Effect Using Mushroom Waste Substitute With Wheat Bran To Aged Laying Hens Diet On Histological And Physiological Performance

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Abstract

This study was conducted at animal production farm belong to College of Agriculture, University of Anbar, from 7th April to 21st July , 2025. The study aimed to investigate using of oyster mushroom culture products grown on crushed Reed Plant (*officinarum*) as a substitute with wheat bran in diets of aged laying hens and its effect on productive performance. The experiment lasted for four periods (28 days/period). One hundred - eight of Lohmann Brown (50-weeks old) were used in study were divided into six experimental groups, with three replicates (six hens per replicate). The experimental treatments were as follows: T1: standard diet without any additives. T2: standard diet with 20% wheat bran replaced by mushroom culture waste ; T3: standard diet with 40% wheat bran replaced by mushroom culture waste ; T4: standard diet with 60% wheat bran replaced by mushroom culture waste ; T5: standard diet with 80% wheat bran replaced by mushroom culture waste ; T6: standard diet with 100% wheat bran replaced by mushroom culture Waste were cultivated by inoculating mycelium on wheat seeds. Reed Plant (*Saccharum officinarum*) straw (80%) and wheat straw (20%) were used. After drying and grinding, the inoculated bags were placed in an incubation chamber until the mushrooms reached maturity. A significant decrease ($P \leq 0.05$) was observed in blood Glucose concentration, AST, LDL, Cholesterol, and PCV in treatment T6, which used 100% mushroom residue powder, compared to the control treatment T1. A significant increase ($P \leq 0.05$) was observed in total plasma protein, albumin, and RBC concentrations in treatment T6. A significant increase ($P \leq 0.05$) was also observed in hemoglobin levels in treatment T4. White blood cell counts showed a significant increase ($P \leq 0.05$) in treatment T2. Histological measurements showed significant increases in villus thickness, villus diameter, and villus depth in both treatments T6 and T5. It can be concluded that birds fed mushroom residue exhibit greater antioxidant resistance, higher protein levels, and enhanced immunity.

Keywords : Mushroom Culture Waste, Wheat Bran, Physiological Performance , Histological , Biochemical , Laying Hens.

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Introduction

Global demand for fruit foods is increasing due to population growth, highlighting the need for basic agricultural practices and Productive performance of laying hens not affected by some feeding programs (1). Each part plays a pivotal role in the agricultural economy by transforming diverse foodstuffs into highly desirable sources for human consumption (6). Agricultural waste is defined as the residues generated during the industrial processing of agricultural products, or the byproducts or residues resulting from this. Waste can be divided into two types: agricultural waste and industrial waste, respectively (9). Agricultural waste consists of residues and stalks, while industrial waste is the waste produced by food and beverage industries, such as bran, husks, residues, and Reed Plant (*Saccharum officinarum*) residues. Generally, the remaining portion of industrial waste is disposed of in landfills or lost, leading to various optional solutions and causing harm, which concerned about, far removed from human concerns, and is environmentally friendly (10). These by-products can serve as a food source, in addition to being a source of carbon. Furthermore, this type of byproduct provides the nutrients required for mushroom growth through fermentation (14). Edible mushrooms are highly nutritious and can be used as food sources. They are rich in high-quality protein, carbohydrates, various vitamins, minerals, and other nutrient ،They are characterized by low glycoproteins and low cholesterol (7) . These edible mushrooms contain a variety of bioactive organic compounds, such as succharides and dietary fiber ،These compounds possess antioxidant, antitumor, and other physiological functions (20). Based on above, the study aimed to determine the effect of using mushroom cultivation residues on ground Reed Plant (*Saccharum officinarum*) as a substitute for bran in the diets of old laying hens and its effect on physiological and histological performance.

MATERIALS AND METHODS

Collection of Mushroom Cultivation Waste

The study was conducted at College of Agriculture, University of Anbar. The fungal inoculum was propagated on wheat seeds according to (13) method. High-quality wheat grains, free from insects and pathogens, were used. 2.5 gm. of calcium carbonate (limestone $CaCO_3$) and 2.5 g of calcium sulfate (gypsum $CaSO_4 + 2H_2O$) were added to each 125 gm. of grain (wet weight). The grains were then sterilized using an autoclave. Eighty percent of growing medium (Reed Plant straw and wheat straw) was used, after drying and grinding them. The growing medium (Reed Plant straw and wheat straw) was packed into bags. The inoculant bags were placed in an incubation chamber until mycelium had fully developed. The remaining growing medium was then dried, ground, and

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stored in bags in suitable locations until field trials could be conducted.

Laboratory Experiment

Preliminary chemical analysis was performed on mushroom culture residues, which included a mixture of sugarcane Reed Plant (officinarum) straw and wheat straw and mycelium filaments after inoculation with oyster mushrooms, to determine their nutritional value. This analysis was carried out in the laboratories of the Scientific Research Authority, Environment and Water Research Center, as shown in Table 1. Tests were also conducted on the powder of the mushroom culture residue mixture to determine its chemical composition and the types and proportions of amino acids it contains, as shown in Tables 1 and 2.

Table 1. Chemical Analysis of Mushroom Culture Waste(Reed Plant (officinarum) straw and wheat straw) and mycelium

Ingredients	%
Protein	10
Fat	3.5
Ash	7.45
Moisture	6.7
Fiber	6.5
Carbohydrate	60
Metabolizable Energy	1300

Table 2. Type and Quantity of Amino Acids in Mushroom Culture Waste Powder

Amino Acids	(mg/100gm)
Aspartic acid	11.80
Glycine	15.65
Lysine	14.70
Serine	13.66
Threonine	15.40
Isoleucine	15.99
Alanine	14.70
Valine	13.65
Tyrosine	12.40
Arginine	15.02
Cysteine	13.98
Methionine	15.80
Proline	13.60
Histidine	12.80
Lucien	14.80
Glutamic acid	12.98
Phenylalanine	14.80

Diets and Feeding

This field experiment was conducted at College of Agriculture, University of Anbar. The experiment lasted 16 weeks, divided into four experimental periods (28 days / period). One hundred - eight of laying hens (Lohmann Brown) aged 50 weeks, were used. The hens were raised using a cages rearing system, with three hens per cage, were distributed into six experimental groups, each containing three replicates, and each replicate containing six hens. The experimental groups were as follows: T1: standard feed without any additives. T2: Wheat bran replaced with 20% mushroom culture waste. T3: Wheat bran replaced with 40% mushroom culture waste. T4: Wheat bran replaced with 60% mushroom culture waste. T5: Wheat bran replaced with 80% mushroom culture waste. T6: Wheat bran replaced with 100% mushroom culture waste Based on dry weight For oyster mushroom and wheat bran farming.

Table 3. Ingredient and Chemical composition Calculated of Experimental Diets						
Ingredients	**Treatments**					
	T1	**T2**	**T3**	**T4**	**T5**	**T6**
Yellow Corn	35	35	35	35	35	35
Wheat	15	15	14.5	14	13.5	13.5
Barley	5	5	5	5	5	5
Soybean Meal*	18	18	18.5	19	19.5	19.5
Protein Concentrate	5	5	5	5	5	5
Mushroom Culture Waste	0	2	4	6	8	10
Wheat Bran	10	8	6	4	2	0
Dicalcium Phosphate	1.75	1.75	1.75	1.75	1.75	1.75
Limestone	7	7	7	7	7	7
Oil	2.25	2.25	2.25	2.25	2.25	2.25
Salt	1	1	1	1	1	1
Total	100	100	100	100	100	100
Calculated Chemical analysis **						
Metabolizable Energy (Kcal/kg feed)	2650	2650	2646	2643	2639	2639
Crude protein %	17.46	17.35	17.41	17.48	17.55	17.44
Calcium %	3.41	3.41	3.41	3.41	3.41	3.41
Lysine %	0.93	0.93	0.95	0.96	0.97	0.97
Cysteine + Methionine	0.7	0.7	0.7	0.71	0.71	0.71
Available phosphorus	0.66	0.66	0.67	0.67	0.67	0.67

Physiological tests of blood plasma after the end of the field experiment. Blood samples were drawn, and at the end of the experiment, 6 birds were randomly selected from each treatment (two birds per replicate) . Blood samples were collected through the birds' wing vein, and test tubes containing the anticoagulant substance (EDTA) were placed in it to conduct biochemical tests on the blood plasma. Samples were collected and placed in a centrifuge at 3000 rpm for a quarter of an hour to separate the plasma from the cellular fraction.

The samples were then placed in a freezer at -20°C for blood biochemical tests. These tests were conducted in the central laboratory of the College of Agriculture, Anbar University. After slaughtering the birds, they were dissected and a piece of the small intestine was taken. Histological section samples were taken from the end of the jejunum before the vestigial scar to the point of the vitelline diverticulum. 5 cm was cut from the end of the jejunum. Samples were taken from all replicates from each of the experimental treatments. One bird was taken for each replicate and the samples were kept in tubes containing 10% formalin for one day. Histological examinations were conducted at the College of Veterinary Medicine, University of Baghdad

Statistical Analysis System (17) was used to analyze the data and examine the effect of different treatments on the studied traits using a completely randomized design (CRD). Significant differences between means were

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compared using Duncan's multiple range test (5).

RESULTS AND DISCUSSION

Figure (1) shows the effect of using different proportions of mushroom culture residues in diet of laying hens on the biochemical blood characteristics. The results showed that the glucose value showed a significant superiority (P≤0.05) for treatment T4 over treatment T6 and did not differ significantly from treatments T1, T2, T3 and T5 . As for the uric acid value and ALT value, no significant differences were observed between the experimental treatments. As for the AST value, a significant decrease (P≤0.05) was found for treatment T6 compuved with treatments T1 , T2 , T3 , T4 and T5

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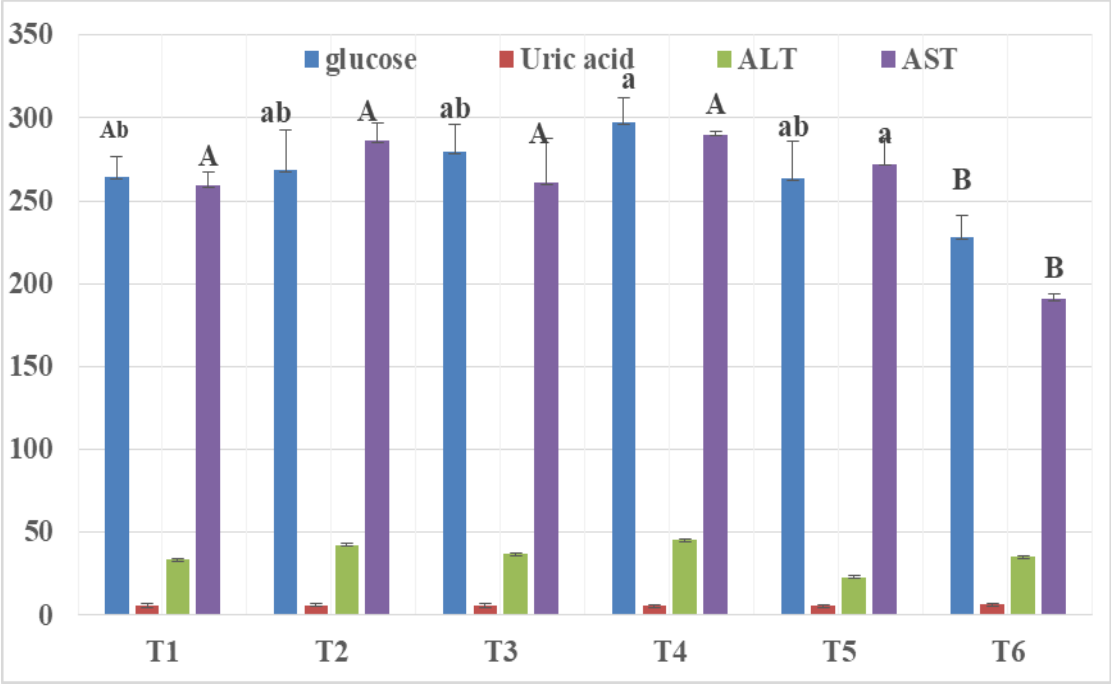


Figure (2) shows effect of using different proportions of mushroom culture residues in diets of laying hens on blood biochemical characteristics. The results for protein and albumin values showed a significant superiority (P≤0.05) for treatment T6 over treatments T1, T2, T3, T4, and T5. As for globulin values, no significant differences were observed between the experimental treatments.

Figure 1 . Replacing Wheat Bran with Mushroom Culture Waste and Crushed Reed Plant to Laying Hen Diet´s on some Biochemical Traits

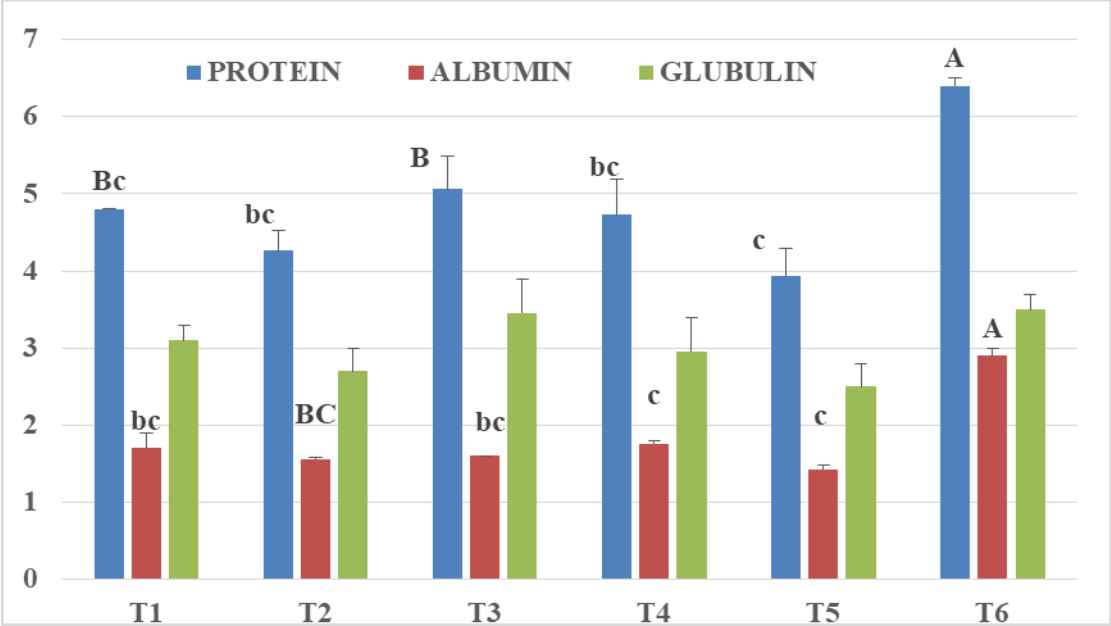


Figure 2. Replacing Wheat Bran with Mushroom Culture Waste and Crushed Reed to Laying Hen Diet´s on Protein , Albumin and Globulin

Table (4) shows the effect of using different proportions of mushroom culture waste in diets of laying hens on biochemical blood characteristics. The results showed values of of heterophylls and lymphocytes and the ratio between heterophylls and lymphocytes. No significant differences were observed between the different experimental treatments.

Table 4. Replacing Wheat Bran With Mushroom Culture Waste and Crushed Reed Plant to Laying Hen Diet´s on Lymphocyte , Heterophil and H/L Ratio

Variable	Treatments						Significant
	T1	T2	T3	T4	T5	T6	
Lymph	± 50.00	± 50.00	±47.00	± 41.00	± 44.33	±48.00	N.S
Hetero	± 39.00	± 41.00	± 43.3	± 47.66	± 44.66	± 40.66	N.S
H/L	± 0.77	± 0.82	± 0.95	± 1.17 0.06	± 1.02	± 0.91	N.S

*Values represent the mean ± Standard Error.
NS: Not statistically significant.
a, b, c: Different letters in the same row indicate statistically significant differences between treatments at the (P≤ 0.05) level.
T1: Control. T2: replacement of wheat bran with 20% mushroom culture waste. T3: replacement of wheat bran with 40% mushroom culture waste. T4: replacement of wheat

Table (5) shows the effect of using different proportions of mushroom culture residues in diets of laying hens on lipids parameters. The results for cholesterol showed a significant increase (P≤0.05) in treatments T1 and T2 compared to treatments T3 and T6, and did not differ significantly with treatments T4 and T5. No significant differences were observed between the experimental treatments for triglycerides, HDL, and VLDL. However, a significant decrease (P≤0.05) in LDL was found in treatments T3 and T6 compared to treatments T1, T2, and T4, which did not differ significantly with treatment T5

Table 5 . Replacing Wheat Bran with Mushroom Culture Waste and Crushed Reed Plant to Laying Hen Diet´s on Cholesterol, Triglycerides , HDL , LDL and VLDL

Variables	Treatments						Significant
	T1	T2	T3	T4	T5	T6	
Cholesterol	±254.66 9.33	253.33 ± 20.89	±198.33 0.66	229.33 ± 5.66	225.33 ± 0.33	208.00 ± 5.00	0.05
Triglycerides	149.33 ± 8.33	148.33 ± 14.33	± 138.33 10.66	132.00 ± 1.00	127.33 ± 12.33	125.66 ± 6.33	N.S
HDL	± 42.66 3.33	45.66 ± 2.02	± 45.66 2.33	± 5.66 0.66	46.00 ± 5.00	± 49.33 0.33	N.S
LDL	182.33 ± 7.66 A	178.00 ± 20.52 A	125.00 4.00 ± C	157.33 ± 6.66 Ab	154.00 ± 3.0 Abc	133.66 ± 4.33 Bc	0.05
VLDL	± 29.66 1.66	29.66 ± 2.72	± 27.66 2.33	26.33 ± 0.33	25.33 ± 2.33	25.00 ± 1.00	N.S

Values represent the mean ± Standard Error.

NS: Not statistically significant.

a, b, c: Different letters in the same row indicate statistically significant differences between treatments at the (P≤ 0.05) level.

T1: Control. T2: replacement of wheat bran with 20% mushroom culture waste. T3: replacement of wheat bran with 40% mushroom culture waste. T4: replacement of wheat bran with 60% mushroom culture waste . T5: replacement of wheat bran with 80%

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mushroom culture waste. T6: replacement of wheat bran with 100%

Table (6) shows the effect of using different proportions of mushroom culture residues in diets of laying hens on blood characteristics. The results of white blood cell value showed a significant superiority (P≤0.05) for treatment T2 over treatments T4 and T5 which did not differ significantly with treatments T1, T2, T3 and T6. As for the uric acid value and the ALT value, no significant differences were observed between the experimental treatments. As for AST value, a significant decrease (P≤0.05) found for treatment T6 over treatments T1, T2, T3, T4 and T5.

Table 6 . Replacing Wheat Bran with Mushroom Culture Waste and Crushed Reed Plant to Laying Hen Diet´s on WBC, RBC , PCV and Hb

Variables	Treatments						Significant
	T1	T2	T3	T4	T5	T6	
WBC	± 22.56 0.96	± 23.33 1.96	±19.80 2.30	17.40 ± 0.10	18.23 ± 1.10	± 18.76 0.63	0.05
RBC	± 1.87 0.06 b	± 2.28 0.13	± 2.87 0.29	± 2.87 0.22	±2.72 0.30	± 2.58 0.05	0.05
PCV	±21.23 0.63	± 23.86 0.26	±23.93 1.16	± 27.76 0.93	27.03 ± 1.76	± 25.03 0.83	0.05
Hb	± 6.40 0.20 c	±7.30 0.10 bc	± 7.30 0.40 bc	± 8.60 0.30 a	± 8.33 0.56 ab	±7.66 0.26 ab	0.05

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Values represent the mean ± Standard Error.
NS: Not statistically significant.
a, b, c: Different letters in the same row indicate statistically significant differences between treatments at the (P≤ 0.05) level.
T1: Control. T2: replacement of wheat bran with 20% mushroom culture waste. T3: replacement of wheat bran with 40% mushroom culture waste. T4: replacement of wheat bran with 60% mushroom culture waste . T5: replacement of wheat bran with 80% mushroom culture waste. T6: replacement of wheat bran with 100%

Histological Examination

Figure (3) shows the effect of using different proportions of mushroom culture residues in the diet of laying hens in histological examination. The results for villus length showed a significant improvement (P≤0.05), as treatments T5 and T6 were significantly superior to treatments T1, T2, T3, and T4 in the same trait. As for villus thickness, a significant improvement was found (P≤0.05), as treatment T3 was superior to treatment T2 and did not differ significantly from treatments T1, T4, T5, and T6 in the same trait. As for crypt depth, a significant improvement was found (P≤0.05), as treatment T5 was superior to treatments T1, T2, and T3 and did not differ significantly from treatments T4 and T6 in the same trait.

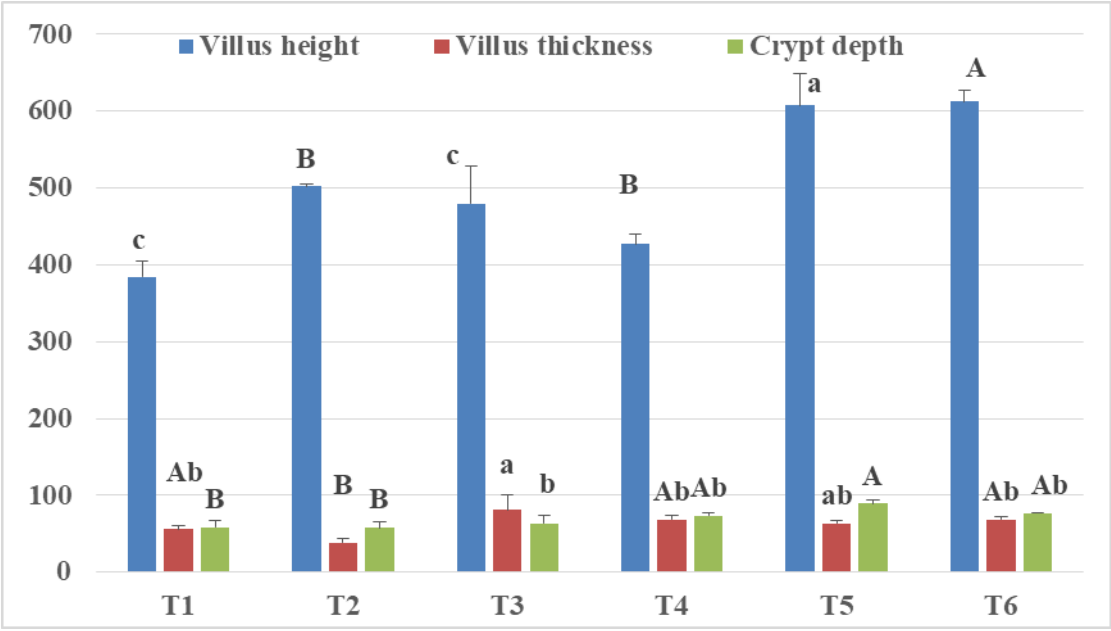


Fig. 3 . Replacing Wheat Bran with Mushroom Culture Waste and Crushed Reed Plant to Laying Hen Diet´s on Villus height , Villus thickness and Crypt depth

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This improvement may be attributed to Mushroom culture waste contains high levels of beta-glucans, a type of soluble fiber that increases the viscosity of digestive contents, leading to reduced carbohydrate absorption from intestines. This, in turn, decreases glucose flow into the bloodstream. Additionally, the formation of a gelatinous layer acts as a physical barrier, reducing contact between digestive enzymes and starches, thus slowing their conversion into simple saccharides (16). This improvement may be attributed to Furthermore, the bioactive compounds in mushrooms culture act as natural inhibitors of alpha-glucosidase and alpha-amylase these enzymes are responsible for breaking down complex saccharides to glucose, and their inhibition results in less free glucose available for absorption in the small intestine This also improves insulin sensitivity and activates the AMPK pathway (15). The significant improvement in total protein and albumin concentrations when using mushroom culture waste is attributed to the direct correlation between egg production and total egg protein content This is because most egg components are transported via the blood to ovary bound, reflecting positively on the health of birds, which in turn is reflected in egg production (18) .The reduced AST levels are attributed to the presence of compounds, flavonoids, and ergothioneine from mushroom remains These compounds reduce oxidative stress in liver cells, protecting them from damage Furthermore, the bioactive compounds enhance liver enzyme systems responsible for detoxification, thus reducing the leakage of aspartate aminotransferase (AST) into the bloodstream This helps improve the functional state of the liver (18). The ability to absorb cholesterol depends on the nature of the feed components. The decrease in cholesterol concentration is attributed to the unsaturated fatty acids and phenols in mushroom culture residue powder, which inhibit cholesterol synthesis due to their role as natural antioxidants, thus helping to suppress free radical activity. Additionally, the soluble dietary fiber in the mushroom residue leads to the formation of viscous gels, such as glucans, which prevent cholesterol absorption (8). The reduction in LDL cholesterol levels in laying hens after adding mushroom culture (Pleurotus ostreatus) products to the feed is often attributed to decreased energy and fat metabolism in the liver. This is because the chicken liver is a major center for fat synthesis and transport to the ovary for yolk formation. Furthermore, the high fiber content leads to decreased energy metabolism and inhibits the liver from synthesizing compensatory fats, thereby reducing the production of very low-density lipoproteins (VLDL), which are partially converted to low-density lipoproteins (LDL) in the blood. (15).

This improvement may be attributed to Mushroom culture waste is an important source of micronutrients essential for red blood cell formation and growth, including iron and copper.

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These two minerals are key components of hemoglobin, the protein responsible for oxygen transport in red blood cells. Iron is the central atom in the heme group of hemoglobin, while copper plays a role in iron metabolism and utilization (2). Mushrooms culture waste are high in iron, an essential element for hemoglobin formation and protection from oxidation. The antioxidants they contain protect red blood cell membranes from oxidative stress. Red blood cells are particularly vulnerable to oxidative damage due to the high oxygen levels and the presence of lipids in their membranes. These antioxidants neutralize reactive oxygen species, preventing lipid oxidation. This leads to longer red blood cell lifespan and maintains high hemoglobin levels, cell volume, and hemoglobin concentration Beta-glucans found in mushrooms culture are known to enhance various types of immune cells, improving their function and strengthening the immune system. This effect can lead to a stronger immune response without increasing the total number of white blood cells, but rather by improving the balance of different types of white blood cells (21).

The improvement in intestinal tissue is attributed to mushroom culture residues, which are rich sources of bioactive compounds and contain high levels of dietary fiber, β-glucans, and chitin, in addition to phenolic compounds and residual fungal enzymes. Numerous studies have shown that incorporating these residues into diets positively impacts digestive health, particularly the morphology of the small intestine (12). The indigestible fibers in mushroom culture residues help improve the balance of intestinal microbiota. These fibers act as prebiotics, promoting the growth of beneficial bacteria and reducing the proliferation of harmful microorganisms (11). When these fibers are fermented by intestinal microbes, short-chain fatty acids are produced, most notably butyric acid. This acid is a major energy source for intestinal lining cells and plays a significant role in stimulating cell division and regeneration (19). In addition, mushroom culture residues contain antioxidant compounds that contribute to reducing intestinal inflammation and oxidative stress, thus helping to protect the intestinal mucosa and reducing intestinal villus erosion. As a result of these interactive effects, an improvement in the length and thickness of the small intestine villi can be observed, as the increased length and thickness of the villi enhance the absorption surface area and nutrient utilization efficiency (3). We can conclude that the beneficial effect of mushroom culture residues on intestinal villi is not direct, but rather occurs through improving intra-intestinal conditions and supporting the vital activities of intestinal cells. This makes it an important component in animal nutrition and feed (18).

Conclusion

This study suggests that mushroom culture waste can be a good source of natural feed additives that improve the concentration of certain physiological parameters in blood plasma and histological condition of intestines of laying hens. This mushroom waste can be used as a natural feed supplement, as well as a substitute for wheat bran in laying hen feed.

REFERENCES

1. Abood, N.K. and B.H. Mousa. (2023). Feeding Programs and

ISSN 2072-3857

Addition High Levels of Calcium, Phosphorus and Vitamin D3 to Aged Laying Hens' Diets on Productive Performance. Iop Conference Series Earth and Environmental Science. 1252(1), 012112.

2. Adams, A., Seecharran, D., and Ansari, A. (2022). Growth of oyster mushroom using sawdust and agriculture waste as substrates. *Mushroom Research*, *31*(1), 73-80.
3. Alhamdani, H.A., Shahooth, M.A., Ali, N.A.-L., Mousa, B.H. and T.M. Al-Thuwaini. (2025). Individual and Synergistic Effect of Adding Zeolite and Yeast to Reduce the Negative Impact of Aflatoxin B1 on the Physiological and Immune Performance of Laying Hens. Journal of Animal Health and Production. 13(1), pp. 38–44.
4. Bormon, C. C., Akib, G., Rifat, A., Hossain, M., Uddin, N., Hossain, F. M. A., ... and Mahfuz, S. U. (2024). Effects of oyster mushroom (*Pleurotus ostreatus*) stem residue supplementation on growth performance, meat quality and health status of broilers. *Poultry Science*, *103*(10), 104054.
5. Duncan, D.B. (1955). Multiple rang and Multiple F tests. Biometrics 1-42.
6. Effiong, M. E., Umeo Kwakoski ochi, C. P., Afolabi, I. S., and Chinedu, S. N. (2024). Assessing the nutritional quality of *Pleurotus ostreatus* (oyster mushroom). *Frontiers in nutrition*, *10*, 1279208
7. Kalaf, H. H., Abed, I. A., & Alkobaisy, J. S. (2024, July). Breeding Two Species of *Pleurotus spp*. Fungi on Different Local Agricultural Wastes. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1371, No. 8, p. 082012). IOP Publishing.
8. Karageorgou, A., Mouiki, D., Lolou, D. M., Hager-Theodorides, A. L., Theodorou, G., Massouras, T., and Goliomytis, M. (2024). The influence of dietary supplementation with oyster mushroom waste on laying hens' performance, egg quality and immune parameters. *Poultry Science*, *103*(12), 104320
9. Kumar, P., Raj, A., and Kumar, V. A. (2024). Approach to reduce agricultural waste via sustainable agricultural practices. In *Valorization of biomass wastes for environmental sustainability: green practices for the rural circular economy* (pp. 21-50). Cham: Springer Nature Switzerland.
10. Kumla, J., Suwannarach, N., Sujarit, K., Penkhrue, W., Kakumyan, P., Jatuwong, K., and Lumyong, S. (2020). Cultivation of mushrooms and their lignocellulolytic enzyme production through the utilization of agro-industrial waste. *Molecules*, *25*(12), 2811.
11. Mousa, B.H., Nafaa, H.H. and O.K. Attallah. (2018). Comparison of adding locally prepared probiotic and organic acid (acetic acid) to diets and effects on performance of laying hens. Biochemical and Cellular Archives. 18, pp. 1245–1249.

ISSN 2072-3857

12. Nasir, J. A., Chand, N., Naz, S., Alhidary, I. A., Khan, R. U., Batool, S., and Losacco, C. (2023). Dietary oyster mushroom (*Pleurotus ostreatus*) waste inhibits experimentally induced Eimeria tenella challenge in Japanese quails model. Animals, 13(21), 3421.
13. Oei, P. 2005. Small-scale mushroom cultivation (oyster, shiitake and wood ear mushrooms). Digigrafi, no 40 Wageningen, Netherlans ; pp. 86.
14. Rungjindamai, N., Trakunjarunkit, K., Posalee, T., & Limpanya, D. (2024). Utilization of agricultural waste for the cultivation of *pleurotus mushrooms* in Thailand. *Journal of Pure and Applied Microbiology*, *18*(2), 941-950.
15. Salahuddin, M., Balan, V., Stamps, K. G., Abdel-Wareth, A. A., Mohammadi, M., Kim, W. K., . and Lohakare, J. (2025). Harnessing mushrooms for poultry nutrition: boosting health, immunity, and productivity. *Poultry Science*, *104*(8), 105223.
16. Singla, A., Gupta, O. P., Sagwal, V., Kumar, A., Patwa, N., Mohan, N., and Singh, G. (2024). Beta-glucan as a soluble dietary fiber source: Origins, biosynthesis, extraction, purification, structural characteristics, bioavailability, biofunctional attributes, industrial utilization, and global trade. *Nutrients*, *16*(6), 900.
17. SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
18. Törős, G., El-Ramady, H., Béni, Á., Peles, F., Gulyás, G., Czeglédi, L., and Prokisch, J. (2024). Pleurotus ostreatus mushroom: a promising feed supplement in poultry farming. *Agriculture*, *14*(5), 663.
19. Yu, C., Dong, Q., Chen, M., Zhao, R., Zha, L., Zhao, Y., and Ma, A. (2023). The effect of mushroom dietary fiber on the gut microbiota and related health benefits: A review. *Journal of Fungi*, *9*(10), 1028.
20. Zhang, S., Ma, D. W., Yang, X., Chen, L., Xia, H., Wang, S. K., and Liao, W. (2025). The effect of chitosan on blood lipids in patients with hypercholesterolemia and hyperlipidemia: a systematic review and meta-analysis. *Food Med Homology*, *2*(4), 9420061.
21. Zhong, X., Wang, G., Li, F., Fang, S., Zhou, S., Ishiwata, A., and Ding, F. (2023). Immunomodulatory effect and biological significance of β-glucans. *Pharmaceutics*, *15*(6), 1615.

ISSN 2072-3857