-like receptor 2 (TLR2)	F-GTGGCCATGTCGATCAGCAGAAAC R-TCAGCGGAGAGTCACAGATGTAGC	56	202	NM_204,278	(Selim et al., 2021)
28S rRNA	F-CAGGTGCAGATCTTGGTGGTAGTA R- GCTCCCGCTGGCTTCTCC	58	273	JN639848	(Hager-Theodorides et al., 2014)

2.9. Statistical analysis

The statistical investigation was achieved by the SAS software (SAS Institute Inc., Cary, NC, USA), SPSS for Windows (Version 22, IBM Corp., NY) (SAS, 2011). For all variables of behavior patterns, productive performance, serum, and tissue measurements; One-Way ANOVA was used for raw data examination. If treatment impacts were significant, Duncan's *post hoc* test was performed for post hoc examines. General significance value was set up as $P < 0.05$. Groups levels are given as mean ± standard deviation.

3. Results

3.1. Behavioral patterns

Table (4) illustrates the significant impact of QRT supplementation on the exhibited behavior patterns of birds. The addition of QRT at various doses led to a marked ($P < 0.05$) improvement in feeding, drinking, crouching, comfort behavior, and body care behavior compared to control birds. Although, QRT supplementation led to a marked ($P < 0.05$) reduction in walking and standing idle behaviors. Furthermore, the higher dose of QRT (600 ppm) showed a greater percentage of birds engaging in feeding (20.98±3.28), drinking (14.66 ±1.54), and crouching (88.92±13.75), while showing reduced walking (9.24±4.73) and standing idle (4.64±0.94) behaviors compared to the lower dose (200 ppm). There were no marked variations in body care and comfort behavior between different QRT doses (200, 400, 600 ppm).

Table 4
Effect of quercetin (QRT) on the proportion (%) of broiler chickens performing different behavioral patterns.

Behavioral patterns	Control	QRT200	QRT400	QRT600	*P*-value
Feeding	14.03 ± 1.85^{c}	16.54 ± 0.14^{b}	18.32 ± 1.24^{ab}	20.98 ± 3.28^{a}	<0.001
Drinking	9.84 ± 0.96^{c}	11.66 ± 0.43^{b}	13.3 ± 0.75 ^{ab}	14.66 ± 1.54^{a}	<0.001
Standing idle	10.43 ± 0.91^{a}	7.07 ± 1.09^{b}	7.43 ± 1.91^{b}	4.64 ± 0.94^{c}	<0.001
Crouching	82.03 ± 11.72^{c}	84.53 ± 16.22^{b}	84.49 ± 15.39^{b}	88.92 ± 13.75^{a}	<0.001
Walking	14.33 ± 3.82^{a}	12.33 ± 3.22^{b}	11.77 ± 3.78^{b}	9.24 ± 4.73^{c}	<0.001
Preening	8.43 ± 0.77^{b}	9.83 ± 1.47^{a}	10.29 ± 1.52^{a}	10.78 ± 2.26^{a}	<0.001
Ruffling	5.78 ± 1. 93^{b}	7.78 ± 1. 93^{a}	7.47 ± 0.19^{a}	7.98 ± 0.08^{a}	<0.002
Shaking	4.89 ± 0.89^{b}	6.24 ± 0 43^{a}	6.52 ± 0.93^{a}	6.94 ± 0.58^{a}	<0.003
Wing stretch	5.23 ± 0.53^{b}	8.49 ± 0.24^{a}	8.53 ± 0.87^{a}	8.88 ± 0.30^{a}	<0.001
Leg stretch	5.36 ± 0.84^{b}	7.12 ± 0.57^{a}	7.54 ± 0.34^{a}	7.51 ± 0.34^{a}	<0.001
Wing and leg stretch	4.23 ± 1.35^{b}	6.41 ± 1.02^{a}	6.46 ± 0.44^{a}	6.68 ± 0.43^{a}	<0.002

The data are shown as the mean and standard deviation. Means bearing different litters within the same raw are significantly different ($P < 0.05$).

3.2. Productivity enhancement

In Table (5), QRT supplementation revealed a substantial increase in FI, BW, and BWG, accompanied by a marked reduction in FCR. Notably, higher dose of QRT (600 ppm) showed superior performance in all productive measurements compared to the lower dose (200 ppm), resulting in the best outcomes in terms of BW (2145.75±214.63a), FI (2983.25 ± 27.27), FCR (1.63 ± 0.04), and BWG (1823.81±193.28).

3.3. Humoral immunity

The mean values for titers of total and IgM antibody in supplemented birds were markedly ($P < 0.05$) improved compared to control ones (Fig. 1). Interestingly, there was a linear dose response observed for QRT on the titers of IgY ($P < 0.05$). Increasing levels of dietary QRT led to an improvement in production of anti-SRBCs IgY antibodies with the best recorded value at the dose of 600 ppm of QRT (0.88±0.04).

3.4. Indexes of immune organs

Relative to control birds, the indices of spleen, thymus, and bursa of Fabricius substantially improved with increasing QRT supplementation, with the most notable results observed at a dose of 600 ppm ($P < 0.05$) (Fig. 2) in the indices of spleen (2.18±0.18), thymus (3.88±0.24), and bursa of Fabricius (2.21 ± 0.23).

Table 5
Effect of quercetin (QRT) on productive performance of broiler chickens (from week 2 till week 5 of age).

Productive performance	Control	QRT200	QRT 400	QRT 600	*P*-value
Average initial weight (g)	320.84 ± 21.24	317.67 ± 19.81	318.07 ± 25.11	321.94 ± 21.25	0.78
Average final body weight (g)	1858.15 ± 94.83c	1985.75 ± 116.33b	1997.25 ± 204.83ab	2145.75 ± 214.63a	<0.001
Total body weight gain (g)	1537.31 ± 73.59^{c}	1668.08 ± 96.52^{b}	1679.18 ± 179.72^{ab}	1823.81 ± 193.28^{a}	<0.001
Feed intake (g)	2773.89 ± 39.89^{c}	2817.56 ± 33.29^{b}	2879.74 ± 89.37^{ab}	2983.25 ± 27.27^{a}	<0.001
Feed Conversion Ratio (FCR)	1.80 ± 0.03^{a}	1.69 ± 0.05^{b}	1.71 ± 0.06^{b}	1.63 ± 0.04^{c}	<0.001

The data are shown as the mean and standard deviation. Means bearing different litters within the same raw are significantly different ($P < 0.05$).

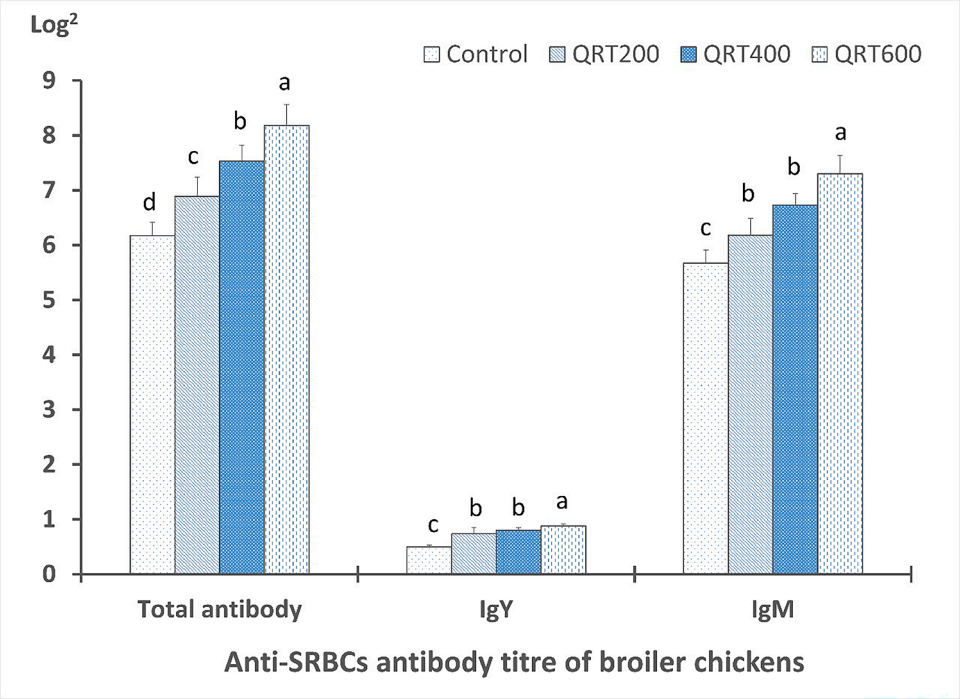

Fig. 1. Effect of quercetin (QRT) on anti-SRBCs antibody titer of broiler chickens.
IgY = immunoglobulin Y; IgM = immunoglobulin M. The data are shown as the mean and standard deviation ($n = 10$). Means bearing different litters within the same parameter are significantly different ($P < 0.05$).

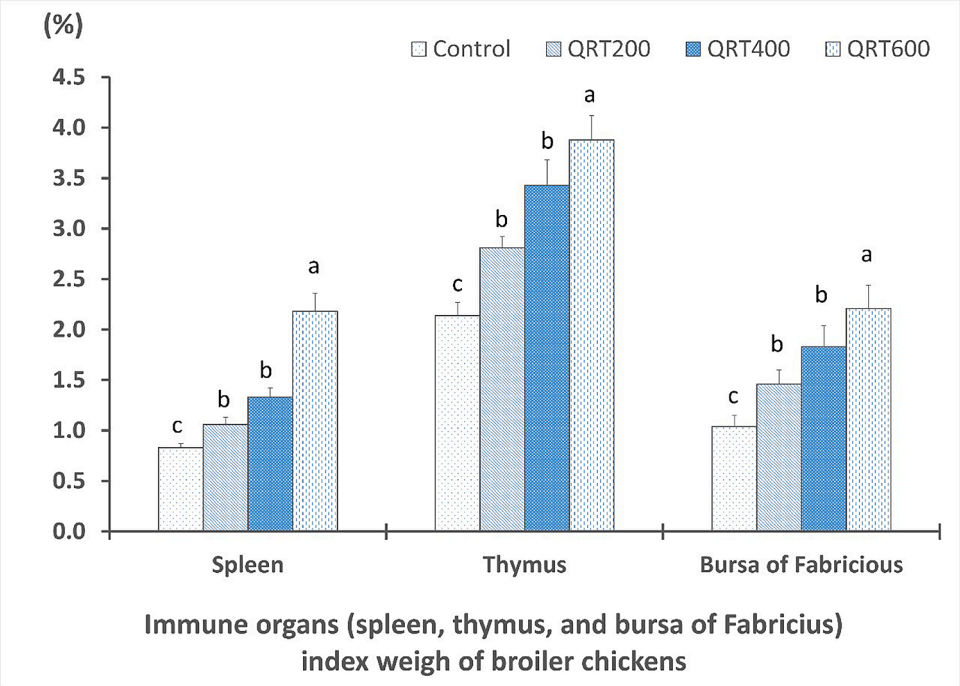

Fig. 2. Effect of quercetin (QRT) on broiler chickens' immune organs (spleen, thymus, and bursa of Fabricius) index weight.
The data are shown as the mean and standard deviation ($n = 10$). Means bearing different litters within each organ are significantly different ($P < 0.05$).

3.5. Immunoglobulin enhancement

Administration of QRT to broilers at doses of 200, 400, or 600 ppm resulted in a marked ($P < 0.05$) improvement in IgG and IgM concentrations relative to control birds. Moreover, the content of IgA showed a marked ($P < 0.05$) improvement in a dose-dependent manner (Fig. 3) with the 600 ppm treatment had the highest values for IgA (0.075 ± 0.003), IgG (0.161 ± 0.023) and IgM (0.188±0.014).

3.6. Splenic oxidant/antioxidant balance

The data expressed in Table (6) show that QRT administration

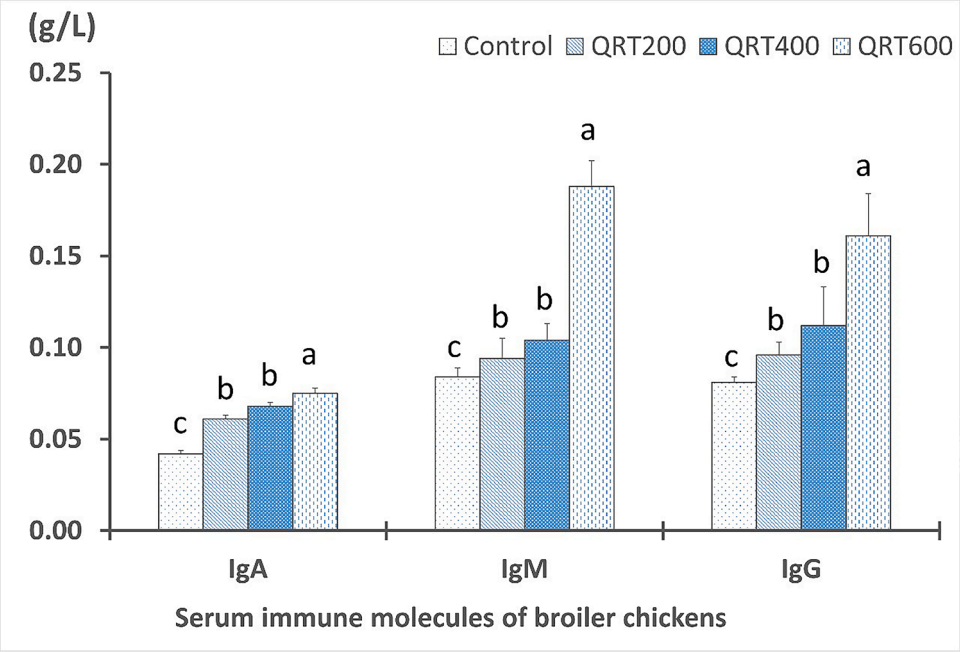

Fig. 3. Effect of quercetin (QRT) on serum immune molecules of broiler chickens.
IgA = immunoglobulin A; IgM = immunoglobulin M; IgG = immunoglobulin G. The data are the mean and standard deviation ($n = 10$). Means bearing different litters within the same raw are significantly different ($P < 0.05$).

Table 6
Effect of quercetin (QRT) on the splenic oxidative-antioxidant balance of broiler chickens.

Oxidant/ antioxidant markers	Control	QRT200	QRT400	QRT600	*P*-value
MDA (nmol/mg protein)	3.34± 0.18^{a}	2.78 ± 0.11^{b}	2.08 ± 0.11^{c}	1.54 ± 0.07^{d}	<0.001
SOD (U/mg protein)	158.15 ± 11.23^{c}	189.33 ± 12.76^{bc}	224.25 ± 17.73^{b}	265.25 ± 16.54^{a}	<0.001
GPx (U/mg protein)	12.54 ± 0.89^{c}	16.38 ± 1.02^{b}	19.98 ± 1.06^{b}	23.51 ± 1.54^{a}	<0.001
CAT (U/g protein)	21.32 ± 1.89^{c}	27.06 ± 1.29^{b}	30.74 ± 1.87^{b}	35.87 ± 2.07^{a}	<0.001

The data are shown as the mean and standard deviation ($n = 10$). Means bearing different litters within the same raw are significantly different ($P < 0.001$).

significantly ($P < 0.001$) enhanced the activities of SOD, GPx, and CAT in broilers compared to control birds. Additionally, MDA values were substantially ($P < 0.001$) reduced in QRT-treated broilers compared to control non-supplemented ones. The 600 ppm QRT-treated birds showed the best results in a dose-dependent manner with the most reduced MDA (1.54 ± 0.07) and improved activates of SOD (265.25 ± 16.54), GPx (23.51 ± 1.54) and CAT (35.87 ± 2.07).

3.7. Expression of the splenic immunity genes

Expression values of splenic INF-γ and TLR2 were up-regulated ($P < 0.001$) in the QRT-administered broilers compared to the control ones. Furthermore, the mRNA expression of splenic TNF-α and IL-4 was significantly up-regulated ($P < 0.001$) in QRT-treated birds compared to control group (Fig. 4). Moreover, there was a marked dose-dependent enhancement in the expression of immunity-related genes, with the 600 ppm-treated group showing the most notable ($P < 0.001$) results by improving the expression values of IL-4 (2.91 ± 0.15), INF-γ (3.62 ± 0.24), TLR2 (2.65 ± 0.17), and TNF-α (2.15 ± 0.13). These results emphasize the significant and dose-dependent improvements in gene expression achieved through QRT supplementation, supporting its potential as a valuable dietary intervention for modulating the immune response in broiler chickens.

4. Discussion

Quercetin is a natural flavonoid that is integrated in various vegetables and fruits, such as apples, red onions, parsley, tea, capers, red grapes, and broccoli (Xiao et al., 2017). It retains a widespread variety of pharmacological benefits, counting antioxidative, anti-inflammatory, anti-diabetic, and lipid-modulatory capacities (Kobori et al., 2016). QRT, along with other flavonoids, is being explored as a potential antioxidant additive for chicken's ration. Although the research on the impacts of QRT on cell-mediated immune response in broiler chickens is limited, its immunomodulatory properties have been well-documented in other species (Dang et al., 2022; Hager-Theodorides et al., 2014; Saeed et al., 2019; Yang et al., 2020).

In our experiment, QRT supplementation enhanced ingestive behaviors (feeding and drinking) of the chickens, which might be due to its metabolic prebiotic capability to regulate the gut microbiota and promote growth of useful bacteria, ultimately improving the broiler chickens performance (Abdel-Latif et al., 2021). Additionally, QRT supplementation increased crouching, body care, and comfort behaviors, which can be attributed to its antioxidant properties (Wenk, 2003). Quercetin acts as a potent anti-inflammatory and antioxidant agent (Anand David et al., 2016), and its antioxidative properties make it an attractive feed additive in poultry nutrition, as it can reduce lipid peroxidation and improve the shelf life and organoleptic characteristics of poultry products (Fernández-Quintela et al., 2019; Sierzant et al., 2022). We propose that QRT alleviates the effects of oxidative stressors present in the birds' environment, which can lead to an increase in stress markers in the blood and lipid peroxidation. These factors can disrupt normal behavioral patterns and show poor welfare in birds. Our experiment proved an increase in standing idle and walking behaviors, as well as a decrease in body care and comfort behaviors in the control group. However, the addition of QRT as a potent antioxidative agent alleviated these adverse effects and helped the birds show normal behavioral patterns and improve their welfare by increasing body care

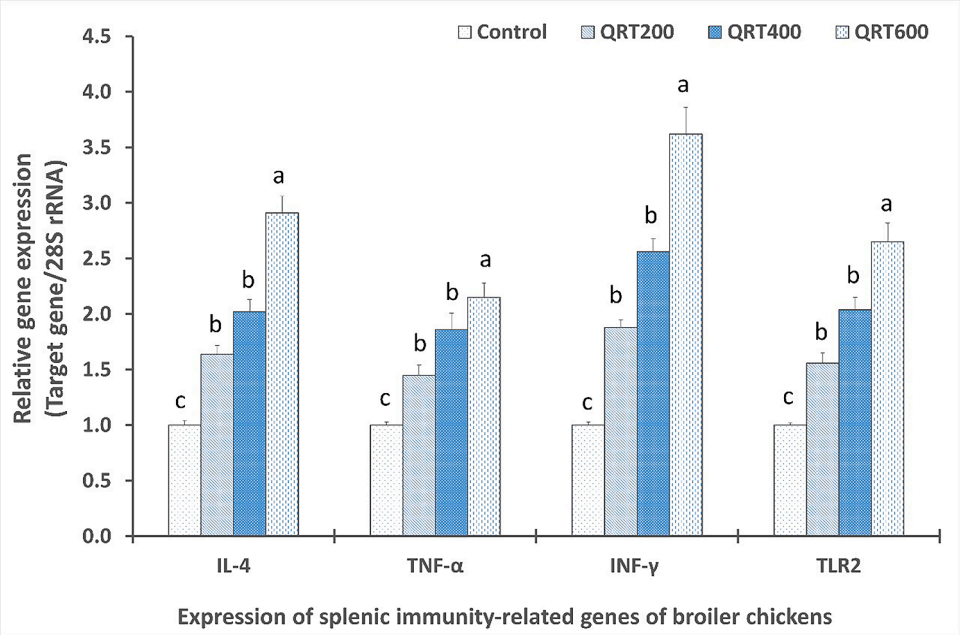

Fig. 4. Effect of Quercetin (QRT) on the expression of splenic immunity-related genes of broiler chickens.
IL-4, interleukin 4; TNF-α, tumor necrosis factor-α; INF-γ, interferon-gamma; TLR2, Toll-like receptor 2. The data are shown as the mean and standard deviation (n = 10). Means bearing different litters within each gene expression are significantly different ($P < 0.001$).

behaviors and comfort behaviors while decreasing standing idle and walking behaviors.

Several researches have displayed that flavonoids, including quercetin, have favorable impacts on broilers chickens performance (Kishawy et al., 2019; Serra et al., 2021). Although, there is limited data on the impact of QRT on broilers growth performance. In laying hens, supplementation of the diet with QRT improved feed conversion ratio (FCR) and laying rate (Liu et al., 2014). These useful impacts were attributed to phytoestrogen activity of QRT. Similarly, in fish, QRT supplementation improved the specific growth rate, and higher dosages were found to be more effective. The growth-promoting effects of QRT in fish were attributed to increased digestive enzyme activity, immune ability, and antioxidant capacity (Kong et al., 2022). In broilers, previous studies have reported a quadratic impact of QRT on gaining of body weight, with the greatest body weight gain observed at a dose of 800 mg QRT/kg (Abdel-Latif et al., 2021). This growth-promoting effects may be attributed to the up-regulation of the receptor of hepatic growth hormone and growth hormone, leading to increased concentrations of insulin-like growth factor-1 and so endorsing the growth of animal (Dang et al., 2022). QRT supplementation can also enhance the production of protein in muscles and initiate growth mechanisms (Saeed et al., 2019). However, conflicting results have been reported, with some studies showing no marked impact of QRT addition on the growth of poultry (Yang et al., 2020). These variations in outcomes could be attributed to differences in animal and management conditions. Other factors may also contribute to the findings related to growth performance (Goliomytis et al., 2014).

The weight of immune organs can serve as an indicator of immune function. Decreased weight of immune organs reflects immunosuppression, while increased weight indicates an enhancement of immunity (Dang et al., 2022). The spleen, bursa of Fabricius, and thymus are the main tissues related to cellular and humoral immunity. Maturation and development of these organs are more efficient in healthy broilers compared to distressed ones, and their growth reflects the functioning and reaction of immune system (Dang et al., 2022; Hager-Theodorides et al., 2014; Saeed et al., 2019; Yang et al., 2020). QRT supplementation increased the relative weight of immune organs, particularly bursa of Fabricius and spleen in poultry (Saeed et al., 2019). However, (Yang et al., 2020) reported non-noteworthy differences in relative weights of thymus, spleen, and bursa following QRT supplementation. Increased lymphoid organs indexes in broilers are frequently considered markers of enhanced B and T lymphocytes proliferation, which implies enhanced immunity (Hager-Theodorides et al., 2014). The thymus is the primary site of proliferation and maturation for thymocytes, specially T-lymphocytes, the bursa of Fabricius is important for the proliferation of B-cell, and spleen, as the biggest peripheral lymphoid organ, plays an essential role in the immunity procedures (Kong et al., 2022).

To evaluate the potential effects of QRT on humoral immune reactions of chickens, we conducted an experiment where the chickens were immunized with sheep red blood cells (SRBCs) (Hager-Theodorides et al., 2014). Measurement of titers of antibody in the blood plasma 6 days after immunization is a primarily conducted procedure in vivo measurement for assessing humoral immunity in poultry. Immunization with SRBCs triggers the stimulation of B cells and the release of antibodies against SRBC, which are indicators of the humoral immune response. QRT administration showed a marked increase in IgM antibody titers, in addition to a substantial dose-dependent enhancement in total antibody and IgY values in an initial reaction to SRBCs immunization. These findings suggest that supplementation of QRT in the diet improves the reaction of humoral immunity in a dose-dependent rhythm. Furthermore, dietary QRT supplementation also increased immunity tissues relative weight, particularly the spleen and bursa of Fabricius, indicating an improvement of the immunity status (Saeed et al., 2019). The improved reaction of humoral immunity was supported by enhanced values of IgG and IgA in poultry following QRT supplementation (Zhang & Kim, 2020). Additionally, the composition of the immune system, activated by the complexes of antigen-antibody reaction, reveal an anti-inflammatory role with the plasma presence of inactive precursors. Therefore, QRT supplementation appears to have a beneficial effect on the humoral immune response and immune organ development in chickens.

Redox balance has a fundamental role in poultry production. Supplementation of QRT improved activities of splenic SOD, CAT, and GPx, while reducing MDA levels, showing lower lipid peroxidation. Goliomytis et al. (2014) recorded a similar improvement in MDA levels when the birds were supplemented QRT at a value of 1000 mg/kg. The

procedure behind this improvement involves the antioxidant properties of flavonoids, which scavenge radicals and generate phenolic acids, thereby enhancing the activity of other antioxidants (Serra et al., 2021). Furthermore, QRT supplementation linearly enhances the antioxidant ability of immune organs, impacting the function and production of immunomolecules and cytokines, ultimately supporting overall health of the bird (Liu et al., 2014).

In terms of immunological-related gene expression, the focus of the study was on the spleen and the influence of QRT supplementation on gene expression. Specifically, IL-4, IFN-γ, TNF-α, and TLR2 were examined due to their roles in inflammation and involvement in the immunomodulatory signaling pathway. The results showed increased expression of these immunity-related genes with QRT supplementation, particularly IL-4, TLR2, TNF-α, and IFN-γ. The up-upregulation of these genes enhances B cell proliferation and differentiation, with B and T lymphocytes playing crucial roles in the immune response by differentiating into Th1 and Th2 cells for cellular and humoral immunity, respectively (Selim et al., 2021). IL-4, TNF-α, and IFN-γ, also act as indicators to enhance the immune reaction by stimulating T-helper, natural killer cells, and cytotoxic macrophages, and promoting T-cell differentiation and proliferation (Jacob & Pescatore, 2017). Within a certain rang, an increase in the context of IL-4, TNF-α, and IFN-γ can help the immunity of chronically stressed or non-diseased broilers. Toll-like receptors (TLRs) have a crucial role in innate immunity through initiating signaling cascade for cytokine synthesis and up-regulating co-stimulatory molecules (Broom & Kogut, 2019). The study revealed an increase in splenic TLR2 expression, consistent with the findings of Yang et al. (2020), who recorded an increased expression of TRAF-2, TNF-α, and TNFRSF1B with QRT supplementation, suggesting that QRT improves the functions of immunity status through NF-κB signaling pathway initiated by TNF-α. Additionally, Ying et al. (2020) recorded a marked enhancement in serum TNF-α values with QRT supplementation, proposing that QRT protects the immune cells from the harmful impact of oxidative stress by reducing and lipid peroxidation and excessive free radical release in challenged broiler chickens.

Despite the promising results, it is important to acknowledge certain limitations of this study. Firstly, while the sample size of 400 broilers was substantial, a larger cohort could have provided more robust statistical power and potentially revealed subtler effects of quercetin supplementation. Secondly, while efforts were made to control environmental factors, variations in individual bird genetics, microbiome composition, and subtle differences in housing conditions could have influenced the results. Finally, the study focused on a specific breed (Cobb 500), and the results may not be fully generalizable to other broiler breeds or poultry species. Future studies addressing these limitations could further strengthen our understanding of quercetin's role in broiler health and productivity.

5. Conclusion

Quercetin administration to broiler chickens' diet showed remarkable effects on behavior, immunity status and performance. The modulation of splenic immunity-related genes, such as IL-4, IFN-γ, TNF-α, and TLR2 expression, along with increased antioxidant activity, contributes to an improved immune status. Particularly, the group receiving 600 ppm of quercetin for 42 days proved the most favorable outcomes. Overall, these findings underscore the importance of incorporating quercetin into poultry diets to perfect the behavior, performance, and immune health of birds.

Ethics approval and consent to participate

The present study was conducted in compliance with the guidelines for animal welfare set by the National Research Council, and it was allowed by Resident Commission of Ethics for the Us and Care of Laboratory Animals at Alexandria University, Egypt (approval number: 2023/013/265). The research was conducted at the Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Sara Elsayed El-Kazaz: Visualization, Resources, Methodology, Conceptualization. **Mona Hafez Hafez:** Writing – original draft, Validation, Resources, Methodology, Formal analysis, Conceptualization. **Ghadeer M. Albadrani:** Visualization, Project administration, Funding acquisition. **Muath Q. Al-Ghadi:** Visualization, Resources, Funding acquisition. **Mohamed M. Abdel-Daim:** Resources, Investigation, Funding acquisition. **Yasser Said El-Sayed:** Writing – review & editing, Visualization, Validation, Supervision.

Declaration of competing interest

The authors declare no conflict of interests. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Data availability

The data and materials are available on request.

Acknowledgements

This study was supported by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R30), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. This research was funded by the Researchers Supporting Project number (RSPD2024R811), King Saud University, Riyadh, Saudi Arabia.

References

Abdel-Latif, M. A., Elbestawy, A. R., El-Far, A. H., Noreldin, A. E., Emam, M., Baty, R. S., … Abd El-Hamid, H. S. (2021). Quercetin dietary supplementation advances growth performance, gut microbiota, and intestinal mRNA expression genes in broiler chickens. *Animals : an open access journal from MDPI, 11*(8), 2302. https://doi.org/10.3390/ani11082302

Aebi, H. (1984). Catalase in vitro. *Methods Enzymology, 105*, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Anand David, A. V., Arulmoli, R., & Parasuraman, S. (2016). Overviews of biological importance of quercetin: A bioactive flavonoid. *Pharmacogn Review, 10*, 84–89.

AOAC. (2005). *Official methods of analysis of the association of analytical chemists international* (18th Edition). Gaithersburg, MARY LAND, USA: Association of Analytical Communities.

Bomski, H. (1995). Biernacki's reaction. In H Bomski (Ed.), *Basic hematology laboratory analyses* (pp. 120–124). Warsaw, Poland: National Institute of Medical Publications.

Broom, L. J., & Kogut, M. H. (2019). Deciphering desirable immune responses from disease models with resistant and susceptible chickens. *Poultry Science, 98*, 1634–1642.

Buege, J. A., & Aust, S. D. (1987). Microsomal lipid peroxidation. In S. Fleischer, & L. Packer (Eds.), *Methods in enzymology* (pp. 302–310). New York: Academic press.

Dang, X., Cho, S., Wang, H., Seok, W. J., Ha, J. H., & Kim, I. H. (2022). Quercetin extracted from Sophora japonica flower improves growth performance, nutrient digestibility, cecal microbiota, organ indexes, and breast quality in broiler chicks. *Animal Bioscience, 35*, 577–586.

El-Kazaz, S. E., & Hafez, M. H. (2020). Evaluation of copper nanoparticles and copper sulfate effect on immune status, behavior, and productive performance of broilers. *Journal of Advanced Veterinary and Animal Research, 7*, 16–25.

Fernández-Quintela, A., Milton-Laskibar, I., Aguirre, L., Gómez-Zorita, S., González, M., Portillo, M. P., & Walrand, S. (2019). *Effects of Quercetin on Mitochondriogenesis in Skeletal Muscle: Consequences for Physical Endurance and Glycemic Control. Nutrition and Skeletal Muscle* (pp. 505–516). 10.1016/B978-0-12-810422-4.00028-2.

Goliomytis, M., Tsoureki, D., Simitzis, P. E., Charismiadou, M. A., Hager-Theodorides, A. L., & Deligeorgis, S. G. (2014). The effects of quercetin dietary

supplementation on broiler growth performance, meat quality, and oxidative stability. *Poultry Science, 93*, 1957–1962.
Hager-Theodorides, A. L., Goliomytis, M., Delis, S., & Deligeorgis, S. (2014). Effects of dietary supplementation with quercetin on broiler immunological characteristics. *Animal Feed Science and Technology, 198*, 224–230.
Jacob, J., & Pescatore, A. (2017). Glucans and the Poultry Immune System. *American Journal of Immunology, 13*, 45–49.
Kishawy, A. T. Y., Amer, S. A., Abd El-Hack, M. E., Saadeldin, I. M., & Swelum, A. A. (2019). The impact of dietary linseed oil and pomegranate peel extract on broiler growth, carcass traits, serum lipid profile, and meat fatty acid, phenol, and flavonoid contents. *Asian-Australasian Journal of Animal Sciences, 32*, 1161–1171.
Kobori, M., Takahashi, Y., Sakurai, M., Akimoto, Y., Tsushida, T., Oike, H., & Ippoushi, K. (2016). Quercetin suppresses immune cell accumulation and improves mitochondrial gene expression in adipose tissue of diet-induced obese mice. *Molecular Nutrition & Food Research, 60*, 300–312.
Kong, Y., Tian, J., Niu, X., Li, M., Kong, Y., Li, R., … Wang, G. (2022). Effects of dietary quercetin on growth, antioxidant capacity, immune response and immune-related gene expression in snakehead fish, Channa argus. *Aquaculture Reports, 26*, 101314. https://doi.org/10.1016/j.aqrep.2022.101314
Lakhanpal, P., & Rai, D. K. (2007). Quercetin: A versatile flavonoid. *Internet Journal of Medical Update - EJOURNAL, 2*(2). https://doi.org/10.4314/ijmu.v2i2.39851
Liu, H. N., Liu, Y., Hu, L. L., Suo, Y. L., Zhang, L., Jin, F., Feng, X. A., Teng, N., & Li, Y. (2014). Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. *Poultry Science, 93*, 347–353.
Mchunu, Z. G., Mthana, M. S., & Mthiyane, M. N. (2024). Dietary effects of Sclerocarya birrea caffra seed cake replacing soyabean meal on physiology, meat and bone quality of indigenous chickens. *Veterinary and Animal Science, 25*, Article 100364. https://doi.org/10.1016/j.vas.2024.100364
NRC. (1994). *Nutrient requirements of poultry: Ninth revised edition (1994)*. Washington, DC: The National Academies Press.
Olsson, M., & Oldenborg, P. A. (2008). CD47 on experimentally senescent murine RBCs inhibits phagocytosis following Fcgamma receptor-mediated but not scavenger receptor-mediated recognition by macrophages. *Blood, 112*, 4259–4267.
Rao, D. D., Senzer, N., Wang, Z., Kumar, P., Jay, C. M., & Nemunaitis, J. (2013). Bifunctional short hairpin RNA (bi-shRNA): Design and pathway to clinical application. *Methods of Molecular Biology, 942*, 259–278.
Reiter, K., & Bessei, W. (1999). The behaviour of broilers in response to group size and stocking density. *Arch Geflügelk, 64*, 93–98.
Saeed, M., Naveed, M., Arain, M. A., Arif, M., Abd El-Hack, M. E., Alagawany, M., Siyal, F. A., Soomro, R. N., & Sun, C (2019). Quercetin: Nutritional and beneficial effects in poultry. World's. *Poultry Science Journal, 73*, 355–364.
Sarrigeorgiou, I., Stivarou, T., Tsinti, G., Patsias, A., Fotou, E., Moulasioti, V., Kyriakou, D., Tellis, C., Papadami, M., Moussis, V., Tsiouris, V., Tsikaris, V., Tsoukatos, D., & Lymberi, P. (2023). Levels of circulating IgM and IgY natural antibodies in broiler chicks: Association with genotype and farming systems. *Biology, 12*(2), 304. https://doi.org/10.3390/biology12020304
SAS Institute. (2011). *Using jmp student edition for windows and macintosh: The user's guide to statistics with jmp student edition*. Cary, NCUSA: SAS Institute.
Selim, S., Abdel-Megeid, N. S., Abou-Elnaga, M. K., & Mahmoud, S. F. (2021). Early nutrition with different diets composition versus fasting on immunity-related gene expression and histomorphology of digestive and lymphoid organs of layer-type chicks. *Animals : an open access journal from MDPI, 11*(6), 1568. https://doi.org/10.3390/ani11061568
Serra, V., Salvatori, G., & Pastorelli, G. (2021). Dietary polyphenol supplementation in food producing animals: Effects on the quality of derived products. *Animals : an open access journal from MDPI, 11*(2), 401. https://doi.org/10.3390/ani11020401
Sierzant, K., Korzeniowska, M., Polbrat, T., Rybarczyk, A., & Smolinski, J. (2022). The use of an optimised concentration of quercetin limits peroxidation of lipids in the meat of broiler chickens fed a diet containing flaxseed oil rich in omega-3. *Animal : an international journal of animal bioscience, 16*, 100603. https://doi.org/10.1016/j.animal.2022.100603
Sun, Y., Oberley, L. W., & Li, Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry, 34*, 497–500. https://doi.org/10.1093/clinchem/34.3.497
Wenk, C. (2003). Herbs and botanicals as feed additives in monogastric animals. *Asian Australian Journal of Animal Science, 16*, 282–289.
Xiao, L., Liu, L., Guo, X., Zhang, S., Wang, J., Zhou, F., Liu, L., Tang, Y., & Yao, P. (2017). Quercetin attenuates high fat diet-induced atherosclerosis in apolipoprotein E knockout mice: A critical role of NADPH oxidase. *Food and Chemical Toxicology, 105*, 22–33.
Yang, J. X., Maria, T. C., Zhou, B., Xiao, F. L., Wang, M., Mao, Y. J., & Li, Y. (2020). Quercetin improves immune function in Arbor Acre broilers through activation of NF-kappaB signaling pathway. *Poultry Science, 99*, 906–913.
Ying, L., Chaudhry, M. T., Xiao, F., Mao, Y., Wang, M., Wang, B., Wang, S., & Li, Y. (2020). The effects and mechanism of quercetin dietary supplementation in streptozotocin-induced hyperglycemic arbor acre broilers. *Oxidative Medicine & Cellular Longevity, 9585047*. https://doi.org/10.1155/2020/9585047
Zahoor, I., Ghayas, A., & Basheer, A. (2018). Genetics and genomics of susceptibility and immune response to necrotic enteritis in chicken: A review. *Molecular Biology Reports, 45*, 31–37.
Zhang, S., & Kim, I. H. (2020). Effect of quercetin (flavonoid) supplementation on growth performance, meat stability, and immunological response in broiler chickens. *Livestock Science, 242*, Article 104286.