Animal Science Papers and Reports vol. 42 (2024) no. 1, 51-64
DOI: 10.2478/aspr-2023-0022
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of the Polish Academy of Sciences, Jastrzębiec, Poland

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Dietary supplementation of *Agaricus bisporus* by-products on development, egg production, egg quality, and antioxidant capacity of yolk in laying quails

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(Accepted February 12, 2024)

The effect of dietary supplementation with *Agaricus bisporus mushrooms* powder (ABP) from by-products on the development, egg production, egg quality, and antioxidant yolk capacity of Japanese laying quail was assessed. A total of 100 female quails 20-week-old were allotted to 5 treatments (5 replications, 4 females each) and received a basal diet supplemented with six graded levels of ABP (0 g/kg, 2.5 g/kg, 5.0 g/kg, 7.5 g/kg and 10.0 g/kg) according to ABP0, ABP25, ABP50, ABP75, and ABP100 groups for 70 days. The results indicated no adverse impact ($P>0.05$) of dietary ABP on production performance and egg internal quality. A quadratic effect was described for egg-breaking strength ($P<0.05$) and eggshell thickness ($P<0.01$), showing the highest values at a dose of 7.5 g/kg ABP. Compared to the control, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) yolk reduction was higher

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(quadratic) in the 5.0 g/kg ABP diet ($P<0.001$), while malondialdehyde (MDA) levels decreased linearly in all groups fed with ABP ($P<0.001$) as compared to the control group. The results suggest that the eggs from ABP quails could have an added value that would improve their marketability. The favourable findings of the current research demonstrate that including mushroom by-products in animal feed could reduce animal feed costs and reduce environmental damage.

KEY WORDS: circular economy / egg quality / Japanese quail / lipid oxidation / mushrooms

Currently, more than 11.8 million tons of mushrooms are produced annually worldwide. Among those, *Agaricus bisporus* is the most commonly cultivated mushroom supposing more than 40% of the world's mushroom population [Kumar *et al*. 2022]. *Agaricus bisporus* or "button mushroom" is a fruiting body of mushroom and has been acknowledged as an important ingredient for its nutritional and therapeutic properties [Khan *et al*. 2018, Altop *et al.* 2022, Kumar *et al*. 2022]. Traditionally, *A. bisporus* has been employed for medicinal purposes due to its composition of natural antibiotics, secondary metabolites, and bioactive compounds which have an important play in health promotion [Mahfuz and Piao 2019, Usman *et al*. 2021, Kumar *et al*. 2022]. Previous studies have confirmed the biological properties of this mushroom such as anticancer, antimicrobial, antioxidant, or antidiabetic activity and low toxicity of its intake [Usman *et al*. 2021].

Moreover, *A. bisporus* provides an excellent source of proteins with all the essential animals, adequate minerals, vitamins, carbohydrates, and low levels of fat [Usman *et al*. 2021]. This fact, combined with its sensory characteristics such as flavor and taste, has led to an increase in its demand for the last six to seven decades [Usman *et al*. 2021]. Nevertheless, the rise in mushroom production involves an increase in waste generated due to its transport and manufacturing [Altop *et al.* 2022]. Most of the food waste comes from damaged or spoiled products that cannot be used for human intake. However, these wastes retain the same nutritional properties (including bioactive compounds) as the initial product [Georganas *et al*. 2020]. Waste food is a common preoccupation, which causes considerable economic and environmental damage. In this sense, Special attention has been given to the utilization of food waste to provide valuable compounds that could be used as an ingredient in animal feed [Georganas *et al.* 2020, Sarmiento-García *et al*. 2023a]. Even though decreasing waste is a priority, their reincorporation into the food chain becomes crucial for establishing a circular economy [Georganas *et al*. 2020]. The residue of the *A. bisporus* shoot constituted 10% to 13% of its total mushroom weight, which is a similar composition to that of its cap [Yang *et al.* 2021]. Hence, the favorable composition of mushrooms and their waste by-products could be a promising alternative for being included as an ingredient in animal feed to reduce production costs and environmental pollution [Mahfuz and Piao 2019, Altop *et al*. 2022].

Mushrooms have been proposed as an interesting livestock ingredient that promotes health and development. In particular, several of these studies have focused on the effect of the inclusion of mushrooms in poultry diets [Giannenas *et al*. 2010, Khan *et al.* 2018, Yang *et al.* 2021, Kumar *et al*. 2021, Altop *et al*. 2022], which is

related to the physiological characteristics of these animals. Birds are sensitive to stress factors and infectious diseases. Different infections lead to decreased growth rates, reduced egg production, and mortality, causing great economic damage to the poultry sector [Mahfuz and Piao 2019]. Hence, trying to improve the immune system for preserving the health of poultry using natural ingredients has become a priority for many researchers [Gül *et al*. 2022, Sarmiento-Garcia *et al*. 2023b]. In this sense, including mushrooms in the diet of avian poultry could offer several benefits to enhance their health status, their performance, or the quality of their products [Giannenas *et al*. 2010, Altop *et al*. 2022].

Over the past years, the breeding of Japanese quail (*Coturnix coturnix Japonica*) has increased worldwide, due to its easy handling, fast production, and the demand for its eggs and meat. [Sarmiento-García *et al.* 2023a]. Moreover, Japanese quail farming proved to be economically sustainable and highly productive [Vargas-Sánchez *et al.* 2019]. Therefore, it reflects an opportunity to look for strategies to increase production by increasing quality and reducing price. Poultry nutrition is one of the most crucial aspects of modifying productive parameters or quality products and constitutes a large part of animal production costs [Kumar *et al.* 2021]. In contrast to conventional avian species, there has been less research concerning the nutrition of this species [Sarmiento-García *et al.* 2023b] and the available data are lacking in precision [Vargas-Sánchez *et al.* 2019]. Even though mushrooms contain bioactive compounds that could enhance avian production or its products, the effective use of ABP in poultry nutrition remains limited [Yang *et al*. 2021], this research aimed to assess the performance, egg quality parameters, and yolk antioxidant capacity of laying quails fed with different levels of *Agaricus bisporus* in the diet.

Material and methods

Experimental design

The study was performed on an animal farm located at Selçuklu, Konya, Türkiye (38°1’36″, 32°30’45″), hence there was no particular requirement for laboratory animal breeding. Nonetheless, the criteria established by the European policy regarding the protection of animals were applied in the experimental period [Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes, 2010].

One hundred (100) Japanese female laying quails (*Coturnix coturnix japonica*) weighing similar (268±10.5 g) and 20 weeks of age, which were purchased from a commercial farm (Konya, Türkiye), were included in this study. Quails were assigned randomly to five dietary experimental groups in a completely randomized design. Each experimental group consisted of five replicate groups, with four females per cage. All the cages had the same dimensions (30 cm wide and 45 cm long), environmental conditions (a controlled temperature (20±2.0ºC), relative humidity (55±5%), and a 16-hour lighting schedule), and all were fitted with an automatic feeder to dispense

feed and water ad libitum. The birds were fed on the experimental ration for 70 days (from 20 weeks old to 27 weeks old).

Agaricus bisporus mushroom powder (ABP) obtained from a local company was used to prepare the experimental diets (Kurucum Gıda, Ltd. Şti., Isparta, Türkiye). The ABP (including mushroom shoots) as powder had been discarded by the company for human consumption.

After that, ABP was introduced into the basal diet at inclusion rates of 0, 2.5, 5.0, 7.5, and 10.0 g/kg for treatments to obtain; ABP0 (control), ABP25, ABP50, ABP75, and ABP100, respectively. Considering the low content (≤1%) and the attempt to favourably impact animal product characteristics, ABP was evaluated as a feed additive, not a feedstuff. The level of ABP addition did not modify the nutrient content of the basal diet, so all diets could be described as isoenergetic (2902 kcal/kg Metabolizable energy) and isonitrogenous (199.9 g/kg crude protein). The basal diet submitted in the mash form was developed to satisfy the nutrient needs of laying quails as provided by the National Research Council [1994]. The procedures described by [AOAC, 2006] were followed to determine the chemical composition of the basal diet. The moisture content (2001.12) was obtained by drying at 105ºC in an oven, the protein content was measured by Kjeldahl (990.03), fat was recovered from the samples in a Soxhlet with petroleum ether (2003.06), and ashes were determined by incineration and drying the water (942.05). Table 1 presents the basal diet's ingredients and its chemical composition.

Table 1. Ingredients and chemical composition of the basal laying quails diet

Ingredients	g/kg	Chemical composition	g/kg
Corn	537.0	Metabolizable energy (kcal ME/kg)	2902
Soybean meal (460 g/kg CP)	348.0	Dry matter	891.3
Meat-bone meal (450 g/kg CP)	27.3	Crude protein	19.99
Sunflower oil	27.5	Crude fibre	28.1
Limestone	52.0	Crude fat	64.0
Salt	3.5	Moisture	108.7
Premix[1]	2.5	Calcium	25.0
DL methionine	2.2	Available phosphorus	3.5
		Lysine	10.6
		Methionine	4.6
Total	1000.0	Methionine+cystine	8.7

[1] Premix provided the following per kilogram of diet; manganese (manganese oxide) – 80 mg, iron (ferrous carbonate) – 60 mg, copper (cupric sulphate pentahydrate) – 5 mg, iodine – 1 mg, selenium – 0.15 mg, vitamin A (trans-retinyl acetate) – 8.800 IU, vitamin D3 (cholecalciferol) – 2.200 IU, vitamin E (tocopherol) – 11 mg, nicotine acetate – 44 mg, Cal-D-Pan – 8.8 mg, vitamin B2 (Riboflavin) – 4.4 mg, thiamine – 2.5 mg, vitamin B12 (cyanocobalamin) – 6.6 mg, folic acid – 1 mg, biotin – 0.11 mg, choline – 220 mg.

Evaluation of performance parameters

The quails (n=100) were weighed (±0.01 g) at the beginning (268±10.5 g) and at the end (282±18.2 g) of the study using a precision weighing balance (±0.01 g) to

evaluate body weight gain. The trial diets were dispensed by weighing each subgroup, and then feed intake was estimated using the difference between the supplied feed quantity and the leftovers of each pilot unit, and dividing this value by the total of quails and the duration of the experiment [Sarmiento-García *et al.* 2023a].

To assess the effect on egg production, all eggs were harvested simultaneously at 10:00 a.m. every day. The rest of the parameters such as egg weight, egg mass, and feed conversion ratio were determined using the previous values and the methods proposed by [Sarmiento-García *et al.* 2023a].

Evaluation of egg quality parameters

To establish the internal and external quality parameters, all eggs (n=240) from the last three days of the trial were gathered, which were analyzed at room temperature at the Egg Quality Laboratory (Faculty of Agriculture, Selcuk University, Konya, Türkiye). Cracked, broken, and damaged eggs were reported across the experimental period and expressed as a percentage of total whole eggs. The strength of the eggs was checked with increasing pressure using the Egg Force Reader (Orka Food Technology Ltd., Ramat Hasharon, Israel). A digital micrometer (Mitutoyo, 0,01 mm, Japan) was employed to determine the thickness of the eggshell from its three parts (pointed, equator, and blunt).

The eggs (n=240) were exposed to air-drying at ambient temperature for three days to assess the internal quality of the eggs, then they were broken on a clean glass surface and the eggshell residues were removed. Subsequently, the albumen was removed from the yolk. Shell weight was calculated in relation to egg weight. Albumen and yolk heights were evaluated using a height gauge, and length and width with a 0.01 mm digital calliper (Mitutoyo, Japan). These results were utilized to calculate the albumen index as can be observed in the following equation (1)

$$\text{Albumen index} = \frac{\text{Albumen height}}{\frac{\text{Albumen width} + \text{Albumen length}}{2}} \text{ x } 100 \quad (1)$$

The next equation (2) was used for determining yolk index

$$\text{Yolk index} = \frac{\text{Height of yolk}}{\text{Diameter ofyolk}} \text{ x } 100 \quad (2)$$

Haugh unit (3) was established as reported by Stadelman and Cotterill [1995]

$$\text{Haugh unit} = 100 \text{ x} \log(\text{albumen height} + 7.57 - 1.7 \text{ x Egg Weight}^{0.37}) \quad (3)$$

Colourimetric analysis was developed by placing the samples in Petri dishes allowing the preservation of the egg yolks' integrity. Egg yolks (n=240) have been analyzed with a pre-calibrated Konica Minolta digital colourimeter (Minolta Chroma Meter CR 400 (Minolta Co., Osaka, Japan) as previously has been shown by Titcomb

et al. [2019]. Colour space (frequently denoted as L* a* b*) has been established to assess changes in yolk colour. The L*, a* and b* parameters show lightness (-0/+100, dark/white), redness (-a/+a, green/red) and yellowness (-b/+b, yellow/blue), respectively.

Antioxidant capacity of the yolk

The modified thiobarbituric acid reactive substance assay (TBARS) test described by Kilic and Richards [2003] and Olgun *et al*. [2022] was conducted on each sample to assess lipid peroxidation. The method was applied in triplicate. Two grams of the yolk (n=100) were weighed and mixed with 12 ml of the trichloroacetic acid (TCA) solution using an UltraTurrax (IKA, USA) for 20 s. The mixture was transferred to tubes and 3 ml of thiobarbituric acid (TBA) solution (0.02 M) was included and mixed. Tubes were put in a water bath for 40 min to develop a pink colour, and then, samples were cooled and centrifuged for 5 min at 2000 rpm. The supernatant was used to determine the absorbance values using a spectrophotometer (PerkinElmer, USA) at a 530 nm wavelength. The blank comprising 1 mL of TCA extraction solution and 1 mL of TBA solution was employed as a benchmark for the spectrophotometric measurement (Perkin Elmer, USA) at a wavelength of 530 nm against a blank containing 1 mL of TCA extraction solution and 1 mL of TBA solution. TBARS value has been estimated based on a standard curve of malondialdehyde (MDA). TBARS value (6) was given as μmol MDA/g yolk using the following equation (4):

$$TBA\ Value = \frac{\left(\frac{absorbance}{k} x \frac{2}{1000}\right) x\ 6.8}{sample\ weight} X\ 100 \quad (4)$$

Free radical scavenging properties were tested by the effect of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) suppression according to a modified method reported by Sacchetti *et al*. [2005]. All analyses were duplicated for three independent measures. Two g of yolk were separated and dissolved in 25 ml of 95% methanol.; then, the elimination process was performed in an ultrasonic bath for 20 min. The solution was filtrated and recovered in 0.1 mL glass tubes. 2.9 mL of DPPH (Sigma-Aldrich) was added to the solution, and then it was shaken vigorously for 25 seconds. The mixture was placed in a spectrophotometer (Perkin Elmer Precise UV/VIS Spectrometer) at 517 nm wavelength to determine the absorbance, after standing for 30 min at ambient temperature. The control was developed similarly, replacing the sample solution with 95% ethanol. Equation (5) from Sacchetti *et al*. [2005] was applied to calculate the radical-scavenging activities of samples, which were shown in the percent suppression of DPPH:

$$DPPH\ inhibition = \frac{[(Control\ absorbance - Sample\ absorbance)}{Control\ Absorbance} x\ 100 \quad (5)$$

Statistical analysis

A one-way ANOVA was employed to evaluate all data studied using the SPSS 23.0 software (IBM SPSS Statistic 2017). Each bird was the experimental unit. A P-value of less than 0.05 was found to be statistically significant, while a P-value of less than 0.10 was found to be a trend. If there are significations, among means, orthogonal polynomial contrasts were carried out to determine the significance of linear and quadratic models. It was tried to predict the outcome of the variable's behavior at an ascending ABP rate.

Results and discussion

All animals were alive and with no signs of illness when the experiment was completed. Table 2 describes the mean values of the performance and egg parameters from experimental groups. Performance parameters (including final body weight, body weight gain, feed intake, and feed conversion ratio) were not affected ($P>0.05$) by the experimental treatments during the entire 70-day study period. Similar trends were observed for egg production parameters (including egg production, egg weight, and egg mass) showing similar values between the experimental and the control groups ($P>0.05$).

Table 2. Effect of ABP supplementation on performance and egg production parameters of laying quails

Parameter	Treatment groups					S.E.M	Probability		
	ABP0	ABP25	ABP50	ABP75	ABP100		P	L	Q
Initial body weight	274.6	273.4	272.8	265.6	276.8	6.88	0.864	0.525	0.463
Final body weight	290.6	280.6	283.2	277.6	280.7	7.51	0.816	0.374	0.551
Body weight gain	16.00	7.20	10.40	12.00	13.90	4.154	0.376	0.175	0.990
Egg production	86.56	86.62	87.57	87.53	90.52	1.154	0.449	0.105	0.440
Egg weight	12.87	13.14	13.09	12.91	12.84	0.323	0.960	0.801	0.538
Egg mass	11.14	11.40	11.47	11.31	11.62	0.386	0.948	0.528	0.936
Feed intake	31.85	33.98	34.12	32.30	32.77	0.612	0.091	0.939	0.046
FCR	2.87	3.01	2.98	2.88	2.82	0.082	0.614	0.469	0.209

ABP – *Agaricus bisporus* mushrooms; FCR – feed conversion ratio; S.E.M. – standard error means. P – Anova effect; L – linear effect; Q – quadratic effect.

Agaricus bisporus mushrooms was added at the concentration of ABP0 – 0 g/kg, ABP25 – 2.5 g/kg, ABP50 – 5.0 g/kg, ABP75 – 7.5 g/kg and ABP100 – 10.0 g/kg.

Body weight gain and egg weight were expressed are in g. Egg production was expressed in percentage (egg/100 quails). Egg mass and feed intake were expressed in g/day/quail. Feed conversion ratio was g feed/g egg.

The external and internal traits in the experimental groups are described in Table 3. No detectable treatment effects ($P>0.05$) were demonstrated on the damaged eggs and eggshell weight, being the experimental values similar to the control. Meanwhile, ABP supplementation significantly affects egg-breaking strength ($P<0.05$) and eggshell thickness ($P<0.01$), showing a quadratic effect. In both cases, the maximum values were recorded for the ABP75 group. For egg-breaking strength, the lowest value was set in the ABP100 group, while for the eggshell thickness, all experimental groups (except ABP75) showed similar values that did not differ among them.

Regarding the internal parameters of the egg, Table 3 shows that ABP supplementation did not affect ($P>0.05$) the albumen or yolk index and Haugh unit. In addition, no significant effect ($P>0.05$) of ABP supplementation was described on yolk colour parameters (including L*, a*, and b*), which remained similar between the experimental diets and the control group.

Table 3. Effects of ABP supplementation on external and internal egg quality traits in laying quails

Parameter	Treatment groups					S.E.M	Probability		
	ABP0	ABP25	ABP50	ABP75	ABP100		P	L	Q
Damaged egg	0.48	0.46	0.17	0.28	1.01	0.337	0.520	0.438	0.168
Egg-breaking strength	1.46^{b}	1.52^{ab}	1.46^{b}	1.69^{a}	1.33^{c}	0.056	0.008	0.595	0.027
Eggshell ratio	8.37	8.33	8.26	8.86	8.06	0.199	0.135	0.909	0.287
Eggshell thickness	217.30^{b}	227.60^{b}	228.00^{b}	242.10^{a}	222.80^{b}	3.770	0.006	0.065	0.008
Albumen index	2.88	2.68	2.85	2.61	2.73	1.150	0.687	0.416	0.659
Yolk index	46.84	47.15	47.18	47.83	48.61	0.807	0.647	0.150	0.645
Haugh unit	90.71	89.43	93.07	90.63	92.60	1.106	0.184	0.185	0.924
L*	51.72	53.11	50.80	80.81	51.77	0.682	0.207	0.359	0.600
a*	1.85	1.28	1.70	2.38	1.63	0.398	0.497	0.631	0.960
b*	34.42	35.08	32.22	33.46	34.41	0.784	0.162	0.532	0.142

ABP – *Agaricus bisporus* mushrooms; S.E.M. – standard error means. P – Anova effect; L – linear effect; Q – quadratic effect.
[ab]Means with different superscripts in the same row were significantly different at P<0.05).
Agaricus bisporus mushrooms was added at the concentration of ABP0 – 0 g/kg, ABP25 – 2.5 g/kg, ABP50 – 5.0 g/kg, ABP75 – 7.5 g/kg and ABP100 – 10.0 g/kg.
Eggshell breaking strength was expressed in kg. Eggshell ratio and damaged egg rate were expressed as percentage. Eggshell thickness was expressed in µm.

Table 4. Effect of ABP supplementation on the antioxidative capacity of yolk

Parameter	Treatment groups					S.E.M	Probability		
	ABP0	ABP25	ABP50	ABP75	ABP100		P	L	Q
DPPH (% reducing)	5.793^{c}	10.350^{ab}	10.650^{a}	9.975^{ab}	8.125^{b}	0.483	0.001	0.088	<0.001
MDA concentration (µmol MDA/kg)	3.998^{a}	2.992^{b}	2.693^{b}	2.502^{b}	2.530^{b}	0.143	<0.001	<0.001	0.013

ABP – *Agaricus bisporus* mushrooms; DPPH – 2,2 diphenyl-1-picrylhydrazyl. MDA – malondialdehyde. S.E.M. – standard error means. P – probability; L – linear effect; Q – quadratic effect.
[abc]Means with different superscripts in the same row were significantly different at P<0.05.

As the results demonstrated in Table 4, ABP inclusion affects ($P<0.05$) the oxidative status of quail´s yolk in terms of DPPH reduction and MDA concentration. The scavenging activity of yolk protein hydrolysates on DPPH radicals demonstrated a quadratic behaviour, being the maximum of that variable described with the inclusion of 5.0 g/kg of *Agaricus bisporus* powder in the ration. Moreover, the MDA value was

linearly affected ($P<0.01$) with mushroom inclusion, showing ABP supplementation groups (ABP25, ABP50, ABP75, and ABP100) lower MDA concentrations than the control group.

Reducing feed costs is an important concern for the livestock sector [Kumar *et al*. 2021]. In this sense, reusing by-products rich in bioactive substances as feed ingredients would seem to be an appropriate solution. These ingredients could be beneficial for the animal's productive development and immune status [Yang *et al*. 2021, Sarmiento-García *et al*. 2023b]. In this sense, the by-products generated due to the increased production of edible mushrooms are presented as an opportunity for animal feed [Lee *et al*. 2015, Mahfuz *et al*. 2018b, 2019].

In the current research, no differences were described between experimental diets in performance parameters such as final body weight, body weight gain, feed intake, and feed conversion ratio during the experimental period. Egg production (including egg mass or egg weight) was similar to the control group. Those facts make ABP a viable approach to use as quail feed. This result is similar to the past study by Yang *et al*. [2021], who did not find any differences in egg production and feed conversion ratio when *Flammulina velutipes* was added to the laying hens diet. Similarly, Mahfuz *et al*. [2018a] did not report any differences in the performance laying parameters among experimental diets containing mushroom stem waste, which are similar to the findings obtained by Odekunle *et al*. [2021]. In contrast with the current results, Kumar *et al*. [2021] described an enhancement in body weight of broiler chicks with similar intake values to the control group when animals received mushrooms in the diet, which is in line with those shown by Giannenas *et al*. [2010], who reported an improvement in final body weight, body weight gain and FCR of broiler fed dried mushroom (10 to 20 g/kg) for 6 weeks. Partially in accordance, Shamsi *et al.* [2015] demonstrated an improvement in body weight gain and feed conversion ratio, but a decreased feed intake in broiler feed with *A. bisporus* (20 g/kg) supplementation, except when *A.bisporus* cap was included. Altop *et al*. [2022], described a decrease in feed intake in all broilers that have been supplemented with *A. bisporus* (at levels of 10 g/kg to 20 g/kg) during the trial (0-42 days) which is in line with those reported by Freitas *et al.* [2019]. It has been described that the content of fibre (which ranges from 10 to 50% of dry matter in mushrooms) could reduce the feed intake in birds. Hence, the differences observed between studies could be attributed to the mushroom species and the dose included [Bederska-Łojewska *et al*. 2017], although even within the same species there would be differences depending on the part of the mushroom used as an ingredient (stem, top, or both) or the processing procedure [Altop *et al*. 2022]. Moreover, it is important to point out that improvements in production parameters were observed in broiler chicks and not in laying hens. It has been shown that the inclusion of mushroom extracts could increase the beneficial bacteria that enhance the fermentation processes in the gut. The end products of fermentation, such as short-chain fatty acids, lower the intestinal pH, reduce harmful bacteria, and stimulate beneficial bacteria. This is particularly noticeable in young birds, who are

more vulnerable to intestinal disease pathogens. In addition, the fact that the nutrient requirements of older birds are less demanding and have a better-developed digestive tract and organs makes the effects of fermentation less evident [Toghyani *et al*. 2012]. This fact would reinforce the differences between previous studies and the current results.

The addition of ABP increased the egg-breaking strength and the eggshell thickness with the inclusion level of 7.5 g/kg compared to the rest of the experimental diets. Although Mahfuz *et al.* [2018b] did not find differences in eggshell thickness when a mushroom diet was offered to the laying hens, those authors described higher calcium retention and lower calcium excretion compared to the control groups. Previous authors have described that enhanced calcium availability could be a potential strategy for improving eggshell quality and reducing damaged eggs [Sarmiento-García *et al*. 2022]. Based on the above information, it is possible that the observed improvement in eggshell thickness and resistance to egg breakage could be due to the increased availability of calcium for shell development in the ABP75 group.

Supplementation by ABP had no adverse effects on the egg's internal parameters which is in agreement with the findings of Lee *et al*. [2015] and Mahfuz *et al.* [2018b] who supplemented laying hens' diet with mushroom stem waste. Freitas *et al*. [2019] did not describe differences in egg internal parameters when laying hens were supplemented with *A. blazei.* Odekunle *et al*. [2021] found no differences in internal egg parameters when oyster mushroom was included in the diet. The absence of differences was maintained in both the late-lay (38-43 weeks of age) mid-lay (28-35 weeks) and early-lay (20-27 weeks of age) stages. Regarding yolk colour, feeding experimental quails on diets with ABS resulted in the absence of differences in yolk colour compared to the control. These results coincided closely with those published by Hwang *et al.* [2012] who did not described any differences in yolk colour when shiitake (*Lentinula edodes)* was added to the laying hens´s diet. Similarly, Odekunle *et al*. [2021] described that the inclusion of oyster mushrooms did not affect yolk colour parameters. Yolk colour is one of the most important factors for egg purchase decisions [Sarmiento-Garcia *et al*. 2023a]. The lack of differences in yolk colour among the experimental diets offers the opportunity to include mushroom by-products in the diet of laying quail. Contrarily, Mahfuz *et al.* [2018b] described a high-coloured yolk in laying hens fed with *F. velutipes*. These authors speculated that the difference from previous studies could be because this mushroom species is rich in carotenoids, which are responsible for the colour and aroma appreciated by consumers.

Egg yolks present a high-fat content, resulting in vulnerability to lipid oxidation, which leads to the deterioration of the product and shortened shelf life [Sarmiento-Garcia et al., 2023b]. Therefore, the development of strategies to reduce oxidation processes is essential. In the current research, the antioxidant ability of egg yolk was influenced by ABP supplementation. As for DPPH increase, the ABP50 group recorded the best value, while MDA concentration was reduced in all groups supplemented with ABP. Nevertheless, it was observed that the values recorded for both parameters improved

in all cases concerning the control, suggesting an improvement in the antioxidant capacity of the yolk when ABP is included in the diet of quails as described [Yang *et al*. 2021] in laying hens. The results obtained could be explained by the phenolic compounds present in ABP. These compounds can give electrons, neutralizing reactive oxygen species and protecting cells from harm; they also can chelate metals that induce reactive oxygen species [Bederska-Łojewska et al., 2017]. Nevertheless, there are other components present in the mushrooms that improve the antioxidant capacity as reported in the current research. For example, it has been described that *A. bisporus* has a high content of trace elements including copper, zinc, and selenium. The most common superoxide dismutase (SOD) is a complex copper-zinc enzyme involved in free radical removal in the organism and catalyses the breakdown of superoxide anions into hydrogen peroxide or molecular oxygen. Including ABP in the diet may enhance SOD production and the antioxidant capacity of the body. Similar findings have been described in the meat. Vargas-Sánchez *et al*. [2018] described a reduction in MDA values of quail meat that had been supplemented with *P. oestratus* in comparison with the control group. Giannenas *et al*. [2010] proposed that the inclusion of *A. bisporus* (at levels of 10 to 20 g/kg) reduced MDA value in the broiler breast (around 33.3%) which is similar to those reported in turkeys. As well, Lee *et al*. [2015] in their study proposed that *Pleurotus eryngii* stem residue could lead to improve serum antioxidant capacity compared to the control group. Moreover, the increase in MDA production is related to an increase in fat content [Vargas-Sánchez *et al*. 2018]. This fact could also suggest a decrease in the fat content of eggs, which is an interesting option in the purchase of eggs. Therefore, it could be hypothesized that the inclusion of ABP in the diet of laying quail could improve egg shelf life by reducing susceptibility to lipid oxidation. This result could lead to a preference for egg purchasing as well as enrich the quality of the product [Yang *et al.* 2021].

The current findings demonstrated that the inclusion of *Agaricus bisporus* mushroom by-products into laying quail diets does not comprise the performance, egg production, or egg internal parameters. Nevertheless, a dose of 7.5 g/kg of ABP would promote eggshell thickness and eggshell-breaking strength. Moreover, the inclusion of ABP in the quail diet would improve the antioxidant capacity of the yolk, in which a dose of 5.0 g/kg improved the maximization of this parameter. Therefore, the inclusion of mushroom by-products can be considered an opportunity in the feeding of laying quails that would contribute to the reduction of feed costs and the reuse of by-products leading to a circular economy.

Financial Support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Ethical Approval

The authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page. The European National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

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