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ORIGINAL PAPER

Dietary micelle quercetin: influence on performance, egg quality, and blood biochemical parameters in Hy-Line Brown laying hens

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ABSTRACT. The study aimed to evaluate the effects of quercetin micelles (MQ) on performance, egg quality, and blood profile of laying hens. A total of 288 Hy-Line Brown laying hens were randomly assigned to one of three dietary groups, with eight replicates of 12 hens each. The hens were housed individually in pens with free access to feed and water. During a 12-week feeding period, the hens were fed a basal diet supplemented with varying concentrations of MQ: 0% (control group), 0.03% (Treatment 1), and 0.06% (Treatment 2). The results indicated that the incorporation of MQ into the hens' diets did not significantly affect feed conversion ratio, egg production, and feed intake ($P > 0.05$). However, egg weight significantly increased with 0.06% MQ supplementation ($P < 0.05$). Moreover, at week 8, hens fed a diet with 0.06% MQ had significantly higher Haugh unit (HU) values compared to both the control and 0.03% MQ groups, along with greater eggshell strength and thickness compared to the control group ($P < 0.05$). By week 12, supplementation with 0.06% MQ resulted in further improvements in HU, albumen height, eggshell strength, and thickness ($P < 0.05$). In addition, the 0.06% quercetin diet was associated with reduced aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase activities, as well as cholesterol levels ($P < 0.05$). However, quercetin had no significant effect on the activity of lactate dehydrogenase, or on albumin and triglyceride levels ($P > 0.05$). In summary, adding 0.06% MQ to the diet of laying hens can improve their performance, egg quality, and positively affect certain blood parameters.

Introduction

Laying hens play an important role in the global poultry industry, contributing significantly to the production of eggs – the primary source of high-quality protein for human consumption. Ensuring the optimal health and performance of these birds is essential to meet the rising global demand for eggs (Korver, 2023). However, at the farm level, some producers use high-risk chemicals such as antibiotics, somatotropin, and preservatives to boost production and control pathogenic diseases. Notably, the use of antibiotics for this purpose is banned in Europe. This practice poses significant hazards to human health. Consequently, research efforts have focused on identifying dietary alternatives to antibiotics to improve production performance, egg quality, and overall health of laying hens. Among many nutritional supplements under investigation, quercetin, a flavonoid abundant in many plant foods, has gained attention for its potential health-promoting properties (Shorobi et al., 2023).

Quercetin (3,3′,4′,5,7-pentahydroxyflavone), a flavonoid naturally found in plants, fruits, and vegetables such as onions, buckwheat, and broccoli, is widely recognised for its antioxidant, antiinflammatory, and immune-modulating properties, extensively studied for their health benefits in humans (Hagde et al., 2022). In poultry, quercetin supplementation at 400 mg per kg has been shown to positively affect feed intake, eggshell weight, and egg shelf-life (Simitzis et al., 2018). Additionally, dietary inclusion of quercetin has been shown to exert antioxidant effects that may support the well-being of laying hens exposed to various stressors (Formica and Regelson, 1995). When included at a concentration of 400 mg per kilogram in the basal diet, quercetin has been associated with increased laying rate, improved egg quality, and lower yolk cholesterol levels (Liu et al., 2013). Despite its promising therapeutic potential, the clinical application of quercetin is limited due to its poor bioavailability. To address this constrain, micelles – nano-sized particles formed by amphiphilic molecules – are utilised to improve the solubility and bioavailability of supplements with low water solubility (Ahammad et al., 2024a). Quercetin micelles increase the stability and absorption of quercetin in the gastrointestinal tract, improving its effectiveness as a therapeutic agent (Hagde et al., 2022). However, data on the effects of micellar quercetin specifically in laying hens are currently lacking.

We hypothesised that supplementing laying hens' diets with quercetin micelles (MQ) would improve production performance and egg quality, as well as induce beneficial changes in blood biochemical profile. This study provides a comprehensive analysis of the effects of dietary MQ inclusion on the production performance, egg quality, and biochemical markers of Hy-Line Brown laying hens.

Material and methods

The Animal Care and Use Committee of Dankook University in Cheonan, Republic of Korea, approved the research protocol (DK-1-2229) for this study.

Birds, husbandry, and experimental diets

A total of 288 Hy-Line Brown laying hens, aged 28 weeks, were randomly assigned to three dietary treatment groups, each consisting of eight replicates per treatment and twelve adjacent pens per replicate. The hens were housed in individual pens and fed from a shared feed trough.

The 12-week trial was conducted in individual pens measuring 38 cm (width) × 50 cm (length) × 40 cm (height). The facility was maintained at 6 °C and a lighting schedule of 16 hours of light and 8 hours of darkness. Each pen was equipped with nipple drinkers and removable trough feeders to provide unrestricted access to feed and water. The experimental basal diet for laying hens was formulated in mash form according to NRC (1994) standards, and ingredients and nutrient composition are detailed in Table 1. The dietary groups included: (1) CON (basal diet); (2) TRT1 – CON + 0.03% MQ; and (3) TRT2 – CON + 0.06% MQ supplementation. The quercetin utilised in our study, with a purity of 97%, was sourced from Sigma-Aldrich (St. Louis, MO, USA). Quercetin was micellised with various emulsifiers, resulting in a product containing a minimum of 20% micellar quercetin and at least 2% quercetin. Tween 80 was selected as the surfactant due to its compatibility and efficiency in forming stable micelles.

Table 1. Composition of laying hen diets (as-fed basis)

Items	CON
Ingredient, %	
maize	53.11
DDGS (30% CP)	20.01
palm kernel meal (16% CP)	1.85
soybean meal (46% CP)	10.99
sesame meal (46% CP)	2.00
tallow	0.94
MDCP	0.06
limestone	10.32
salt	0.05
methionine (99%)	0.05
lysine (50%)	0.27
vitamin mix[1]	0.10
mineral mix[2]	0.10
choline (50%)	0.10
phytase (500 unit)	0.05
Total	100.00
Calculated value, %	
crude protein	16.02
crude fat	5.03
crude fibre	4.24
crude ash	4.57
calcium	4.10
phosphorus	0.51
available phosphorus	0.20
lysine	0.75
methionine + cystine	0.94
metabolisable energy, kcal/kg	2650
linoleic acid	2.43

CON – basal diet, DDGS – dried distillers grains, CP – crude protein, MDCP – mono dicalcium phosphate; [1] vitamin mix provided per kg of diet: IU: vit. A 10 800, vit. D_3 4 000, vit. E 40; mg: vit. K_3 4, vit. B_1 6, vit. B_2 12, vit. B_6 6, vit. B_{12} 0.05, biotin 0.2, folic acid 2, niacin 50, D-calcium pantothenate 25; [2] mineral mix provided per kg of diet: mg: Fe 100 (as ferrous sulphate), Cu 17 (as copper sulphate), Mn 17 (as manganese oxide), Zn 100 (as zinc oxide), I 0.5 (as potassium iodide), Se 0.3 (as sodium selenite)

Sampling and laboratory analysis

The performance of laying hens was evaluated at weeks 0, 6, and 12 by measuring parameters such as egg production, egg weight, feed intake, and feed conversion ratio (FCR). Daily records of egg production, egg weight, and feed intake were carried out for each replicate, and production percentage, FCR, and egg weight were calculated based on these records.

Egg quality evaluations were carried out after weeks 4, 8, and 12, with 720 eggs (240 eggs per treatment and 30 eggs per replicate), free of shell defects and cracks, were selected at 17:00. Quality measurements were conducted the same day at 20:00.

Eggshell colour was determined using an eggshell colour fan and visual evaluations. Haugh unit (HU), eggshell strength, and yolk colour were assessed using a digital egg tester (DET6500, NABEL Co., Ltd, Kyoto, Japan). Eggshell thickness was measured using a dial pipe gauge (Ozaki MFG Co., Ltd., Tokyo, Japan).

At the end of the trial, 5 ml of blood was drawn from 48 hens (16 hens per treatment, 2 hens per replicate) via wing vein puncture. Blood samples were deposited in K2-EDTA heparinised tubes (BD Vacutainer, Plymouth, UK), and centrifuged at 3000 rpm for 15 min to separate plasma, which was stored in Eppendorf tubes at −20 °C until analysis. The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH), as well as albumin, triglyceride, and cholesterol levels in the plasma were determined using a Konelab 20 automatic blood biochemistry analyser (Thermo Fisher Scientific, Vantaa, Finland), and commercial diagnostic kits according to the manufacturer's guidelines.

Statistical analysis

All data in this experiment were analysed following a completely randomised design using one-way ANOVA. Tukey's range test was employed to determine significant differences between means, with each replicate treated as an independent experimental unit. Data variability was measured using the standard error of the mean (SEM). Statistical significance was set at a $P < 0.05$.

Results

As presented in Table 2, the dietary inclusion of quercetin had no significant effect on performance parameters such as FCR, egg production, and feed intake in laying hens ($P > 0.05$); however, egg weight was significantly increased

Table 2. Effect of micelle quercetin supplementation on laying performance in laying hens

Items	Groups			SEM	*P*-value
	CON	TRT1	TRT2		
Weeks 0–6					
egg production, %	87.6	89.4	89.5	1.0	0.6380
feed intake	109.6	109.7	109.7	0.9	0.2382
egg weight, g	62.1^b	63.6^b	66.2^a	0.8	0.0012
FCR	1.98	1.94	1.94	0.02	0.1264
Weeks 7–12					
egg production, %	87.3	88.9	88.9	1.5	0.4486
feed intake	109.6	109.7	109.8	0.7	0.1146
egg weight, g	63.4^b	64.7^b	65.9^a	0.7	0.0097
FCR	1.99	1.95	1.95	0.03	0.4574
Overall weeks 0–12					
egg production, %	87.5	89.2	89.2	1.1	0.1197
feed intake	109.6	109.7	109.7	0.8	0.4456
egg weight, g	62.7^b	64.2^b	66.1^a	0.9	0.0194
FCR	2.06	1.97	2.00	0.02	0.1943

FCR – feed conversion ratio, CON – basal diet, TRT1 – CON + micelle quercetin 0.03%, TRT2 – CON + micelle quercetin 0.06%, SEM – standard error of the mean; ^{ab} – means in the same row with different superscripts are significantly different at $P < 0.05$

Table 3. Effect of micelle quercetin supplementation on egg quality in laying hens

Items	Groups			SEM	*P*-value
	CON	TRT1	TRT2		
Week 4					
egg shell colour	11.7	11.8	11.8	0.2	0.8375
Haugh units	85.9^b	87.0^b	91.8^a	1.1	0.0004
yolk colour	7.8	8.0	7.9	0.1	0.3645
albumen height, mm	8.3	8.5	8.5	0.2	0.4893
eggshell strength, kg/cm^2	3.98	4.12	4.42	0.21	0.1424
eggshell thickness, mm^{-2}	38.9	39.3	38.4	0.6	0.5282
Week 8					
eggshell colour	11.4	11.6	11.0	0.3	0.3657
Haugh units	84.0^b	86.7^b	91.1^a	1.5	0.0027
yolk colour	8.9	8.6	8.6	0.1	0.0785
albumen height, mm	8.7	8.7	8.9	0.2	0.4824
eggshell strength, kg/cm^2	4.06^b	4.17^{ab}	4.42^a	0.12	0.0390
eggshell thickness, mm^{-2}	40.7^b	41.3^{ab}	42.5^a	0.5	0.0095
Week 12					
egg shell colour	12.1	11.9	11.7	0.2	0.2741
Haugh units	83.3^b	88.9^a	89.7^a	2.0	0.0239
yolk colour	8.2	8.3	8.4	0.1	0.4021
albumen height, mm	10.0^b	11.4^{ab}	11.6^a	0.5	0.0484
eggshell strength, kg/cm^2	4.61^b	4.78^{ab}	5.21^a	0.19	0.0287
eggshell thickness, mm^{-2}	39.9^b	40.3^{ab}	42.1^a	0.7	0.0241

CON – basal diet, TRT1 – CON + micelle quercetin 0.03%, TRT2 – CON + micelle quercetin 0.06%, SEM – standard error of the mean; ^{ab} – means in the same row with different superscripts are significantly different at $P < 0.05$

by the addition of 0.06% quercetin ($P < 0.05$). Table 3 presents egg quality traits, where at week 8, eggshell strength and thickness were significantly higher in the TRT2 group compared to the control group, and HU was higher in TRT2 compared to both the control and TRT1 groups ($P < 0.05$). Additionally, at week 12, HU, albumen height, eggshell strength, and thickness in TRT2 were higher compared to the control ($P < 0.05$). Regarding blood profile parameters (Table 4), quercetin exerted a significant effect on AST, ALT, and ALP activity, and cholesterol levels ($P < 0.05$). Specifically, these markers were reduced in the TRT2 group compared to the control group. However, LDH activity, and albumin and triglyceride levels were not affected by dietary quercetin supplementation ($P > 0.05$).

Table 4. Effect of micelle quercetin supplementation on blood profile in laying hens

Items	Groups			SEM	*P*-value
	CON	TRT1	TRT2		
Finish (week 12)					
AST, U/l	215[a]	209[a]	194[b]	4	0.0056
ALT, U/l	4.63[a]	4.00[ab]	3.63[b]	0.37	0.0460
ALP, U/l	447[a]	438[ab]	415[b]	8	0.0115
LDH, U/l	1209	1184	1172	87	0.7663
albumin, g/dl	2.49	2.35	2.31	0.12	0.3373
triglycerides, mg/dl	1580	1574	1543	126	0.8377
cholesterol, mg/dl	167[a]	148[ab]	137[b]	8	0.0185

AST – aspartate aminotransferase, ALT – alanine aminotransferase, ALP – alkaline phosphatase, LDH – lactate dehydrogenase, CON – basal diet, TRT1 – CON + micelle quercetin 0.03%, TRT2 – CON + micelle quercetin 0.06%, SEM – standard error of the mean; [ab] – means in the same row with different superscripts are significantly different at $P < 0.05$

Discussion

In this study, we investigated the effects of MQ supplementation on key performance parameters in laying hens, including egg production, FCR, feed intake, and egg weight. Our findings were consistent with those of Simitzis et al. (2018), who reported no significant alterations in egg production and FCR in laying hens administered quercetin at 200, 400, and 800 mg per kg feed. Similarly, Liu et al. (2014) observed no significant effects on egg production, although they recorded a decrease in the FCR value with increasing dietary quercetin levels (0.2, 0.4, and 0.6 g quercetin/kg diet). On the other hand, our study contradicts some previous findings concerning other flavonoid-rich supplements (Li et al., 2023). Additionally, the supplementation of 0.06% micelle silymarin in other flavonoid-rich supplements significantly reduced egg count, egg production, egg weight, and FCR (Ahammad et al., 2024b). Liu et al. (2013) demonstrated that dietary supplementation with 0.2 and 0.4 g/kg quercetin significantly increased the laying rate and decreased the feed-egg ratio. In addition, quercetin in the diet was shown to significantly increase the laying rate, particularly in the group supplemented with 0.4 g/kg, while also reducing the feed:egg ratio (Yang et al., 2018). In contrast, our findings indicated that MQ supplementation had no significant effect on egg production, feed intake, or FCR, although it did significantly influence egg weight. In general, flavonoids have been demonstrated to modulate lipid metabolism, improve nutrient absorption, and enhance antioxidant defence in poultry (Sandoval et al., 2020). Consequently, dietary supplementation with flavonoids may promote more efficient nutrient utilisation and storage, leading to increased egg weight without significantly altering egg production or feed intake. Additionally, flavonoids have been found to improve ovarian function, eggshell quality, and reduce oxidative stress in poultry, all of which could contribute to increased egg weight (Dai et al., 2021). The current study also showed that MQ supplementation improved egg quality, although the precise mechanisms by which MQ exerts these effects in laying hens remain to be fully elucidated and require further research.

Egg quality, as assessed through parameters such as eggshell colour, HU, yolk colour, egg weight, albumin height, eggshell thickness, and eggshell strength, is essential for meeting consumer expectations, maintaining market competitiveness, and adhering to industry standards. In this study, quercetin supplementation positively affected egg quality, particularly HU, albumen height, eggshell strength, and eggshell thickness. HU is a key indicator of egg quality, reflecting both freshness and shelf life (Li et al., 2017). The observed increase in HU values as a result of quercetin supplementation indicates a beneficial effect on egg albumen quality (de Menezes et al., 2012). This rise can be attributed to the improved albumen protein composition and structural integrity resulting from quercetin's antioxidant and hepatoprotective properties. Furthermore, the current study revealed that quercetin supplementation led to an increase in albumen height, particularly noticeable in week 12. Elevated albumen height, likely due to the aforementioned effects of quercetin, reflects improved liver function, positively affecting protein synthesis and secretion, and thus albumin quality (Vargas-Mendoza et al., 2014). The uterus plays a crucial role in eggshell formation and development, contributing significantly to its final structure and quality (Hashemi Jabali et al., 2018). In this regard, Mirbod et al. (2017), proposed that the favourable effects of herbal bioactive compounds on protein metabolism and systemic antioxidant potential might support the oviduct or uterine health status, resulting in improved eggshell quality. The hepatoprotective properties of quercetin may enhance calcium metabolism and its utilisation in laying hens, ultimately leading to higher eggshell mineralisation (Vargas-Mendoza et al., 2014). The mechanisms underlying quercetin's action may contribute to increased eggshell thickness and strength. Several studies have explored the influence of quercetin on egg quality in laying hens. Liu et al. (2013) observed that specific levels of quercetin significantly improved HU, eggshell strength, eggshell thickness, and yolk protein content, indicating a variable effects of this compound (0.2 and 0.4 g/kg) on egg quality. Feeding a diet enriched with 300 mg/kg quercetin led to notable improvements in egg quality parameters, including increased eggshell strength, albumen height, and HU, while concurrently reducing the accumulation of abdominal fat (Wei et al., 2023). Moreover, supplementation with 0.06% micelle silymarin resulted in a linear increase in Haugh units, egg weight, and eggshell strength throughout the trial period (Ahammad et al., 2024a). Conversely, Liu et al. (2014) found no significant effects of quercetin on egg quality parameters when administered at increasing concentrations of 0.2, 0.4, and 0.6 g/kg diet. Similarly, Simitzis et al. (2018) reported no changes in egg quality with quercetin supplementation levels of 200, 400, and 800 mg/kg feed. Consistently, Ying et al. (2015) also demonstrated that non of the administered quercetin doses (0, 0.2, 0.4, and 0.6 g/kg) affected egg quality over an 8-week period. The positive impact of quercetin supplementation on egg quality may thus depend on its solubility and absorbability. In the present study, the micelle form likely contributed to the higher absorption of quercetin in the blood.

The current work focused on the effects of micellar quercetin supplementation on blood biochemical parameters in laying hens, showing a significant influence on specific hepatic enzymes (AST, ALT, and ALP) and cholesterol levels, while LDH activity, as well as albumin and triglyceride levels were not significantly affected. The activity of AST was previously shown to decrease with the inclusion of 0.100% MQ (Ahammad and Kim, 2024). These significant alterations in AST, ALT, and ALP activities following micelle quercetin supplementation underscore the potential hepatic benefits of this flavonoid (Hezaveh et al., 2019). AST and ALT enzymes are important indicators of hepatic health, reflecting the liver's enzymatic activity and integrity (Giannini et al., 2005). Significant reductions in their activity levels suggests a possible improvement in liver function and reduced cellular damage, as their elevated activity is often indicative of liver stress or damage (Shireen et al., 2008). This liver-protective effect is in line with earlier studies indicating that quercetin can mitigate liver damage and improve liver enzyme activity (Formica and Regelson, 1995). Moreover, the decline in ALP activity levels suggests a positive effect on bone health and overall phosphorus metabolism (Li et al., 2018). This decrease observed in the present study may indicate improved phosphorus utilisation in laying hens, potentially leading to strengthened bone health, and consequently eggshell thickness. The significant reduction in cholesterol levels observed with micelle quercetin supplementation supports existing research on quercetin's potential to modulate lipid metabolism and lower cholesterol levels (Seiva et al., 2012).

In contrast to the effects on AST, ALT, and ALP activity, and cholesterol levels, micellar quercetin supplementation did not lead to significant changes in LDH activity, or albumin, and triglyceride levels. The activity of LDH, a cytoplasmic enzyme indicative of tissue damage and stress, remained unchanged, suggesting minimal cellular damage induced by the supplementation (Koukourakis et al., 2006). In addition, the concentration of albumin, which is essential for osmotic regulation and transportation of molecules (Belinskaia et al., 2021), remained stable, indicating no significant impact on protein metabolism. The steady levels of triglycerides are noteworthy, suggesting that micelle quercetin did not affect lipid metabolism in laying hens. This finding is in agreement with some previous studies that reported no significant effect of quercetin on triglyceride levels (Serban et al., 2016). Due to the limited research available on blood biochemical parameters in laying hens, direct comparisons are difficult. However, future investigations should delve deeper into the mechanisms underlying these effects and explore the long-term implications of micelle quercetin supplementation in poultry diets.

Conclusions

Our study demonstrated that quercetin micelle supplementation at a level of 0.06% had a significant impact on laying hen performance, egg quality, and biochemical blood profile. Based on these findings, we recommend dietary supplementation of 0.06% quercetin as an effective strategy for increasing egg production, their weight, and quality, while maintaining favourable blood parameters in reared poultry.

Conflict of interest

The Authors declare that there is no conflict of interest.

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