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Enhancing Growth Performance, Immunity, and Gut Morphology in Quails through Oyster Mushroom Stem Waste Supplementation

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ABSTRACT

The aim of this study was to determine the effect of oyster mushroom stem waste supplementation on the grown performance of quails. For this a total of 240 days old quails were purchased from a reputed hatchery. Equal number of chicks was assigned to four groups and each group was further replicated three times. Each replicate consisted of 20 chicks. Group PO-0 was considered as control and was fed with commercial ration with no supplementation. Group PO-1 was fed with commercial ration supplemented with 10 g mushroom waste/kg of feed. While group PO-2 and group PO-3 was fed with commercial ration supplemented with 20g and 30g of mushroom waste/kg of feed respectively. The study revealed that there was no notable impact on feed intake. However, there was a significant increase ($P<0.05$) in weight gain observed in the PO-3 group, accompanied by a considerably lower feed conversion ratio (FCR) in the same group. No instances of mortality were recorded in either the control or experimental groups. Nevertheless, there was a noteworthy improvement in dressing percentage observed in the PO-3 group. Additionally, the antibody titers against NDV, IBV, and IBDV were significantly higher ($P<0.05$) in the PO-3 group. Similarly, the histological features of the cecum, including villus height, width, and their respective ratio, were significantly higher in PO3 compared to the control group. In conclusion, this study demonstrates that supplementing the diet with various levels of oyster mushroom stem waste led to improved growth performance, enhanced immunity, and improved gut morphology in quails, all without affecting liver and kidney functions. The most favorable outcomes were achieved when oyster mushroom stem waste was incorporated at a 3% level in the feed.

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JN conducted the experiment. NC designed the study. AH analysis the data. RUK wrote, revised and submitted the manuscript. All authors read and approved the final version of the manuscript.

Key words
Mushroom, Japanese quails, Growth, Histomorphology

INTRODUCTION

The increasing worry over antibiotic resistance has sparked a heightened focus on limiting or even banning their usage in poultry farming methods (Nasir *et al*., 2023). The poultry industry faces substantial economic hurdles due to immunosuppression and diseases, especially when vaccines yield less-than-optimal results. Considering the financial losses tied to gastrointestinal infections and the strict controls on antibiotic incorporation into feed, there is a rising need for natural substitutes to lessen dependence on growth-promoting agents (Hafeez *et al*., 2023).

Medicinal mushrooms, as they are commonly known, present a potential alternative to antibiotics in poultry diets (Fard *et al*., 2014; Mahfuz *et al*., 2017). Mushrooms are favored for their health-enhancing properties and medicinal qualities, making them a popular choice (Tang *et al*., 2016). Mushrooms serve as an excellent source of protein, vitamins, minerals, and unsaturated fatty acids (Tang *et al*., 2016). Mushrooms are characterized by their richness in proteins, carbohydrates, and fiber, coupled with low fat content (Mahfuz *et al*., 2017). Traditionally, mushrooms have been cultivated for both human consumption and for their pharmacological properties in various countries. Presently, there is a wealth of scientific literature highlighting the health-promoting advantages of incorporating mushrooms in the diets of farm animals. However, research on their effects on productive performance and overall health status remains somewhat restricted (Mahfuz *et al*., 2017). While it has been suggested that mushrooms can be advantageous for the well-being of chickens, determining the optimal inclusion levels in diets is still a subject of ongoing debate.

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discussing the health-enhancing advantages of incorporating mushrooms into the diets of farm animals. However, the understanding of their effects on productivity and overall health remains somewhat restricted (Mahfuz *et al*., 2017). While it has been theorized that mushrooms could be advantageous for the well-being of chickens, the optimal inclusion levels in diets are still a subject of debate. Given this context, the current review underscores the potential of mushrooms as a natural dietary supplement capable of enhancing growth performance, immunity, intestinal histology and blood metabolites in broiler chickens.

MATERIALS AND METHODS

Chemical analysis of stem waste of oyster mushroom

The collected stem waste of mushroom was cleaned and dried in oven at 45°C for 24 h. The dried material was ground to powder form by using electric grinder machine (Moulnix, 600, LM-240, France) to particle size of 1mm in the laboratory of department of animal nutrition, The University of Agriculture, Peshawar-Pakistan. Chemical analyses of stem waste of oyster mushroom powder for protein, crude fiber fat content and ash was performed as directed. Dry matter content was performed by (method 930.15), method used for ash was (method 942.05), similarly, for determination of CP (method 984.13), whereas ether extract was determined through (method 920.39). Analyses of all the above-mentioned contents were done according to standard procedures of AOAC (2005).

Experimental animals

A total of 240 days old quails were purchased from a reputed hatchery. Equal number of chicks was assigned to four groups and each group was further replicated three times. Each replicate consisted of 20 chicks. Group PO-0 was considered as control and was fed with commercial ration with no supplementation. Group PO-1 was fed with commercial ration supplemented with 10 g mushroom waste/kg of feed. While group PO-2 and group PO-3 was fed with commercial ration supplemented with 20g and 30g of mushroom waste/kg of feed respectively. Chicks were reared on floor. Feed and clean water was offered ad-libitum. All the birds were kept together for seven days for adaptation. Birds were fed commercial ration for those seven days. After adaptation periods birds were divided in groups in manner aforementioned. Temperature was kept 95 degrees Fahrenheit during first week, which was decreased by 5˚F per week up to 75 ˚F.

Performance traits

Throughout the experimental trials, data on various performance parameters including feed intake, weight gain, feed conversion ratio, mortality, and dressing percentage were meticulously recorded, and the results were subsequently analyzed.

Dressing percentage

In order to determine the dressing percentage of the quails, three birds were randomly selected from each replicate. Each bird was individually weighed on an electric balance to obtain their live weight. Subsequently, all the birds were humanely slaughtered, and the skinning process was carried out. The edible parts, including the breast, wings, thighs, and legs, were weighed. The dressing percentage was then calculated using the following formula:

Dressing % = (dressed weight × 100) / (live weight).

Mortality

Deceased birds were collected and the daily count of mortalities was recorded. Additionally, the cause of mortality was determined by conducting postmortem examinations on the birds.

Antibody titre

To assess the immune status of the quails, antibody titers against New Castle disease, infectious bronchitis, and infectious bursal disease viruses were measured. At the conclusion of the experimental trials, three milliliters of blood were drawn from three birds per replicate. Serum was extracted from the blood samples through centrifugation. The antibody titer was then determined by analyzing the collected serum using an ELISA kit, following the method outlined by Marquardt *et al*. (1980).

Gut morphology

On the 42nd and final day of the experimental trials, three quails from each replicate were chosen and humanely slaughtered. Sections from the ilium of the intestine were carefully excised. These sections were then incised, allowing for the removal of intestinal content, followed by thorough washing with a saline solution. Subsequently, the tissue underwent a series of processing steps, starting with fixation in 10% buffered formalin. This was followed by a dehydration process using varying concentrations of alcohol, typically in ascending order. The tissue was then subjected to clearing with xylene, succeeded by infiltration with molten paraffin. Embedding the tissue in paraffin blocks facilitated the subsequent step of sectioning.

After embedding, the tissue sections were prepared for examination under the microscope. To quantify the characteristics of the villi, measurements were taken from the top of the villus to the apex of the lamina propria. Villus

width was assessed at three different locations: the base, middle, and top. The average of these three measurements was recorded as the villus width. Additionally, the depth of the crypt, measured from the point of villus-crypt junction to the base of the crypt, was recorded. The villus height to crypt depth ratio was determined by dividing the villus height by the crypt depth.

Table I. Ingredients and chemical composition of basal feed.

	Starter phase (1-21 days)	Finisher phase (22-42 days)
Ingredients		
Yellow corn	53.21	60.75
Soybean meal (48% CP)	37.92	25.00
Corn gluten meal (34% CP)	2.00	7.10
Corn oil	2.20	2.80
Dicalcium phosphate	2.30	2.05
Limestone	0.83	0.68
NaCl	0.45	0.50
Vitamin and minerals premix[1]	0.50	0.50
DL-Methionine	0.20	0.10
L-Lysine HCl	0.22	0.37
L-Threonine	0.11	0.10
Choline chloride	0.05	0.05
Chemical composition		
ME, kcal/kg	3000	3150
Crude protein, %	22.5	21.30
Methionine, %	0.55	0.44
Lysine, %	1.42	1.23
Sulfur amino acids, %	0.96	0.80
Threonine, %	0.95	0.85
Calcium, %	1.05	0.90
Available phosphorus, %	0.50	0.45

[1]Provided per kg of diet: vitamain A, 8000 IU; vitamain D3,2000 IU; vitamain E,15 IU; vitamain K3, 1.5 mg; thiamine, 2 mg; riboflavin, 5 mg; pyridoxine, 5 mg; vitamain B12, 0.02 mg; folic acid, 0.7 mg; nicotinic acid, 40 mg; pantothenic acid, 12 mg; biotin, 0.2 mg b Provided per kg of diet: copper, 10 mg (as copper sulfate); iron, 90 mg (as ferrous sulfate); manganese, 100 mg (as manganese sulfate); zinc, 100 mg (as zinc sulfate); selenium, 0.3 mg (as sodium selenite); iodine, 0.5 mg (as calcium iodate).

Liver and kidney functions tests

To assess potential toxic effects of *Pleurotus ostreatus* waste on quails, kidney and liver function tests were conducted. On the forty-second day, approximately 2 milliliters of blood were collected from the jugular vein of birds into ethylene-diamine tetra-acetic acid (EDTA) tubes. Three birds were randomly selected from each replicate for blood sampling. Subsequently, the collected blood underwent centrifugation at 4000 rpm for three minutes to separate the serum from the rest of the blood components.

For liver function evaluation, the quantification of various enzymes produced by the liver, including alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), was carried out. Commercially available ELISA kits were employed for the determination of these enzymes (MyBio Source, USA). Additionally, kidney function tests, specifically urea and creatinine levels, were assessed using the collected serum in accordance with the manufacturing guidelines provided by commercially available kits from MyBio Source, USA.

Statistical analysis

The data was organized in an Excel spreadsheet and subsequently subjected to statistical analysis using analysis of variance (ANOVA) with a complete randomized design (CRD). To discern significant differences between means, the LSD test was applied utilizing the latest software, Statistix 8.1.

RESULTS

Chemical analysis of stem waste of oyster mushroom is shown in Table II. Stem waste of oyster mushroom consists of carbohydrates 31.50%, crude protein 19.34%, moisture 89.78%, fats 2.31% and ash 10.06%.

Table II. Chemical analysis of stem waste of oyster mushroom.

Composition	Percentage
Carbohydrates	31.50
Crude protein	19.34
Moisture	89.78
Fats	2.31
Ash	10.06

The inclusion of different levels of stem waste from oyster mushrooms in the diet did not have a significant impact on feed intake, as indicated in Table III. Notably, feed intake remained consistent on a weekly basis, as well as during the starter, finisher, and overall phases. It is worth mentioning that numerically higher feed intake was observed in group PO-3, which received a diet containing 3% of stem waste from oyster mushrooms. In contrast, the control group (PO-0), which did not receive any stem waste, exhibited relatively lower feed intake.

Table III. Impact of various levels of stem waste powder of oyster (*Pleurotus ostreatus*) mushroom on feed intake, body weight and FCR of quails, body weight gain/ quail and FCR of quails.

Phases	PO-0	PO-1	PO-2	PO-3	p value
Feed intake					
Week-2	48.66±1.20	45.33±1.45	43.33±2.33	48±1.52	0.1728
Week-3	92.00±2.08	93.66±1.76	92.66±1.20	93.33±1.20	0.8857
Starter phase	140.67±1.20	139±3.21	136±1.15	141.33±2.02	0.3355
Week-4	118.67±0.66	123.67±1.45	126.33±2.96	127±2.30	0.0722
Week-5	143.67±3.52	147±0.57	144±1.73	146±1.15	0.1738
Week-6	155±2.64	154±1.73	157.33±2.90	159.67±1.33	0.2263
Finisher phase	417±2.02	424.67±0.88	430±5.50	432.67±4.05	0.0678
Overall	558±3.21	563.67±4.50	566±7.81	574±6.08	0.0679
Body weight gain/ quail					
Week-2	22.66±1.20^{c}	24.00±0.57^{bc}	28.66±2.02^{ab}	29.33 ±1.85^{a}	0.0348
Week-3	35.33±0.88^{c}	38.66±0.88^{bc}	41.00±2.00^{ab}	43.66 ±0.88^{a}	0.0089
Starter phase	58.00±2.08^{c}	62.66±0.33^{bc}	67.00±2.08^{ab}	69.66 ±1.76^{a}	0.0065
Week-4	38.66±0.88^{b}	43.66±0.66^{a}	44.66±2.02^{a}	46.66 ±1.45^{a}	0.0174
Week-5	44.00±1.00^{b}	47.33±0.88^{b}	47.66±1.76^{b}	52.33 ±0.88^{a}	0.0078
Week-6	36.33± 0.84^{b}	38.66±0.76^{b}	39±0.96^{b}	42.33 ±1.38^{a}	0.0047
Finisher phase	119.00±2.00^{c}	129.67±2.72^{b}	131.33±1.20^{b}	141.33 ±1.45^{a}	0.0003
Overall	177.00±1.52^{c}	192.33±2.51^{b}	198.33±3.78^{b}	211.33 ±0.66^{a}	0.0000
FCR					
Week-2	2.1 ±0.25^{a}	1.88 ±0.04^{ab}	1.51±0.07^{c}	1.65±0.13^{bc}	0.0136
Week-3	2.60 ±0.08^{a}	2.42 ±0.09^{ab}	2.26 ±0.12^{b}	2.13±0.02^{b}	0.0312
Starter phase	2.42 ±0.09^{a}	2.21 ±0.06^{ab}	2.03±0.06^{b}	2.02 ±0.03^{b}	0.0086
Week-4	3.05 ±0.07^{a}	2.83±0.07^{ab}	2.84 ±0.15^{ab}	2.73 ±0.06^{b}	0.0174
Week-5	3.24 ±0.10^{a}	3.10±0.05^{a}	3.08±0.14^{ab}	2.78 ±0.06^{b}	0.0551
Week-6	4.30±0.103^{a}	3.97±0.08	4.02±0.14	3.78±0.22	0.1795
Finisher Phase	3.50 ±0.05^{a}	3.27±0.06^{b}	3.27 ±0.04^{b}	3.05±0.03^{c}	0.0016
Overall	3.15±0.04^{a}	2.92±0.04^{b}	2.85±0.05^{b}	2.71 ±0.01^{c}	0.0006

Means in the same row but with dissimilar superscripts are significantly different at P<0.05. PO-0, control group; PO-1, 10g of *P. ostreatus* per kg of feed; PO-2, 20 g of stem waste *P. ostreatus* per kg of feed; PO-3, 30g of *P. ostreatus* contained in per kg of feed.

The weight gain of quails was significantly influenced by the dietary supplementation of different levels of stem waste from oyster mushrooms, as demonstrated in Table III ($p<0.05$). The supplementation of stem waste from oyster mushrooms had a significant impact ($p\leq0.05$) on the weight gain of quails across all recorded stages. In the second week of age, the highest weight gain (29.33g) was observed in group PO-3, which was fed a diet containing 3% of mushroom waste. Conversely, the lowest weight gain (22.66g) was noted in the control group (PO-0) which received no mushroom waste. During the second week, the weight gain in group PO-1 was not significantly different from both the control group and group PO-2. Similarly, the weight gain of group PO-2 was not significantly different from that of group PO-3. In the third week, the highest weight gain (43.66g) was observed in group PO-3, followed by group PO-2 (41g) and group PO-1 (38.66g). The control group exhibited the lowest weight gain (35.33g). Throughout the starter phase, group PO-3 exhibited the highest body weight gain (69.66g), while the control group (PO-0) had the lowest (58.00g). Group PO-1 and PO-2 had weight gains of 62.66g and 67g respectively. In the fourth week, weight gain was higher and equal in groups PO-3 (46.66g), PO-1 (43.66g), and PO-2 (44.66g), with the lowest weight gain recorded in group PO-0. During the fifth week, the highest weight gain (52.33g)

was observed in group PO-3, while the lowest and equal weight gain was recorded in groups PO-0 (44.00g), PO-1 (47.33g), and PO-2 (47.66g). A similar trend was noted in the sixth week, with higher weight gain in group PO-3 (42.33g), and the lowest and equal weight gain in groups PO-0 (36.33g), PO-1 (38.66g), and PO-2. In the finisher stage, group PO-3 recorded the highest weight gain (141.33g), followed by groups PO-1 (129.67g) and PO-2 (131.33g), while the lowest weight gain was observed in group PO-0 (119.00g). Similarly, the overall weight gain was highest in group PO-3 (211.33g), followed by groups PO-1 (192.33g) and PO-2 (198.33g). The lowest overall weight gain (177.00g) was observed in group PO-0.

The feed conversion ratio (FCR) of quails in response to the dietary supplementation with different levels of stem waste from oyster mushrooms is presented in Table IV. The FCR was significantly affected by the inclusion of stem waste from oyster mushrooms at all recorded stages, except for the sixth week. During the second week, the control group exhibited a higher (poorer) FCR of 2.1, followed by PO-1 (1.88) and PO-3 (1.65), while the lowest (better) FCR was observed in group PO-2 (1.51). In the third week, the highest FCR was recorded in group PO-0 (2.60), followed by PO-1 (2.42), while the lowest and similar FCR values were recorded in PO-3 (2.13) and PO-2 (2.26). The trend for FCR during the starter phase was similar to that of the third week, with higher FCR in group PO-0 (2.42), followed by PO-1 (2.21), and the lowest and similar FCR in PO-3 (2.02) and PO-2 (2.03). In the fourth week, the highest FCR was observed in PO-0 (3.05), while the lowest FCR was recorded in PO-3 (2.73). FCR in the fourth week was the same in groups PO-1 (2.83) and PO-2 (2.84), which did not differ from the control group. During the fifth week, higher and equal FCR values were recorded in groups PO-0 (3.24) and PO-1 (3.10), followed by PO-2 (3.08), while the lowest FCR was observed in group PO-3 (2.78). The supplementation of stem waste from oyster mushrooms did not affect the FCR during the sixth week, with values of 4.30, 3.97, 4.02, and 3.78 for groups PO-0, PO-1, PO-2, and PO-3, respectively.

In the finisher phase, the control group exhibited the highest FCR, followed by groups PO-1 and PO-2 with the same FCR of 3.27. A lower FCR was found in group PO-3 (3.05). Overall, the FCR was higher in the control group (PO-0) at 3.15, followed by groups PO-1 (2.92) and PO-2 (2.85), while the lower FCR was recorded in group PO-3 (2.72).

Table IV. Effect of different levels of stem waste powder of oyster (*Pleurotus ostreatus*) mushroom on mortality, dressing percentage, antibody titre (log^{10}), dimension of cecum of Japanese quails and functions of kidneys and liver.

Items	PO-0	PO-1	PO-2	PO-3	p-value
Mortality (%)	1.66±0.66	1.00±0.57	1.00±0.57	0.00±0.00	0.2437
Dressing %	69.36±0.60^{c}	70.35±0.28^{bc}	70.83±0.22^{ab}	71.67^{a}±0.21	0.0140
Antibody titre (Log10CFU/g)					
NDV	3.21±0.19^{c}	3.87±0.27^{b}	4.74±0.51^{a}	4.95±0.56^{a}	0.0012
IBV	3.07±0.23^{c}	3.56±0.36^{b}	4.21±0.42^{ab}	4.73±0.61^{a}	0.0005
IBDV	2.80±0.29^{c}	3.26±0.21^{b}	3.33±0.28^{b}	4.11±0.31^{a}	0.0001
Dimensions of cecum					
Villus length (mm)	0.39±0.01^{c}	0.44±0.02^{bc}	0.48±0.02^{ab}	0.53±0.03^{a}	0.0024
Crypt depth (mm)	0.14±0.01^{a}	0.11±0.01^{ab}	0.09±0.01^{ab}	0.07±0.01^{c}	0.0143
Villus width (mm)	0.16±0.01^{b}	0.19±0.02^{b}	0.20-±0.01^{ab}	0.25±0.01^{a}	0.0344
VH:CD	2.80±1.11^{b}	4.10±0.49^{b}	5.57±0.99^{ab}	7.98±1.33^{a}	0.0152
Functions of kidneys and liver					
ALT (IU/L)	21.72±0.97	19.12±1.34	19.29±1.24	16.553±1.40	0.106
AST (IU/L)	201±18.00	219±17.34	205±6.65	188±16.25	0.601
ALP (IU/L)	1969±79.83	1845±79.18	1935±40.27	1610±132.14	0.784
BU (mg/dl)	3.64±0.24	4.06±3.42	3.11±0.46	4.20±0.37	0.251
CR (mg/dl)	0.59±.02	0.67±0.10	0.66±0.08	0.69±0.12	0.873

Means in the same row but with dissimilar superscripts are significantly different at P<0.05. PO-0, control group, PO-1, 10g of *P. ostreatus* per kg of feed; PO-2, 20 g of stem waste *P. ostreatus* per kg of feed; PO-3, 30g of *P. ostreatus* contained in per kg of feed; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BU, blood urea; CR, creatinine.

Table IV displays the average values of mortality and dressing percentage in quails in relation to the inclusion of various levels of stem waste from oyster mushrooms in their diet. Interestingly, mortality rates were not significantly influenced by the supplementation of different levels of stem waste from oyster mushrooms. It is worth noting that numerically, the control group exhibited a higher mortality rate compared to all the treatment groups. The dressing percentage in quails was notably influenced by the inclusion of stem waste from oyster mushrooms. As the level of stem waste increased, there was a corresponding increase in dressing percentage. The highest dressing percentage was observed in group PO-3 (71.67), while the lowest was in group PO-0 (69.36). Groups PO-1 (70.35) and PO-2 (70.83) exhibited similar dressing percentages.

The mean values of antibody titers for Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and infectious bursal disease virus (IBDV) in quails, in response to the inclusion of stem waste from oyster mushrooms in their diet, are presented in Table IV. The antibody titer for NDV was significantly affected ($p<0.05$) by the inclusion of stem waste from oyster mushrooms. The highest and similar antibody titers against NDV were recorded in groups PO-2 (4.74) and PO-3 (4.95), followed by PO-1 (3.87), while the lowest antibody titer was found in the control group (3.21). The effect of including various levels of stem waste from oyster mushrooms in the diet on the antibody titer against IBV was also found to be significant ($p\leq0.05$). The highest antibody titer was recorded in group PO-3 (4.73), followed by PO-2 (4.21), PO-1 (3.56), while the lowest antibody titer was observed in the control group PO-0 (3.07). The antibody titer against IBV in group PO-2 was not significantly different from that of group PO-3. Furthermore, the antibody titer against IBDV in response to feeding a diet containing various levels of stem waste from oyster mushrooms was also significantly affected ($p<0.05$). The highest antibody titer against IBDV was recorded in group PO-3 (4.11), followed by PO-2 (3.33) and PO-1 (3.26), both of which had the same antibody titer. The lowest antibody titer was observed in the control group (2.80).

The caecum morphology of quails is detailed in Table IV. Villus height and villus width in the caecum, in response to the inclusion of different levels of stem waste from oyster mushrooms in the diet, were significantly affected ($p<0.05$). The highest villus length was recorded in group PO-3 (0.53mm), followed by PO-2 (0.48mm) and PO-1 (0.44mm), while the lowest villus length was measured in group PO-0 (0.39mm). Crypt depth was highest in the PO-0 group (0.14mm), followed by PO-1 (0.11mm) and PO-2 (0.09mm) groups. The lowest crypt depth was found in the PO-3 group (0.07mm). The highest villus height to crypt depth ratio was calculated in the PO-3 group (7.98mm), followed by the PO-2 group (5.57mm), while a lower and similar ratio was recorded in the PO-1 (4.10mm) and PO-0 groups (2.80mm). Additionally, the highest villus width was recorded in group PO-3 (0.25mm), followed by PO-2 (0.20mm), while the lowest and similar villus width was measured in groups PO-0 (0.16mm) and PO-1 (0.19mm).

The values of various indicators for liver and kidney functions, including ALT, AST, ALP, BU, and creatinine, are provided in Table IV. It was observed that feeding different levels of stem waste from oyster mushrooms did not have any significant impact on liver and kidney functions. The values of ALT, AST, ALP, blood urea, and creatinine remained consistent across all groups, indicating no discernible differences in these parameters.

DISCUSSION

The study assessed feed intake (FI) on a weekly, starter, finisher, and overall basis. Interestingly, feeding diets supplemented with varying levels of stem waste from oyster mushrooms did not exert a significant impact on feed intake. Numerically, higher feed intake was observed in the PO-3 group, while the control group exhibited comparatively lower feed intake. These findings align with previous reports (Lee *et al*., 2012; Guimarães *et al*. 2014; Chang *et al*., 2016; Mahfuz *et al*., 2019), who similarly observed that the inclusion of stem waste from *Flammulina velutipes* mushrooms did not affect feed consumption in Japanese quails and broiler chickens. In contrast, Altop *et al*. (2022), observed a decrease in feed intake when including stalk meal from *Agricus bisporous* mushrooms in broiler diets. Similarly, Hammod (2018) found that broilers fed with a diet containing 30 g of *Agricus bisporus* mushroom/kg of feed experienced a decrease in feed intake. On the other hand, Shamsi *et al*. (2015) reported a decrease in dietary consumption of broiler chickens when supplemented with 20% *Agaricus bisporus* mushroom, which contrasts with the current study's findings. Hans *et al*. (2015) found that supplementing broiler diets with fermentation products of *Cordyceps militaris* mushrooms increased feed intake in broiler birds, which contradicts the current study's observations.

The body weight gain (BWG) of quails, both on a weekly and cumulative basis (starter, finisher, and overall), was significantly influenced by the inclusion of different levels of stem waste from oyster mushrooms in their feed. Notably, as the level of stem waste increased, the BWG improved. Group PO-3 exhibited significantly higher BWG compared to the control group. Many reports have concluded improved body weight gain in broilers when mushroom stalk residues were included as feed additives

(Toghyani *et al*., 2012; Guimarães *et al*., 2014; Shamsi *et al*., 2015). This enhancement in BWG could be attributed to the presence of oligosaccharides in the mushroom's cell wall, which may have positively influenced digestibility and nutrient utilization, resulting in improved weight gain (Roy and Fahim, 2019). Additionally, mushrooms may have contributed to a more balanced intestinal microflora, leading to a more efficient use of nutrients in the feed (Toghyani *et al*., 2012).

FCR suggests the ability of quails to transform feed into body weight. Inclusion of stem waste powder of oyster mushroom significantly affected the FCR of quails on weekly basis, and also on starter, finisher and overall basis. Higher FCR was recorded in the control group followed by groups PO-1, PO-2 while lowest (good) FCR was recorded for the group PO-3. Improved FCR in birds receiving diets having different levels of stem waste of oyster mushroom may be due to the possible increases in nutrients and energy utilization or both (Altop *et al*, 2022). In the present study, stem waste of oyster mushroom has increased villus length which results in better absorption of nutrients and hence leads to improved feed conversion ratio of quails. In alignment to the outcomes of current research trials, Altop *et al*. (2022) observed that inclusion of stalk meal of a mushroom *Agricus bisporous* in broilers diet decreased FCR of broilers. In agreement with the current study, Shang *et al*. (2016) reported that feeding diet supplemented with mushroom *Hericiumcaput-medusae* improved impact on FCR of meat type chicken. The current findings are in agreement with Shamsi *et al*. (2015) who found that the FCR of broiler chicken was improved with feeding diet supplemented with 20% of a mushroom called *Agaricus bisporus*. In consistence to the current study Han *et al*. (2015) explored that supplementing broilers diet with fermentation products of *Cordyceps militaris* mushroom has improving impact on FCR of broilers in starter phase.

Mortality is the death rate of birds in the particular population of those birds. Mortality was not affected by feeding diet having different levels of stem waste of oyster mushroom. These results are similar to the previous findings of Cox *et al*. (2010), Kayvani *et al*. (2012), Ba *et al*. (2021) and Fan *et al*. (2020). There was significant effect of feeding ration having different levels of stem waste of oyster mushroom on dressing percentage of quails. Dressing percentage of birds increased with increasing the levels of stem waste of oyster mushroom. Highest dressing percentage was found in the group which received the feed with highest level (3%) of stem waste of oyster mushroom while the lowest dressing percentage was found in the control group which received feed without (0%) stem waste of oyster mushroom. This enhancing impact of mushroom waste on dressing percentage might be due to increasing protein absorption in the ileum of birds as a result of addition of mushroom in the diet (Jahanian and Ashnagar, 2015). Moreover, the mushroom exerts positive effect on the villi length of the birds' gut which helps to in better absorption of nutrients as a result of which the dressing percentage is improved (Baurhoo *et al*., 2007).

In the present study, highest antibody titre against all three viruses was found in group PO-3, which received 3% of oyster mushroom waste in feed. The higher values for antibody titre against the viruses may be due to modulating effect on intestine immune system specially, on Peyer's patches to stimulate the secretion of haematopoietic growth factors, IL-6 and GM-CSF (Koh *et al*., 2002). Both *in vitro* and *in vivo* studies have demonstrated that beta glucan present in large amount in mushrooms increased the antibody titre of animals due its enhanced effect on activating macrophages, T-helper and natural killer cells, which also involves the differentiation and proliferation of T-lymphocytes (Willis *et al*., 2013). There are receptor on phagocytes and Natural killer cells for beta-glucans, which act upon these cells and stimulate immunity of the body (Lowe *et al*., 2001).

In the present study, villus height, width and their ratio were significantly improved while crypt depth decreased signfciantly in the quails supplemented with PO-3. Improved intestinal morphology in response to feeding mushrooms may be due to the antioxidant properties of mushroom (Gungor and Erner, 2020). The beneficial effect of mushroom stalk meal on the intestinal microflora can be another reason for the improvement in intestinal morphology. Jazi *et al*. (2017) noted that gut microflora is quite important and plays a crucial role in improving intestinal morphology in poultry. Moreover, there is positive and direct relation between villus height and microbial population in gut (de los Santos *et al*., 2005; Feng *et al*., 2007).

From the results it was found that there was non-significant effect of stem waste of oyster mushroom on liver and kidney function of quails. Different indicators of liver and kidney function were tested to investigate whether feeding stem waste of oyster mushroom has any toxic effect on quails' body. To evaluate the effect of stem waste of oyster mushroom on liver function, blood levels of AST, ALT and ALP were tested. All the values of liver enzymes were in normal range, as these enzymes serve as index of liver health (Hanley *et al*., 2005). Hence, it is clear from the results that inclusion of stem waste of oyster mushroom has no toxic effect on liver function of quails. Similarly, to find out toxic effect of stem waste of oyster mushroom on kidneys of quails blood urea and blood creatinine values were being found out. It was observed in the results that feeding different levels of stem waste of oyster mushroom

had no effect on the BU and CR level quails. BU and CR levels in all experimental groups were in normal range. The normal range of blood urea level and creatinine level indicates that stem waste of oyster mushroom does not interfere with the renal capacity to excrete metabolites, which further proves that it has no deteriorating impact on kidney function of quails (Jahanian and Ashnagar, 2015).

CONCLUSION

It is concluded from the findings of this study that supplementation of diet with different levels of stem waste of oyster mushroom improved growth performance, immunity and gut morphology in quails without affecting liver and kidney functions. Best results were obtained when stem waste of oyster mushroom was used at the level of 3% in the feed.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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