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Effect of adding a synergistic mixture of Pleurotus ostreatus and beta-glucan to the diet on blood biochemical and immunological parameters of broiler Exposure to heat stress.

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Abstract
This study investigated the effect of adding a synergistic mixture of oyster mushroom and beta-glucan on the immunological and biochemical traits of broiler chickens exposed to heat stress. 300 one-day-old broiler chicks (Ross 308) were randomly distributed to four treatments. The first treatment (control) was fed a standard diet without any addition. The second, third, and fourth treatments were fed on a ration with the addition of the synergistic mixture at the rate of (0.5, 1, 1.5) g / kg feed for the starter ration. The addition to the final ration was (0.250, 0.5, 0.750) g/kg for each of the oyster mushrooms and the same amount of beta-glucan. The results showed a significant improvement ($P<0.05$) for the immunological characteristics (fabricia gland, index gland, spleen weight, corticosterone, H/L). The chemistry is vital for the blood of the treatments to which the synergistic mixture was added, especially the fourth treatment, to which 1.5 g / kg was added to the starter ration and 0.5 g / kg to the growth ration. Accordingly, the synergistic mixture of oyster mushrooms and beta-glucan can be adopted to cope with the heat stress that broilers are exposed to.

Keywords: betaclocan, oyster mushroom, immunological traits, biochemical traits, heat stress, synergistic mixture.

Introduction

Poultry production is the fastest growing and most profitable of all livestock sectors expanding in developed and developing countries (Hailemariam, et al., 2022). Poultry meat is accepted by consumers for its high nutritional quality, delicious taste, low cost, and an important animal protein source for human growth and development (Abd El-Hack, et al., 2022).There are many factors affecting this industry, including: Heat stress, which is a major concern in the poultry industry, affects broilers through health status and body weight (Siddiqui, et al, 2022). Stress also has a negative effect on the quality of poultry meat (Liu, et al., 2022).Growth promoters have been used in the poultry production industry for decades, resulting in a high risk of antibiotic-resistant bacteria being transmitted to humans. Breeding broilers is difficult without the use of antibiotics, so finding an effective nutritional alternative to support growth performance, gut health, and functions without the use of industrial antibiotics is crucial (Soumeh, et al., 2021).Animal nutritionists and producers are beginning to look for alternatives that promote growth, improve meat quality, and promote animal health.Plant extracts are currently receiving a lot of attention as feed additives due to the benefit of being natural.

Especially its extracts, which can be added to broiler feed such as probiotics (Lefter, et al., 2022). Oyster mushrooms are also one of the most important types of fungi that belong to the Basidiid family, which are rich in biologically active compounds such as flavonoids, antioxidants, and antioxidants. For bacteria that balance the gut microflora and thus improve the digestion process, which is positively reflected on feed consumption (Ullah.M, et al., 2020).It has been proven that beta-glucans, which are complex sugars found in the cell walls of some grains, fungi and yeasts, improve intestinal health, increase the flow of new immune cells, increase macrophage function, stimulate phagocytosis, influence intestinal morphology and enhance the number of goblet cells (Schwartz and Vetvicka 2021).

Hasan, et al., (2023) indicated that the study of biochemical blood parameters plays a very important role in assessing the physiological state of wild birds and conducting physiological studies, as well as to understand the basic physiological parameters and how these parameters differ according to age and sex. Hematological and biochemical parameters are good indicators of metabolic status and are affected by various seasonal processes related to moulting, reproduction and migration, in addition to daily fluctuations.

Materials & Methods

This study was conducted in the field of poultry research at the College of Agriculture - University of Basra for the period from 17/1/22 2022 to 22/2/2022. For a period of (35) days, 300 unsexed (Ross 308) broiler chicks, one day old, with an average starting weight of 42 g, were used in this study. Birds were distributed randomly into four treatments, and each treatment was divided into three replications (25 birds / replicate).The chicks were reared on the ground, and sawdust was used as a mattress to cover the floor. The hall was divided into cages with iron barriers, and the dimensions of one repetition were (100 x 200 x 75) cm.

The birds were fed free feed on a starting diet from the age of (1-21) days and a growth diet from (22-35) days as shown in Table (1), as the first female worker was fed (on a standard diet) without any addition.As for the second treatment, the tarzi mixture of 0.5 g / kg feed for the starter and 0.250 g / kg feed for the final was added to it. The third treatment was fed 1 g / kg feed for the starter and 0.5 g / kg feed for the final, and the synergistic mixture was added to the fourth treatment at 1.5 g / 1 kg feed for the starter And 0.750 g / 1 kg of feed for the final.The birds were exposed to heat stress from the age of one day until the end of the experiment (35) days, by exposing all treatments to the temperatures shown in Table (2) for 8 hours per day.

Prepare the synergistic mixture

-The mycelium of the oyster mushroom Pluerotes ostreatus was obtained from the National Center for Organic Agriculture / Plant Protection Department / Ministry of Agriculture - Iraq.

- Beta-glucan was supplied from one of the offices for medical and laboratory supplies, in a package of (500) gm.

- The spindle of the oyster fungus growing on the carrier was added with the same amount of beta-glucan to the feed. It was mixed well, then incubated in polyethylene bags at a temperature ranging from (28-30)^0 and a humidity ranging from (40-50)% for a period of (7-10) days, until the completion of the growth of the mycelium on all the amount of forage (Saadoun and Solaf, 2017).

Studied traits:

1- Immunological properties

1-1 Calculation of the Gland Fabricia Guide

The relative weight of the glandular tissue and spleen was calculated according to the following equation:

Member relative weight %=$\frac{User\ weight\ (gm)}{live\ body\ weight\ (g)} \times 100\%$

The index of the glandular fascia was calculated according to the following equation which was adopted by Hussein & Jassim, (2019). :-

Gland Fabricia Guide=

$=\frac{The\ ratio\ of\ gland\ weight\ to\ body\ weight\ in\ the\ experimental\ equation}{Ratio\ of\ gland\ weight\ to\ body\ weight\ of\ control\ treatment} \times 100\%$

1-2 Measuring the concentration of corticosterone / (Cortisol) in blood serum

Serum corticosterone concentration was estimated using a ready-made test kit (Kit) of American origin and using an Elecsys Cortisol (cobas e 411) device. According to the method described by (Tietz et al., 1999).

1-3 Measurement of the Heterocyte/Lymphocyte Ratio (H/L)

The ratio of heterogeneous cells to lymphocytes was estimated in the Poultry Technology Laboratory of the College of Agriculture, University of Basra, by placing a drop of blood through a capillary tube on a

clean glass slide and spreading it accurately using another glass slide, as it was pulled back at an angle of 45º, after which the slides containing blood smears were placed In methanol for fixation for 3-5 minutes, and after it dried, the slides were dipped in Giemsa dye, diluted (10%) for 15 minutes, and after drying, they were ready for examination under the microscope. L under the microscope according to the method indicated by (Shen & Paterson., 1983), and the H/L ratio was extracted according to the following equation:

$$\text{H/L ratio} = \frac{The\ number\ of\ heterophiles\ is\ H/100\ white\ cells}{Lymphocyte\ count\ L/100\ white\ cells} \times 100\%$$

2- Biochemical characteristics of blood

The concentration of total protein, albumin, glucose, and cholesterol were measured. High-density lipoproteins (HDL) were measured, and the concentration of (LDL) in blood serum was measured using a ready-made assessment kit (Kit) equipped by the French company (Biolabo). The company is in the guide attached to the kit.Very low-density lipoproteins (VLDL) were calculated according to the following equation:

VLDL = Total Cholesterol – (HDL – LDL)

3- Statistical analysis

The use of complete random design (CRD), using the program (SPSS, 2019) in statistical analysis And using the following mathematical model (yij = μ + ti + eij).

Table (1) The components of the diet used in the experiment

the components (%)	starter bush days (21-1)	The final bush days (35-22)
yellow corn	56	58
wheat	4.5	8
Soybean meal (48%)	32	27
protein concentrate *	5	4
limestone	0.7	0.7
Vegetable oil	0.5	1
Vitamin and Mineral Blend**	1	1
the salt	0.3	0.3
the total	%100	%100
Calculated chemical composition***		
Crude protein (%)	22.98	20.82
Kilo calories represented energy / kg feed	2970	3048

* Protein concentrate for feeding broiler chickens (Brocorn-5 special W) produced by (Wafi B.V. Alblasserdam-Holland), chemical composition: 40% crude protein, 5% crude fat, 2.20% crude fiber, 7.10% moisture, 28.30% crude ash , 4.20% calcium, 2.65% phosphorus, 2107, energy (kcal / kg). 3.70%Metonin, Cyclin + Cysteine 0.42%, 4.12%Leisen, Turbetovan 0.42%, Throne 1.70%, sodium 2.50%, chlor mg/kg, iron 2.000 mg/kg, selenium 5.00 mg/kg ** a mixture of minerals and vitamins, chemical composition: 10% crude protein, 2.1% crude fat, 0.34% crude fiber, 2.66% moisture, 51.02% crude ash, 20.08% Calcium, 10.83% Phosphorus, 753.82 kcal kg. Energy ($kcal.g^{-1}$). *** Calculation of the chemical composition of the feed was made according to NRC (1994).

Table (2) The approved periodic temperatures during the experiment period

age / day	1-7	8-21	22-35
temperature	$38\text{-}40^0$	$34\text{-}36^0$	$27\text{-}29^0$

** Note that the period of exposing birds to heat is (8) hours per day, then it is gradually reduced.

Results and discussion

immunological measurements

The results of Table (3) indicate that there is a significant difference ($P < 0.05$) in the relative weight of the Fabricia gland and the Fabricia gland index between (T4) to which the synergistic mixture was added at an amount of (1.5) g / kg feed and the control treatment (T1) and (T2).) to which the synergistic mixture was added by (0.5) g / kg feed, as the fourth treatment recorded the lowest relative weight of Fabricia gland 0.067%, which did not differ significantly with (T3), which recorded a relative weight of 0.070% compared with the first treatment (control) and treatment The second, in which the value of the two transactions amounted to 0.079 and 0.77%, respectively.Whereas, the percentage of the index of Fabricia gland (T4) and the third was 0.84, 0.88%, respectively, compared with the treatments (T1) and (T2), which recorded 1.00 and 0.97% each, respectively, which differed significantly with treatment (T4, T3).Table (3) showed that there were no significant differences in the relative weight of the spleen among all the experimental treatments, while there were arithmetic differences between the treatments. While there was a significant increase ($P < 0.05$) in the corticosterone hormone for the control treatment (T1), as its concentration in the blood serum was 1.62 (µ g / dl) compared with the treatment (T4), which recorded the lowest concentration of the hormone corticosterone, as it reached 1.37 (µ g / dl).

As for heterotrophic cells (H%) and lymphoid cells (L%) and the ratio between them (H/L), it is clear from Table (3) that there are significant differences ($P<0.05$) between the study coefficients. The treatment (T4) recorded the lowest percentage of heterotrophic cells, which amounted to 21.29%, compared with the control treatment (T1 and T2), which recorded the highest percentage of 27.30 and 25.37%, respectively, and which differed significantly with them, while it did not differ significantly with (T3), which It recorded a rate of 23.41%.In the same context, the results of the current study showed that the control treatment (T1) recorded the highest percentage of heterotrophic cells to lymphocytes (H/L), amounting to 0.42%, which did not differ significantly with the treatment (T2, T3) 0.41 and 0.36%, respectively. But it differed significantly with the treatment (T4), which recorded the lowest rate of 0.29%. The study of the Fabricia gland and its index is one of the indicators of the health and immunological status of the birds. Based on the results obtained in this study, which are shown in Table (3), the increase in the relative weight of the Fabricia gland and the index of the gland for the control treatment (T1) can be inferred that this treatment was affected more than the remainder. Treatments of heat stress to which the birds of this study were subjected or a disease that they suffered from. This preference can be attributed to the treatments to which the synergistic mixture was added to the properties it is characterized by.(Khan, et al., (2019) showed that oyster mushrooms play an important role in stimulating growth and immunity in poultry.And feeding broiler chickens on a protein isolated from mushroom and its aqueous extract and ethanol enhanced health via immune modulation (Ullah, et al., 2020). In the same context, (Hassan, R. et al., (2020) indicated that feeding poultry on oyster mushroom cultivation waste at a level of 1% improved performance and the humoral immune response to disease vaccines.

The significant increase ($P < 0.05$) in the concentration of corticosterone hormone for treatment (T1), may be attributed to the effect of heat stress on the birds of this treatment during the breeding period, as heat

stress stimulates the adrenaline gland to secrete this hormone. The level of corticosterone hormone in Serum reflects the well-being of birds (Von Eugen, et al., 2019). These results agreed with the findings of (Scanes, (2016), which confirmed that the increase in the concentration of corticosterone in the blood plasma is due to the birds being affected by stress.Also, these changes in the concentration of this hormone affect the immune response and the change in the size of the mass of the thymus gland, spleen, and gland fabricia. Therefore, this effect can be inferred from the results obtained from this study. As for the ratio (H/L), which was expressed by Al-Daraji et al. (2008), lymphocytes and heterochromatin cells are more sensitive than the rest of the white blood cells in the case of exposure of birds to abnormal conditions, because of their ability to devour pathogens.Also, one of the indicators that gives evidence of the extent of exposure of birds to stress is the ratio between them (H / L), and this ratio is an indicator of the overlap between immunity and environmental physiology. Accordingly, this increase in the ratio (H/L) in the control treatment (T1) can be attributed to the fact that it was affected more than the rest of the treatments, especially treatment (T4) and (T3). heat stress to which birds were exposed.Or perhaps the high ratio (H/L) in treatment (T1) is due to an increase in the concentration of the corticosterone hormone in the blood serum, as shown in the results of this study, as this hormone works to increase the percentage of heterotrophic cells and reduce the percentage of lymphocytes and inhibit their work, which leads to an increase in the stress index (H/L) in birds, while the treatment (T4) and (T3) recorded a significant ($P<0.05$) decrease in the (H/L) ratio. It can be attributed to the high content of the synergistic mixture of biologically active compounds that act as natural antioxidants, including flavonoids and antibacterial compounds that lead to the balance of gut microflora in addition to phenolic compounds, tocopherols and carotenoids (Ullah.M, et al., 2020; Zhou et al., 2010).Which works to improve the health status of birds and contribute to reducing the number of heterotrophic cells and increasing the number of lymphocytes (Oladele et al., 2018).

Table (3) shows the effect of adding the synergistic mixture of Pleurotus ostreatus and beta-glucan to the diet of broilers exposed to heat stress on immunological measurements at 35 days of age (mean ± standard error).

treatments	Weight Gland Fabricia %	Gland Fabricia Index	the weight Relative to spleen %	The corticosterone hormone	H%	L%	H/L
T1	0.079^{a} ± 0.003	1.00^{a} ± 0.048	0.157 ± 0.005	1.62^{a} ± 0.027	27.30^{a} ± 1.18	64.36^{bc} ± 1.09	0.42^{a} ± 0.01
T2	0.077^{ab} ± 0.002	0.97^{ab} ± 0.29	0.155 ± 0.004	1.53^{b} ± 0.018	25.37^{ab} ± 1.14	62.17^{c} ± 0.90	0.41^{a} ± 0.02
T3	0.070^{bc} ± 0.002	0.88^{bc} ± 0.023	0.151 ± 0.003	1.43^{c} ± 0.022	23.41^{ab} ± 2.52	65.36^{b} ± 0.59	0.36^{ab} ± 0.04
T4	0.067^{c} ± 0.002	0.84^{c} ± 0.019	0.149 ± 0.002	1.37^{c} ± 0.024	21.29^{b} ± 1.14	73.16^{a} ± 0.57	0.29^{b} ± 0.01
significant	*	*	N.S	*	*	*	*

(*) The different letters in each column indicate a significant difference (p<0.05). N.S. (**) means that there are no significant differences between the averages.

Biochemical blood measurements

The results of Table (4) showed that there were significant differences (P < 0.05) in all parameters of blood biochemistry for all treatments of the study.We note that there is a significant superiority in total protein and albumin for the fourth treatment (T4) to which the synergistic mixture was added in the amount of (1.5) g / kg, which recorded (3.98 and 2.78 g / dI) for both total protein and albumin, compared with the control treatment (T1), which was recorded The lowest value was (3.63 and 1.82 g/dI) for each, respectively.As for triglycerides, cholesterol, and low-density and very low-density lipoproteins, the results indicate that treatment (T1) has the highest concentration, which was recorded for the above standards (104.45, 140.90, 42.79, 17.26mg/dI), respectively, compared with treatment (T4), which recorded a decrease. Significantly for the aforementioned biochemical parameters, which recorded the lowest concentration of 92.20, 124.51, 32.58, and 13.62 (mg/di) for triglycerides, cholesterol, and low-density and very low-density lipoproteins, respectively.While the fourth treatment recorded the highest concentration of high-density lipoproteins, which are considered important in the body, which recorded 97.69 (mg/dI) compared to treatment (T1), which recorded the lowest concentration, with a value of 91.31 (mg/dI). As Husien, et al., (2021) showed that the high values of total protein in the blood serum indicate that it results from a good metabolism of a large part of the protein in the organs of the body of birds.

Bederska-Łojewska, et al., (2017) showed that adding oyster mushrooms works to bind saponins, proteins, and amino acids resulting from adding oyster mushrooms to poultry diets, which increases the utilization of protein.The decrease in the concentration of triglycerides, cholesterol, low-density lipoproteins, and very low-density lipoproteins in the treatments to which the synergistic mixture was added, compared with the control treatment, is attributed to the properties of oyster mushrooms in containing biologically active compounds in the form of flavonoids, antioxidants, and anthocyanins.Which contributes to preventing fat oxidation and thus reduce the level of triglycerides (Brusslmans et al., 2005) and glucose referred to by (Shirshaab & Jassim 2021).The decrease in cholesterol in the blood serum can be due to the improvement of the intestinal flora, especially in the numbers of lactic acid bacteria, which work to increase the acidity of the intestinal medium, which helps in the decomposition of cholesterol and a decrease in its rate of absorption. It works to inhibit enzymes that help manufacture cholesterol, which leads to a decrease in its concentration. And some lactic acid bacteria depend on the breakdown of fats and the breakdown of cholesterol in order to provide their body with a carbon source during the metabolism process (Al-Dorrah and Al-Darwash, 2011).In the same context, the results obtained in this study are similar to what was reached by (2017) Ekunseitan et al., explaining that the addition of oyster mushroom extract to the water significantly improved (P<0.05) the characteristics of carcasses and HDL and reduced the concentrations of LDL, VLDL, and fats The triple.The results of the current study are similar to what was shown by Laroche and Michaud, (2007), who indicated that β-glucan contributes to the reduction of total cholesterol in the blood and low-density lipoprotein (LDL) cholesterol. It also reduces high-density lipoprotein (HDL) cholesterol. Which differed with the results of the current study.

Table (4) Effect of adding the synergistic mixture of oyster mushroom Pleurotus ostreatus and beta-glucan on the average concentration of biochemical parameters of blood serum of birds subjected to heat stress at the age of 35 days (average ± standard error).

treatments	total protein (g/dI)	the two albums (g/dI)	Triglyceride (mg/dI)	cholesterol (mg/dI)	HDL (mg/dI)	LDL (mg/dI)	VLDL (mg/dI)

T1	**3.63c ± 0.05**	**1.82^{b} ± 0.08**	**104.45^{a} ± 2.38**	**140.90^{a} ± 1.49**	**91.31^{c} ± 1.81**	**41.79^{a} ± 1.86**	**17.26^{a} ± 0.56**
T2	**3.44bc ± 0.04**	**2.21^{b} ± 0.08**	**99.96^{ab} ± 2.06**	**138.71^{a} ± 2.94**	**92.42^{bc} ± 0.61**	**38.85^{ab} ± 0.84**	**16.97^{ab} ± 0.37**
T3	**3.69b ± 0.05**	**2.67^{a} ± 0.13**	**95.03^{b} ± 2.97**	**132.49^{ab} ± 1.65**	**95.87^{ab} ± 0.88**	**34.47^{ab} ± 1.50**	**14.65^{bc} ± 0.53**
T4	**3.98a ± 0.09**	**2.78^{a} ± 0.18**	**92.20^{b} ± 2.92**	**124.51^{b} ± 3.62**	**97.69^{a} ± 1.44**	**32.58^{b} ± 1.48**	**13.62^{c} ± 1.11**
significant	*	*	*	*	*	*	*

(*) The different letters in each column indicate a significant difference ($p<0.05$). N.S. (**) means that there are no significant differences between the averages.

conclusions

It can be concluded that the addition of the synergistic mixture of oyster mushrooms and beta-glucan at the level of 1.5 g / kg feed for the starter ration and 0.5 g / kg for the final ration is the best level, as these treatments showed a significant improvement ($P < 0.05$) for the immune traits (Fabricia gland Gland index, spleen weight, corticosterone, H/L) and blood biochemistry. No negative effect on birds was observed.

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