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# Effects of pepper waste and capsaicin-based commercial feed additives on growth performance, egg production, and quality in Japanese quails

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#### Summary

This study aimed to investigate the effects of diets containing different levels of hot pepper waste and capsaicin-based commercial feed additives (ID Phyt Capcin) on the growth performance, carcass characteristics, blood parameters, and egg production of Japanese quails (Coturnix japonica). The research lasted for a total of 9 weeks, consisting of a 6-week feeding period and a 3-week laying period. The subjects were divided into seven groups, including a control group. Hot pepper waste (HPW) and ID phyt capcin (ID) groups were supplemented with feed additives at levels of 2.5%, 5%, and 7.5%, respectively. The results showed that at the end of the first 3 weeks, the 7.5% HPW group exhibited the highest live weight (BW) (P < 0.05), indicating a positive effect on growth. However, at week 6, no significant difference in BW was observed among the groups (P > 0.05). Body weight gain (BWG) was highest in the 7.5% HPW group during the T1 period (P < 0.05), but in the T2 period, the 2.5% ID and 5% ID groups demonstrated superior BWG (P < 0.05). Daily feed intake (DFI) results revealed that the highest consumption occurred in the 5% and 7.5% HPW groups during the T1 period (P < 0.05), while the lowest consumption was recorded in the 5% ID group. However, no significant differences in DFI were found among the groups during the T2 and T3 periods (P > 0.05). Feed conversion ratio (FCR) results showed significant differences between groups in all periods (P < 0.05). The 5% ID groups consistently exhibited the best feed efficiency (P < 0.05), while the 7.5% HPW group demonstrated lower feed efficiency in the T2 and T3 periods. Carcass and internal organ measurements indicated that HPW increased the gizzard weight (P < 0.05) but significantly reduced heart and spleen weights (P < 0.05). Blood parameters showed that triglyceride (TG) levels decreased in the HPW groups (P < 0.05), while these levels remained higher in the ID groups (P < 0.05). Regarding egg production, the highest production rate was recorded in the 7.5% ID group (P < 0.05), while egg yolk color was significantly superior in the HPW groups (P < 0.05). No significant differences were found among the groups for other egg quality parameters, such as shell thickness, Haugh unit, and hatchability (P > 0.05). These findings suggest that the type and dosage of feed additives can lead to varying effects. HPW positively influenced yolk color and feed intake, while ID enhanced feed conversion efficiency. The study emphasizes that the optimal use of capsaicin-based additives can improve performance and quality, although their long-term effects require further investigation.

Keywords: capsaicin, performance, egg quality, quails, pepper waste

The poultry industry continues to utilize specific feed additives to address various challenges, including disease resistance, heat stress prevention, improving feed efficiency, and promoting growth and production (27, 58, 62). These additives aim to enhance the overall health of poultry by positively influencing the digestive system, metabolism, and immune response, thereby improving animal performance. Additionally, they are designed to improve the quality of animal products, such as eggs and meat (3, 26, 49).

In the poultry industry, producers have historically employed prophylactic antibiotics and growth promoters to enhance production and health. However, these practices have led to the improper use of antibiotics. The indiscriminate use of antibiotics, commonly referred to as "antibiotic misuse", has raised concerns regarding the accumulation of antibiotic residues and the proliferation of antibiotic-resistant pathogens in poultry meat (55, 65). This phenomenon presents a significant public health risk due to antimicrobial resistance, resulting in adverse effects on both human and animal health (16, 19). Given the persistent threat of emerging antibiotic resistance mechanisms across various microorganisms, the European Commission has implemented a ban on the use of antibiotics as growth promoters in animal feed (29). This policy shift has prompted researchers to investigate alternative additives to enhance production and improve overall health outcomes (52). In poultry farming, alternative additives such as organic acids, probiotics, prebiotics, oils, enzymes, and medicinal plants are increasingly utilized (53, 65).

In recent years, the addition of plant extract mixtures to diets has been shown to improve growth performance, nutrient digestion, and digestive enzyme activities, while also modulating the gut microbiota to benefit poultry health (47, 59). Most bioactive compounds in plants consist of secondary metabolites such as terpenoids, phenolics, glycosides, and alkaloids. The composition and concentration of these bioactive components vary depending on biological factors as well as production and storage conditions. One such plant, hot pepper (Capsicum spp.), is among the most important spices in human nutrition (1), cultivated worldwide and utilized in both culinary and traditional medicinal applications (20, 37). In recent years, it has also been extensively studied as a feed additive or a ration component for animals (51). Although many species of *Capsicum* exist, five are particularly economically significant and cultivated worldwide: C. annuum, C. frutescens, C. chinense, C. baccatum, and C. pubescens (22).

Hot pepper has the potential to enhance pancreatic and intestinal enzyme activities, improve bile acid secretion, and increase body weight in poultry. Additionally, it may help reduce heat stress, improve feed digestibility, increase feed intake and feed conversion ratio, decrease mortality rates, optimize carcass characteristics, improve blood parameters, and lower production costs (1, 51). Therefore, due to its natural, non-toxic, residue-free, and easily accessible properties, its use as a phytogenic feed additive could yield positive outcomes (9, 30, 44, 47, 51).

The production of *Capsicum* species has increased by 39% between 2002 and 2022 (31), making it one of the most commercially cultivated vegetables (73). However, approximately 46% of *Capsicum* crops are estimated to be wasted annually (68). Around 26% of *Capsicum* species are not considered marketable and are treated as waste depending on the needs of the producers (17). Hot pepper (*Capsicum* spp.) waste contains capsaicin, which has bactericidal, bacteriostatic, coccidiostatic, and antifungal properties, and is rich in vitamins A and C. This suggests that it could be beneficial for animal health and meat quality, making it suitable for use as a feed additive (52). The ban on antibiotics and the high cost of commercial feed additives have made it necessary to find cheaper, more effective, and safer alternatives. In this context, the use of plant waste as a feed additive offers promising potential in terms of reducing waste, providing economic benefits, and offering reliable products to consumers. The use of hot pepper (*Capsicum annuum* L.) waste and capsaicin-based commercial feed additives is expected to improve quail growth performance, blood parameters, and egg quality, while offering cost advantages. This study aims to investigate the effects of hot pepper waste as an alternative to capsaicin-based commercial feed additives on the performance criteria of Japanese quail (*Coturnix japonica*).

## **Material and methods**

**Ethics.** Approved by Harran University (Şanlıurfa, Türkiye) Animal Experiments Local Ethics Committee with grant number KARAR2022/009/03. The study was conducted at Harran University, Faculty of Veterinary Medicine.

**Housing quails and experimental design.** A total of 315 four-day-old dual-purpose Japanese quails (*Coturnix japon-ica*) were used in this study. The quails were divided into

Tab. 1. The composition and nutritional content of basal diets

Calculated Composition	Basal Diet					
Calculated Composition	<sup>1</sup> Starter (%)	<sup>2</sup> Grower and production (%)				
Yellow Corn	50.00	54.00				
Soybean Meal (44% CP)	18.18	7.60				
Maize gluten meal (43% CP)	12.00	11.50				
Sunflower meal (45% CP)	6.50	8.00				
Soybean, Full Fat	8.50	7.90				
Di-calcium phosphate	1.05	0.70				
CaCO <sub>3</sub>	1.02	2.00				
Salt NaCl	0.40	0.30				
Premix	0.30	0.34				
Limestone	1.30	4.00				
DL-Methionin	0.20	0.30				
L-Lysine	0.55	0.36				
Total	100.00	100.00				
Calculated analysis						
ME (kcal/kg)	2906	2900				
Crude Protein (%)	24.0	20.1				
Calcium (%)	1.26	2.55				
Methionine + cysteine (%)	1.04	1.05				
Lysine (%)	1.46	1.05				
Available phosphorus (%)	0.30	0.22				

Explanations: <sup>1</sup>Premix provides per kg of starter diet: Vit A - 18.0 IU; Vit D3 - 3.96 IU; Vit E - 30 IU; Cobalt - 0.15 mg; Copper - 15.90 mg; Iodine - 0.60 mg, Iron - 205.86 mg; Manganese - 140.11 mg; Selenium - 0.38 mg, Zinc - 125.27 mg.

<sup>2</sup>Premix provides per kg of grower and production diet: Vit A – 20.40 IU; Vit D3 – 4.49 IU; Vit E – 30.0 IU; Cobalt – 0.17 mg; Copper – 15.37 mg; Iodine – 0.68 mg, Iron – 299.95 mg; Manganese – 156.84 mg; Selenium – 0.40 mg, Zinc – 134.32 mg.

seven groups, including a control group, and each treatment group was replicated three times. The quails were selected to have equal average weights and were randomly placed in cages measuring  $50 \times 50 \times 50$  cm, with 15 quails per cage. Each treatment group consisted of a total of 45 quails (15 per replication). The study was conducted between October and November 2023, with additional heating required due to meteorological conditions. The quails were kept at a temperature of  $34^{\circ}C \pm 2^{\circ}C$  during the first 14 days, and then the temperature was gradually decreased from 26°C  $\pm 2^{\circ}$ C to  $24^{\circ}$ C  $\pm 2^{\circ}$ C from day 14 to day 21. Afterward, the temperature was maintained at  $24^{\circ}C \pm 2^{\circ}C$  for the remainder of the experiment. To increase access to feed and water, the light period was extended to 23 hours of light and 1 hour of darkness. The quails had unlimited access to feed and water throughout the experiment. In the experimental design, three treatment groups received 2.5%, 5%, and 7.5% (kg/kg) levels of hot pepper (*Capsicum annuum* L.) waste (HPW) as a feed additive. The other three groups received 2.5%, 5%, and 7.5% (kg/kg) levels of a feed additive containing capsaicin (ID). The feeding trial lasted a total of six weeks (42 days), with starter feed provided during the first week and grower and production feed used for the remaining five weeks. The additives were incorporated into the feeds starting from the first week. Care was taken to minimize feed spillage, and spilled feed was weighed again. At the end of the sixth week, a random sample of quail (n = 126)were euthanized via cervical dislocation for blood analysis and carcass evaluation, and the remaining quails continued in the trial for an additional three weeks (21 days) to assess egg production and quality parameters. The nutritional composition of the base diets is presented in Table 1.

**Feed additives.** In this study, two different capsaicinbased feed additives were utilized. The first additive was Urfa Pepper (*Capsicum annuum* L.), a variety locally recognized in the Şanlıurfa region. Urfa Pepper is widely used in the production of pepper paste and powdered pepper in this area, resulting in the generation of significant amounts of pepper waste as a by-product. For this study, Urfa Pepper waste was randomly collected from the Şanlıurfa region, dried, and ground. Pepper waste (HPW) is composed of unused components such as the stem, seeds, placental tissue, and skin of the pepper fruit. The second additive was a commercially produced capsaicin-based feed additive (ID Phyt Capcin, Profeed). According to the manufacturer, ID phyt

Tab. 2. The composition and nutritional content of feed additive

Ingradiants	Feed Additive						
Ingreatents	HPW (%)	ID (%)					
Dry matter	90.69	98					
Crude Protein	18.98	4.0					
Crude Fiber	30.50	8.8					
Ether Extract	11.16	1.66					
Total Ash	8.73	55					
Calcium	1.22	18					
ME (kcal/kg)	949.88	934.15					
Capsaicinoids (g)	~5.25	5.0					

capcin (ID) is a brownish powder with a strong aroma and contains at least 10 g/kg of encapsulated pepper, palm oil, and dextrose. The additive was produced by homogenizing pepper and dextrose to create a uniform mixture, which was then coated with heated palm oil using the fluidized bed granulation method to produce encapsulated pepper. The nutritional composition of the feed additives is presented in Table 2.

**Performance parameters.** A total of 315 quails were reared to assess performance parameters. The birds were weighed weekly starting from day 4, with their body weights recorded each week. Feed intake and feed conversion ratios were calculated by weighing the remaining feed at the end of each day. All measurements were carried out using a scale with an accuracy of 0.01 g. Performance evaluations were conducted at different time intervals. Body weight (BW) was assessed at the end of the first three weeks (W3) and at the end of the six-week period (W6). Body weight gain (BWG), daily feed intake (DFI), and feed conversion ratio (FCR) were evaluated in three distinct time periods: T1 (0-3 weeks), T2 (4-6 weeks), and T3 (0-6 weeks).

**Blood constituents and carcass characteristics.** Upon completion of the experimental procedure, a total of 126 Japanese quails (3 males and 3 females from each cage) were euthanized for the evaluation of carcass and blood parameters. The weights of the carcass and internal organs were determined using a balance with a precision of 0.01 grams. The weights of the carcass, liver, heart, spleen, gizzard, and abdominal fat (AF) were recorded. Approximately 5 mL of blood was collected from each bird during the euthanasia procedure, as it flowed from the incision. Blood samples were collected in accordance with relevant standards and centrifuged at 3,000 rpm for 15 minutes to separate the serum. The serum samples were subsequently frozen at  $-20^{\circ}$ C for biochemical and hemogram analyses.

Blood parameters, including total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), calcium (Ca), and total protein (TP), were measured spectrophotometrically (Siemens Healthineers Atellica, Erlangen, Germany). Immunoglobulin levels (IgG, IgA, and IgM) were determined using the nephelometric method (Siemens BN2 analyzer, Erlangen, Germany). Hematological parameters, including white blood cells (WBC), monocytes (MONO), neutrophils (NEU), red blood cells (RBC), hemoglobin (HGB), procalcitonin (PCT), mean platelet volume (MPV), and heterophil-to-lymphocyte ratio (H/L), were analyzed using a hematology analyzer (Abbott Diagnostics, Santa Clara, California, USA). All measurements were conducted using commercial kits. The oxidative stress index (OSI) was calculated using the following formula: OSI = (TOS/TAS) (18).

**Egg quality characteristics.** After a six-week feeding trial, 126 quails were sacrificed for blood analysis, and 189 of the initial 315 quails continued in the study. From the remaining quails, a total of 147 quails were selected for the egg trial (5 females and 2 males from each group, with 21 quails per trial group and 7 quails per replicate). The quails that continued in the trial were housed together without changing their cages, and both males and females were kept together to calculate hatchability (HT). The

feeding period was completed at the end of the sixth week, and the first egg-laving was observed in the groups. Consequently, egg measurements started in the sixth week and continued for three weeks. During this period, one egg per day was randomly selected for each replicate and placed in an incubator to calculate hatchability (HT) rates. HT was calculated by dividing the number of hatched chicks by the number of eggs placed in the incubator. Egg weight (EW), shell weight (SW), yolk weight (YW), and albumen weight (AW) were measured using a scale with 0.01 g precision. The length (EL) and width (EGW) of the eggs, as well as the height (AH), width (ALW), and length (AL) of the albumen, and the height (YH) and width (YOW) of the yolk, were measured using a digital caliper with 0.001 mm precision. Shell thickness [EST: Egg Equatorial Shell Thickness, PST: Egg Pointed-End Shell Thickness, BST: Egg Blunt-End Shell Thickness] was assessed using a micrometer with the same precision. Egg production (EP) rate (%) was determined based on the daily egg data recorded for each cage. The color of the egg yolk (YC) was evaluated using the DSM YolkFan color scale (1: very light, 15: dark yellow-orange). Eggs with darker yolks were classified as 16 (orange). Haugh unit (HU) was calculated using the following formula:  $HU = 100 \log [(H + 7.57) - (1.7W^{0.37})].$ Here, H represents albumen height (mm) and W represents egg weight (g) (36). The Haugh unit (HU) was calculated as an indicator of egg quality.

**Statistical analysis.** The statistical analysis of continuous data obtained in this study, including live weight, slaughtercarcass characteristics, egg quality traits, and blood parameters, was conducted using analysis of variance (ANOVA) for variables that met the assumptions of parametric tests. Differences between the treatment groups were assessed at a significance level of 5%. In instances where statistically significant differences were identified among the groups, Duncan's multiple comparison test was employed to determine which specific group or groups were responsible for those differences. All analyses were performed using SPSS 21 (38).

## **Results and discussion**

The results for body weight (BW), body weight gain (BWG), daily feed intake (DFI), and feed conversion ratio (FCR) are presented in Table 3.

**Body weight (BW).** The results showed significant differences between the groups at the end of the third week (W3) (P<0.05), but no differences were observed during the W6 period (P>0.05). The 7.5% HPW group exhibited the highest body weight values during the W3 period, indicating a positive effect of HPW on growth during the first three weeks. The effects of 2.5% ID and 5% HPW were similar to the control group, while the 7.5% ID group had the most negative impact on body weight. This finding suggests that the effect of ID on growth is less pronounced compared to HPW. By the W6 period, differences between the groups had disappeared, and both additives had lost their effects.

Body weight gain (BWG). The BWG data revealed statistically significant differences between the groups during both T1 and T2 periods (P < 0.05). In the T1 period, the highest BWG value was observed in the 7.5% HPW group compared to the control group, indicating that the higher HPW concentration positively influenced weight gain during the first three weeks. However, in the 5% ID, 7.5% ID, and 2.5% HPW groups, a negative change in BWG values was observed compared to the control group, suggesting that the effect of ID on weight gain during the first three weeks is limited compared to HPW. In the T2 period, the results exhibited a different trend. During this period, a negative effect was observed only in the 7.5% HPW group. In contrast, the 2.5% and 5% ID groups showed the highest BWG values compared to the control group. Unlike T1, the effect of HPW on weight gain was more limited in comparison to ID during the T2 period. No significant differences were detected between the groups in the T3 period (P > 0.05).

Criteria	Weeks	Control	2.5% ID	5% ID	7.5% ID	2.5% HPW	5% HPW	7.5% HPW	SEM	P-value
BW	W3	118 <sup>ab</sup>	115ªb	113 <sup>bc</sup>	107º	113 <sup>bc</sup>	117ªb	120ª	0.917	0.004
	W6	206	214	212	201	208	205	203	1.626	0.341
	T1	4.97 <sup>ab</sup>	4.81 <sup>ab</sup>	4.70 <sup>bc</sup>	4.46°	4.72 <sup>bc</sup>	4.94 <sup>ab</sup>	5.07ª	0.038	< 0.001
BWG (g/day)	T2	4.17 <sup>bc</sup>	4.73ª	4.74ª	4.47 <sup>ab</sup>	4.50 <sup>ab</sup>	4.23 <sup>bc</sup>	<b>3.96</b> ⁰	0.047	< 0.001
	Т3	4.57	4.77	4.72	4.46	4.61	4.59	4.52	0.036	0.258
	T1	17.0 <sup>abc</sup>	17.15ªb	15.15°	16.48 <sup>abc</sup>	15.32 <sup>bc</sup>	17.67ª	17.47ª	0.280	0.047
DFI (g/day)	T2	26.96	28.96	28.96	28.15	27.67	28.03	28.24	0.200	0.067
(g/uuy)	Т3	21.98	23.05	22.06	22.32	21.51	22.85	22.86	0.190	0.256
FCR (g feed/g weight)	T1	3.49 <sup>bc</sup>	3.65 <sup>ab</sup>	3.32 <sup>°</sup>	3.78ª	3.30°	3.65 <sup>ab</sup>	3.48 <sup>bc</sup>	0.033	< 0.001
	T2	6.84 <sup>b</sup>	6.22 <sup>℃</sup>	6.19°	6.48 <sup>bc</sup>	6.51 <sup>bc</sup>	6.71 <sup>bc</sup>	7.40ª	0.073	< 0.001
	Т3	4.93 <sup>ab</sup>	4.90 <sup>ab</sup>	4.74 <sup>b</sup>	5.12ª	4.79 <sup>b</sup>	5.03 <sup>ab</sup>	5.14ª	0.040	0.038

Tab. 3. The effect of hot pepper waste and ID phyt capcin as feed additives on the performance of growing Japanese Quails

Explanations: a-b-c – Means within the same row bearing different superscripts differ significantly. No statistically significant difference was observed between groups for the gender \* group interaction (P > 0.05). FCR – feed conversion ratio; BW – body weight; BWG – body weight gain; DFI – daily feed intake; T1 – 0-3 weeks; T2 – 4-6 weeks; T3 – 0-6 weeks; W3 – end of week 3; W6 – end of week 6; SEM – standard error mean; ID – Id Phyt Capcin; HPW – hot pepper waste.

**Daily feed intake (DFI).** The DFI results showed significant differences between the groups during the T1 period (P < 0.05), but no significant differences were observed during the T2 and T3 periods (P > 0.05). In the T1 period, the highest DFI values were observed in the 5% and 7.5% HPW groups compared to the control group, while the lowest values were found in the 5% ID and 2.5% HPW groups. These findings suggest that higher concentrations of HPW during the first three weeks increase feed intake, indicating a positive effect of HPW on feed consumption.

Feed conversion ratio (FCR). The FCR results showed significant differences between the groups across all periods (P < 0.05). In the T1 period, lower FCR values were observed in the 5% ID and 2.5% HPW groups, while the highest FCR value was found in the 7.5% ID group. This indicates that high ID concentrations lead to lower feed efficiency initially. In the T2 period, the 7.5% HPW group exhibited the highest FCR value, while the 2.5% and 5% ID groups had lower FCR values. During this period, ID groups performed better in feed efficiency, while higher concentrations of HPW had a negative impact. In the T3 period, FCR values became more balanced, but higher FCR values were still observed in the 7.5% ID and 7.5% HPW groups. Overall, ID groups showed more efficient feed utilization, while high concentrations of HPW negatively affected feed efficiency in the long term.

**Carcass characteristics.** Following the completion of the experimental procedure, a total of 126 quails were slaughtered, and carcass characteristics were evaluated. Six quails from each group (3 males and 3 females) were analyzed (Tab. 4). The analysis revealed no significant differences between the groups in terms of live weight, carcass weight, liver weight, and abdominal fat percentage (P > 0.05). This suggests that the feed additives had a limited effect on metabolic fat deposition. However, significant differences were observed between the groups regarding heart and spleen weights, with reductions observed in all groups compared to the control group (P < 0.05). The lowest heart weight was found in the 7.5% ID group, while the lowest spleen weight was observed in the 7.5% HPW group. These findings indicate that the presence of high capsaicin levels may limit the development of the heart and spleen. The gizzard weight, however, increased in all groups compared to the control group, except for the 7.5% ID group, with the highest gizzard weights observed in the HPW groups.

**Blood parameters.** Blood samples were collected simultaneously from the 126 quails used for carcass and internal organ measurements. A total of 20 parameters were obtained from the collected blood samples through biochemical and hemogram tests (Tab. 5). The analysis revealed a significant difference between the groups in triglyceride (TG) levels (P < 0.05). Compared to the control group, all HPW groups had lower TG levels, while the 5% and 7.5% ID groups showed similar values to the control group; however, the 2.5% ID group exhibited higher TG levels. No significant differences were observed between the groups in total cholesterol (TC), HDL, and LDL parameters (P > 0.05). Significant differences were found in Total Antioxidant Status (TAS), Total Oxidant Status (TOS), and Oxidative Stress Index (OSI) values (P < 0.05). All ID and HPW groups significantly affected TAS values, with the 7.5% HPW supplementation showing the lowest TAS value. Similarly, the lowest TOS value was recorded in the 7.5% HPW group, while the highest value was observed in the 2.5% HPW group. In contrast to TAS and TOS values, the Oxidative Stress Index (OSI) value was higher in all groups except the 7.5% HPW group compared to the control group, with significant differences observed (P < 0.05). Regarding immunoglobulin levels, no significant difference was found in IgG levels (P > 0.05), whereas significant differences were observed in IgA and IgM levels (P < 0.05). The highest IgM levels were found in the 2.5% ID and 2.5% HPW supplemented groups, indicating that lower levels of capsaicin increased IgM levels. The highest IgA levels were observed in the 7.5% ID and 5% HPW groups. No significant differences were

Parameters	Control	2.5% ID	5% ID	7.5% ID	2.5% HPW	5% HPW	7.5% HPW	SEM	P-value
LBW	211	210	209	198	209	207	201	2.37	0.712
Carcass	145	145	146	135	142	139	136	1.25	0.079
Heart	1.75ª	1.68 <sup>ab</sup>	<b>1.68</b> <sup>ab</sup>	1.48°	1.62 <sup>abc</sup>	<b>1.66</b> <sup>ab</sup>	1.55 <sup>bc</sup>	0.02	0.015
Spleen	0.11ª	0.09 <sup>abc</sup>	0.10 <sup>ab</sup>	0.10 <sup>ab</sup>	0.08 <sup>bc</sup>	0.10 <sup>ab</sup>	0.07°	0.004	0.014
Liver	4.91	4.97	4.52	4.10	4.66	4.70	4.30	0.13	0.582
Gizzard	3.37 <sup>bc</sup>	3.63 <sup>abc</sup>	3.54 <sup>abc</sup>	3.31°	3.75 <sup>abc</sup>	3.84 <sup>ab</sup>	3.99ª	0.06	0.029
AF	2.53	2.32	2.22	1.98	2.17	2.67	1.96	0.09	0.219

Tab. 4. Carcass characteristics of Japanese quails fed diets containing hot pepper waste and ID Phyt Capcin

Explanations: a-b-c – Means within the same row bearing different superscripts differ significantly. No statistically significant difference was observed between groups for the gender \* group interaction (P > 0.05). AF – abdominal fat; LBW – live body weight; ID – Id Phyt Capcin; HPW – hot pepper waste; SEM – Standard error mean.

Parameters	Control	2.5% ID	5% ID	7.5% ID	2.5% HPW	5% HPW	7.5% HPW	SEM	P-value
TAS (mmol/l)	1.72ª	1.33⁵	1.48 <sup>b</sup>	<b>1.44</b> <sup>b</sup>	1.49 <sup>b</sup>	<b>1.44</b> <sup>b</sup>	1.16º	0.032	< 0.001
TOS (µmol/l)	8.35 <sup>ab</sup>	7.36 <sup>bc</sup>	8.06 <sup>b</sup>	7.71 <sup>b</sup>	10.04ª	8.92ab	<b>5.72</b> ⁰	0.250	< 0.001
OSI	<b>4.87</b> <sup>b</sup>	5.82ab	5.56 <sup>ab</sup>	5.78ab	7.04ª	6.22ab	5.21 <sup>b</sup>	0.185	0.049
WBC (× 10³/µL)	19.57	17.0	16.50	16.12	17.20	17.27	17.40	0.480	0.619
H/L	5.58	5.68	5.73	6.05	6.90	6.21	5.61	0.250	0.800
MONO (× 10³/µL)	0.13ª	0.05	0.07 <sup>b</sup>	0.07 <sup>b</sup>	0.06 <sup>b</sup>	0.07 <sup>b</sup>	0.04 <sup>b</sup>	0.006	0.003
NEU (× 10³/µL)	0.12ª	0.11 <sup>ab</sup>	0.07 <sup>bc</sup>	0.08 <sup>bc</sup>	0.09 <sup>abc</sup>	0.07 <sup>bc</sup>	0.05°	0.005	0.002
RBC (× 10 <sup>6</sup> /µL)	3.38	3.26	3.16	3.18	3.18	3.23	3.29	0.035	0.641
HGB (g/dL)	8.05	9.05	8.13	8.18	8.08	7.90	8.41	0.145	0.431
PCT (%)	0.013ª	0.009ab	0.010ª	0.010 <sup>ab</sup>	0.009 <sup>ab</sup>	0.008 <sup>ab</sup>	0.005 <sup>b</sup>	0.001	0.039
MPV (fL)	15.35ª	12.22 <sup>abc</sup>	13.62ab	12.43 <sup>abc</sup>	12.64 <sup>abc</sup>	<b>11.00</b> <sup>bc</sup>	9.38	0.474	0.031
LDL (mg/dL)	35.39	43.11	43.33	39.22	44.28	36.78	39.89	1.010	0.211
HDL (mg/dL)	117.0	111.2	112.2	108.1	116.2	117.0	116.0	1.470	0.570
Calcium (mg/dL)	12.86	13.13	13.41	13.64	12.60	13.10	11.49	0.357	0.772
TC (mg/dL)	186.5	197.1	190.1	181.3	189.8	181.9	181.2	2.500	0.579
TG (mg/dL)	170.4ªb	214.2ª	172.5ªb	170.0 <sup>ab</sup>	146.4 <sup>b</sup>	140.8 <sup>b</sup>	126.6 <sup>b</sup>	6.690	0.013
TP (g/L)	2.26	2.24	2.26	2.33	2.17	2.18	2.17	0.030	0.770
IGG (mg/dL)	0.67	0.97	0.69	0.58	0.73	0.53	0.68	0.037	0.053
IGM (mg/dL)	0.40 <sup>b</sup>	0.85ª	0.43 <sup>b</sup>	0.72 <sup>ab</sup>	0.83ª	0.56 <sup>ab</sup>	0.43 <sup>b</sup>	0.049	0.032
IGA (mg/dL)	0.34 <sup>ab</sup>	0.37 <sup>ab</sup>	0.35 <sup>ab</sup>	0.44ª	0.42 <sup>ab</sup>	0.45ª	0.33 <sup>b</sup>	0.012	0.041

Tab. 5. Hematological and biochemical blood values of Japanese quails

Explanations: a-b-c – Means within the same row bearing different superscripts differ significantly. No statistically significant difference was observed between groups for the gender\* group interaction (P > 0.05). TAS – total antioxidant status; TOS – total oxidant status; OSI – oxidative stress index; WBC – white blood cell; H/L – heterophil/lymphocyte index; MONO – monocyte; NEU – neutrophil; RBC – red blood cells; HGB – hemoglobin; PCT – procalcitonin; MPV – mean platelet volume; LDL – low-density lipoprotein; HDL – high-density lipoprotein; TG – triglyceride; IGG-IGM-IGA – immunoglobulin G-M-A; TC – total cholesterol; TP – total protein; ID – Id Phyt Capcin; HPW – hot pepper waste; SEM – standard error mean.

found in HB, H/L, WBC, and RBC values, indicating that these parameters were not affected by the presence of capsaicin (P > 0.05).

Egg characteristics. All egg characteristics are presented in Table 6. According to the results, significant differences were observed in egg production values between the groups (P < 0.05). The highest values were observed in the groups supplemented with ID, while the HPW groups showed lower values compared to the control group. The 7.5% ID group provided the best results. In contrast, a clear superiority of HPW was observed in yolk color. The presence of ID did not show any effect, while higher values were found in all HPW groups (P < 0.05), with the highest values observed in the 5% and 7.5% HPW groups. Another difference was observed in yolk weight. Except for the 2.5% ID group, all other groups showed higher yolk weight compared to the control group (P < 0.05). Albumin height showed a decreasing trend in all groups compared to the control group (P < 0.05), with the lowest values observed in the groups supplemented with high levels of ID and HPW. No significant differences were observed between the groups in terms of Haugh unit, hatchability, egg shape measurements, and shell thickness (P > 0.05). These results suggest that certain additives have significant effects on specific egg characteristics, but limited effects on other parameters.

Capsicum (hot pepper) can enhance pancreatic and intestinal enzyme activities, improve bile acid secretion, and increase body weight in poultry. Additionally, it may reduce heat stress and improve feed digestibility, feed intake, feed conversion efficiency, mortality rates, carcass characteristics, blood parameters, and production costs (51). However, in this study, the body weight (BW) and body weight gain (BWG) data indicate that the effects of feed additives vary periodically. In the W3 period, the group with the highest BW and BWG in the T1 period was the 7.5% HPW group. This suggests that HPW positively influences growth during the first three weeks. On the other hand, the negative effects of 2.5% HPW and the results of the 5% HPW group being similar to the control group suggest that higher doses may be more effective. The reduction in the effect of 7.5% HPW in the T2 period indicates that this additive has a short-term effect. This finding is consistent with the results of Reda et al. (63), who conducted a study by adding red pepper oil at different levels to their feed. In their study, positive effects on BW and BWG were observed in all groups during the first three weeks, while this effect decreased in the

Tał	<b>6</b> . 6.	The	effect of	of ID	and	HPW	on	egg	quality	characteristics
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	Control	2.5% ID	5% ID	7.5% ID	2.5% HPW	5% HPW	7.5% HPW	SEM	P-value
EP (%)	71.21 <sup>b</sup>	78.18 <sup>ab</sup>	76.03ªb	83.33ª	78.48 <sup>ab</sup>	73.62 <sup>b</sup>	73.94 <sup>b</sup>	0.985	0.021
EW (g)	12.09	11.94	12.48	12.37	12.32	12.01	12.08	0.078	0.447
EGW (mm)	26.07	25.99	26.14	26.35	26.20	26.07	26.06	0.064	0.826
EL (mm)	32.81	32.53	33.41	32.85	33.07	32.67	32.79	0.104	0.397
YW (g)	3.74 <sup>bc</sup>	3.57⁰	<b>4.17</b> <sup>a</sup>	4.04 <sup>ab</sup>	3.90 <sup>abc</sup>	4.02 <sup>ab</sup>	4.05 <sup>ab</sup>	0.051	0.019
YOW (mm)	25.65	25.27	25.89	25.72	26.11	25.94	26.05	0.128	0.619
YC	11.79°	12.2°	12.29°	11.71°	13.87 <sup>b</sup>	15.0ª	14.87ª	0.156	< 0.001
YH (mm)	11.32	11.61	12.0	11.79	12.0	11.59	11.62	0.073	0.139
AW (g)	8.31	7.83	7.85	8.18	7.89	80.03	7.31	0.161	0.766
ALW (mm)	34.04	34.80	36.16	35.82	35.48	34.07	35.09	0.296	0.373
AL (mm)	45.88 <sup>b</sup>	44.91 <sup>b</sup>	49.38ª	46.07 <sup>b</sup>	45.95 <sup>b</sup>	48.16ªb	45.61 <sup>b</sup>	0.405	0.030
AH (mm)	5.87ª	5.27 <sup>b</sup>	4.73⁰	<b>4.74</b> ⁰	5.12 <sup>bc</sup>	<b>4.63</b> ℃	4.72℃	0.073	< 0.001
SW (g)	1.04	1.02	1.02	1.02	1.05	1.03	0.99	0.008	0.637
EST (mm)	0.228	0.223	0.219	0.224	0.232	0.237	0.220	0.002	0.160
PST (mm)	0.241	0.238	0.242	0.235	0.249	0.255	0.245	0.003	0.384
BST (mm)	0.221	0.213	0.211	0.214	0.216	0.213	0.210	0.002	0.730
AST (mm)	0.215	0.200	0.224	0.210	0.232	0.235	0.225	0.004	0.166
SI (%)	79.51	79.92	78.43	80.24	79.28	79.84	79.52	0.272	0.715
HT (%)	72.9	74.7	75.6	74.0	74.5	72.2	76.0	1.026	0.974
HU	90.16	93.29	87.50	87.58	92.20	89.83	90.40	0.830	0.457

Explanations: a-b-c – Means within the same row bearing different superscripts differ significantly. EP - egg production; EW - egg weight; EGW - egg width; EL - egg length; YW - yolk weight; YOW - yolk width; YC - yolk color; YH - yolk height; AW - albumen weight; ALW - albumen width; AL - albumen length; AH - albumen height; SW - shell weight; EST - egg equatorial shell thickness; PST - egg pointed-end shell thickness; BST - egg blunt-end shell thickness; AST - average egg shell thickness; SI - shape index; HT - hatchability; HU - haugh unit; ID - Id Phyt Capcin; HPW - hot pepper waste; SEM - standard error mean.

subsequent weeks. Similarly, in the study by Tayeb et al. (72) on red pepper supplementation, BW and BWG increased in the 2<sup>nd</sup> and 3<sup>rd</sup> weeks, but no significant changes in weight gain were observed in the later weeks. These data are consistent with the findings of our study and support the short-term effects of HPW. In contrast, Özer et al. (57) noted that adding low doses of hot pepper to the rooster diet during the developmental period could reduce weight gain, and they suggested that this inconsistency might be due to variations in the amount of spice used. In the ID groups, BW values were generally close to or lower than the control group. Notably, the 7.5% ID group showed the lowest BW and BWG values during the first three weeks, indicating a negative effect of ID on growth during this period. While BWG remained low in the 2.5% and 5% ID groups in the T1 period, these groups exceeded the control group and reached the highest BWG values in the T2 period, suggesting that ID may be a more effective additive in the long term.

By the sixth week, it was observed that body weight (BW) and body weight gain (BWG) values had reached similar levels across all groups (P < 0.05), and there was no persistent effect of the additives. This finding aligns with some studies that reported HPW supplementation had no effect on final BW and BWG (2, 32,

34, 72). This suggests that the effects of feed additives may change over time and could influence growth performance differently during different periods. The reduction in differences between groups and the stabilization of parameters in long-term evaluations indicate that the effects of additives may vary over time. Despite the similar metabolizable energy (ME) values of both additives (Tab. 2), the differences in their compositions could be a potential reason for the observed differences in BW and BWG. However, considering the inclusion rates of the additives in the diets, it is debated whether these differences are substantial enough to affect the groups to a significant degree.

The DFI data indicate that feed consumption was higher during the T1 period in the presence of high HPW and low ID. This finding is consistent with the results of BW and BWG during the first three weeks. Several studies have suggested that the addition of hot pepper to the feed may increase feed intake during the initial periods (2, 15). Specifically, in the 5% and 7.5% HPW groups, the increased feed consumption during the first three weeks may be attributed to the appetite-enhancing effect of hot pepper in poultry. The inclusion of hot red pepper has been shown to cause significant changes in the energy balance of poultry, thereby increasing appetite and affecting feed intake

(74). Adedoyin et al. (6) reported that chicks fed diets enriched with hot red pepper exhibited higher average feed consumption compared to the control group. Similarly, Sayeed et al. (67) found that the use of plant supplements, such as protexin and hot pepper, significantly increased feed consumption compared to the control group (P < 0.05). Capsaicin and other antioxidants can positively affect the digestive system by enhancing the function of pancreatic enzymes (amylase, protease, and lipase) (7). The increase in these enzymes stimulates bile acid synthesis in the liver, leading to improvements in digestion and nutrient absorption. However, in this study, the increase in feed consumption was only observed during the T1 period, indicating that the appetite-enhancing effect of capsaicin in hot pepper waste and ID supplementation was limited. Although high feed consumption and BWG were observed in HPW groups during the T1 period, this effect diminished in the T2 and T3 periods, and no significant improvement in feed efficiency was achieved. In another study, broilers supplemented with 0.3% hot pepper in their diet showed a decrease in feed consumption (13). This suggests that the appetite-enhancing effect of capsaicin may not have a sustainable impact on growth and feed efficiency in the long term, highlighting the importance of other factors (nutrient content, energy utilization, digestive efficiency, etc.) in influencing feed intake and growth.

Improvements in feed conversion efficiency (FCR) may be observed in poultry diets supplemented with capsaicin; this is likely due to the stimulatory, digestivefacilitating, and antimicrobial properties of capsaicin (12). When examining FCR data for the T1, T2, and T3 periods, it is evident that the HPW groups, with higher feed intake, had higher FCR values, and consequently, exhibited lower feed efficiency. Specifically, during the T1 period, despite the positive effect of increased feed intake on growth in the HPW groups, feed efficiency was negatively impacted. During this period, the ID groups demonstrated more efficient growth with lower FCR values, indicating that they gained more weight with less feed. In the T2 period, despite an increase in feed intake, the HPW groups continued to show higher FCR values, with a decline in efficiency. Conversely, the ID groups continued to grow efficiently with lower feed intake. In the T3 period, despite high DFI values in the HPW groups, FCR values decreased, and the relationship between growth and feed efficiency weakened. The ID groups continued to exhibit more efficient growth with lower feed intake. These findings suggest that the differences observed in our study cannot be solely attributed to the presence of capsaicin but are also linked to the nutritional content of the additives. However, several studies have reported that capsaicin content contributes to the improvement of FCR values (2, 6, 15, 63, 69). Another study mentioned that the inclusion of garlic, black pepper, hot pepper, and their mixtures in broiler diets resulted in positive outcomes for FCR values (60).

In this study, the HPW groups were generally associated with higher feed intake (DFI) and body weight gain (BWG), but exhibited lower feed efficiency (high FCR). Although the HPW groups showed rapid weight gain initially, feed efficiency decreased in the long term, while the ID groups demonstrated more efficient growth with lower feed intake. These findings indicate that high feed intake does not always lead to more efficient feed utilization, and feed efficiency can vary depending on several factors, such as the feed composition and the metabolic responses of the animals.

The analyses revealed no significant differences between the groups in terms of live weight, carcass weight, liver weight, and abdominal fat percentage (P > 0.05). These findings suggest that the effects of the additives on metabolic fat deposition and overall carcass weight are limited. While these results are consistent with some studies in the literature, they also show certain discrepancies. For instance, Reda et al. (63) found that the addition of pepper oil to Japanese quail diets did not affect carcass, liver, heart, or gizzard weights. On the other hand, Afolabi et al. (8) reported that dietary supplementation with 0.1%, 0.2%, and 0.3% red hot pepper had no effect on carcass weight, liver, heart, stomach, or abdominal fat weight, but significantly reduced kidney weight at the 0.3% level. Shahverdi et al. (69) reported that the supplementation of hot pepper or its combination with black pepper significantly increased the weights of various organs (liver, thigh, breast meat, gizzard, heart, and spleen) and reduced abdominal fat percentage. However, the highest values for heart and spleen weights were observed in the control group, suggesting that capsaicinoid content might reduce heart and spleen weights. This finding aligns with a separate study that reported a decrease in heart weight (72). Furthermore, the increase in gizzard weight suggests a potential positive effect of HPW on gizzard development, which is consistent with other studies reporting an increase in gizzard weight in the literature (14, 15). These discrepancies highlight that the effects of additives are not solely dependent on type, dosage, and application duration, but also influenced by factors such as the animals' genetic makeup, physiological responses, and environmental conditions. The results of our study are partially consistent with the current literature and emphasize the need for a comprehensive evaluation of the long-term effects of additives.

The blood analysis results indicated significant differences between the groups in terms of triglyceride (TG) levels (P < 0.05). The supplementation of ID increased TG levels, suggesting its effect on enhancing fatty acid metabolism and consequently increasing the circulating concentrations of lipoproteins. This finding strengthens the idea that both supplements are related to calcium (Ca) content, as their metabolizable energy (ME) values are quite similar. It is well-known that calcium supplementation has beneficial effects on lipid metabolism (64). However, no significant differences were observed between the groups regarding total cholesterol (TC), HDL, and LDL parameters (P > 0.05). These findings differ from several studies in the literature. These studies have reported that the supplementation of red pepper in the diet significantly reduces TG, LDL, and TC levels while increasing HDL levels (4, 60, 61, 63, 69). On the other hand, one study indicated that capsaicin content did not significantly affect TC levels but reduced triglycerides (TG). Additionally, some studies have reported that incorporating spice plants into animal feeds may increase the activity of enzymes responsible for converting cholesterol into bile acids, leading to a decrease in cholesterol and TG levels and, consequently, an improvement in lipid profile (60, 71). The reductions in TG observed in HPW groups may be attributed to this mechanism. Given that HPW is particularly rich in capsaicinoids and phenolic compounds, it could play a regulatory role in lipid metabolism by limiting triglyceride synthesis or enhancing the utilization of fats as an energy source. As a result, a decrease in plasma TG levels was observed. These findings suggest that the beneficial effects of HPW on the blood lipid profile could not only optimize energy utilization but also improve animal health and performance.

Statistical analyses revealed significant differences between groups in total antioxidant capacity (TAS), total oxidant status (TOS), and oxidative stress index (OSI) values (P < 0.05). In HPW groups, TAS values were significantly lower, with the lowest value recorded in the 7.5% HPW group. This finding suggests that HPW may have an inhibitory effect on antioxidant capacity. On the other hand, the higher TAS values in the ID groups indicate better preservation of antioxidant capacity and reduced metabolic stress in these groups. This suggests that ID supplementation may have a more favorable impact on oxidative stress and animal health. Similarly, for TOS values, the lowest value was observed in the 7.5% HPW group, while the highest value was observed in the 2.5% HPW group. This indicates that HPW may control oxidant levels at lower concentrations, but higher levels might lead to increased oxidative stress. Regarding OSI values, the highest value was observed in the 2.5% HPW group, while the lowest value was observed in the control group (P < 0.05). The lowest value in the control group, which was the only group without capsinoids, may indicate that the presence of capsinoids significantly increases oxidative stress. The findings of this study corroborate the idea that the effects of phytogenic feed additives on antioxidant capacity and oxidative stress may vary depending on the type and level of the additive used. For instance, Gül and Cufadar (35) reported that the addition of hot pepper oil to diets did not have a significant effect on TAS, TOS, and OSI values. In contrast, Kirar et al. (41) reported that sumac supplementation increased TAS values while decreasing OSI values in quail diets. Furthermore, Önel et al. (54) observed a reduction in OSI values in quails fed with murt essential oil. These results highlight that the effects of phytogenic feed additives are influenced by both the type of additive and its usage level, and should be evaluated in conjunction with findings from the literature.

When examining immunoglobulin levels, it was found that IgG did not show a significant difference between groups (P > 0.05), while IgA and IgM levels displayed significant differences (P < 0.05). This suggests that the additives used did not have a significant effect on IgG production. The highest IgM levels were observed in the presence of low ID and HPW compared to the control group. IgM plays a role in immune functions such as lysing bacteria and viruses, and it generally serves as the first line of defense in the body (45). These findings suggest that certain additives may activate the immune response early on. In contrast, IgA levels showed the highest values in the presence of high ID and HPW. IgA plays a crucial role, particularly on mucosal surfaces (e.g., respiratory and digestive systems), and these findings may indicate the effect of the additives on this immune pathway (23). These results demonstrate that the additives used have specific effects on the immune system, particularly increasing IgM and IgA levels, which may suggest a stronger immune response. Capsaicinoids, which are components rich in vitamin C and found in the structures of stress hormones, are known to strengthen the immune system and improve disease resistance by reducing the H/L ratio (42). However, no significant difference was observed in the H/L ratio between groups in our findings (P > 0.05). Additionally, Sayeed et al. (67) noted that adding 2% red pepper to quail diets could provide benefits for performance and blood metabolites.

When the data on egg characteristics were analyzed. no significant differences were observed between the groups in terms of egg weight (P > 0.05), while statistically significant differences were found in egg production (EP) values (P < 0.05). Although the 2.5% HPW supplementation was observed to increase egg production compared to the control group, it was generally found that HPW maintained egg production at an optimal level. In contrast, higher egg production was obtained in all ID groups compared to the control group. The different effects of both additives may be attributed to the differences in their contents (Tab. 2). However, it should be noted that physiological differences may also have an impact on these results. In particular, the relationship between triglycerides (TG) levels, and egg production should not be overlooked.

TG levels are critical for the adequacy of lipids, which are structural components of the follicles (25). Since a large portion of follicular fluid consists of lipoproteins and water (46), changes in lipid metabolism can directly affect the egg formation process. Increased lipid levels can serve as an energy source for egg production and provide significant support for follicular development (25). According to the data in Table 5, it is understood that the 2.5% ID supplementation increased TG levels, thereby affecting fatty acid metabolism and raising lipoprotein levels. This finding provides significant evidence that the ID supplementation enhances EP. Moreover, maintaining TG levels at an optimal level in the 5% and 7.5% ID groups contributed to an increase in egg production. This result supports the idea that ID supplementation positively contributes to egg production capacity by maintaining TG levels at appropriate levels. However, this situation strengthens the idea that egg production capacity may be related to the content of the ID supplementation. The superiority of ID supplementation in egg production can also be explained by its high calcium carbonate  $(CaCO_2)$  content (Tab. 2). Calcium plays a significant role in the formation of eggshells and is essential for maximum egg production, quality, and hatchability. It increases mobilization in the intestines and bones, ensuring continuous renewal of blood calcium levels (40). Therefore, the diet of laying hens should contain adequate amounts of calcium in a form that can be efficiently utilized (66). However, when egg quality characteristics were examined, no effect of ID supplementation was observed in terms of shell characteristics, and no significant difference was detected between the groups (P > 0.05). In light of these findings, it can be stated that more specific studies are needed to determine how ID supplementation affects egg production and quality.

The most prominent effect of HPW supplementation was the change in yolk color. The primary cause of this change is known to be the presence of HPW, rather than capsaicin. Yolk color was significantly affected in all HPW groups (P < 0.05), whereas no effect was observed in the presence of ID (P > 0.05). The main reason for this is the carotenoids present in HPW, which influence and enhance yolk color. Animals can only convert or metabolize carotenoids; they cannot synthesize them (70). *Capsicum* species are rich in carotenoids (pro-vitamin A), which contribute to cellular activities and growth. They are also rich in vitamins A, B, C, and certain minerals (11). Like antioxidants, carotenoids neutralize excess free radicals and protect cells from toxic effects (24). Similar to the current study, other researchers have reported improvements in yolk color when phytogenic additives are included in diets (5, 10, 21, 34, 43, 48, 50). Consumers' egg preferences are now based not only on the cholesterol content or fatty acid profile of the yolk but also on the health benefits, including its color (28). Yolk color is an important purchasing criterion for consumers (11). Additionally, the economic impact of these effects on egg production costs and their consideration within the context of sustainable livestock practices must be evaluated. Considering changes in consumer preferences, the use of feed supplements with high carotenoid content could contribute to the development of innovative products in the egg industry, both aesthetically and in terms of health.

The Haugh unit (HU) is a significant indicator for evaluating egg freshness and internal quality, and is commonly associated with shelf life (56). HU and the yolk index are key parameters for determining egg internal quality, with higher values indicating better quality (33). Fresh eggs collected from young flocks typically have HU values of 85 or higher; however, these values tend to decrease due to factors such as age, storage conditions, and temperature (39). In the present study, the HU values of all experimental groups were above 85, indicating that the eggs generally had good quality. However, the lack of a significant difference in HU values between the groups (P > 0.05) suggests that the feed additives did not have a noticeable effect on this parameter.

The results of this study demonstrate that the seasonal effects of feed additives vary in terms of metabolic parameters and performance. HPW initially had a positive effect on growth performance and feed intake, but in the long term, it reduced feed efficiency and decreased TG levels. In contrast, ID supplementation increased TG levels, supporting follicular development and enhancing egg production. One of the most notable effects of HPW was the improvement in yolk color, which is related to the carotenoid content.

Further detailed research is recommended to investigate the long-term effects of both ID and HPW supplements, as well as their applicability in different species. The impact of carotenoid-rich diets on egg quality and consumer preferences should be considered within the context of sustainable livestock practices. Additionally, studies examining the bioavailability of calcium sources and their effects on performance could contribute to the development of new strategies to enhance egg production. The economic dimension of these additives and their impact on production costs should also be considered, and future research should broaden its scope to encompass these factors.

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