The effect of adding the synergistic mixture of *Pleurotus ostreatus* and Beta-Glucan to the diet on the microbial content and characteristics of broiler carcasses subjected to heat stress

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Abstract

This study investigate the effect of adding a synergistic mixture of oyster mushroom and beta-glucan on the microbial content and characteristics of broiler carcasses subjected to heat stress. 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 (35) a day. This study aimed to investigate the effect of a synbiotic mixture of oyster mushrooms and beta-glucans on the microbial content and characteristics of broiler carcasses subjected to heat stress. 300 one-day-old broiler chicks (Ross 308) were randomly distributed to four treatments. The first treatment (control) was fed the standard diet without any addition. As for the second, third and fourth treatment, they were fed on a ration with the addition of the synergistic mixture at the rate of (0.5, 1, 1.5) gm per kg of feed for the starter ration, while the addition to the final ration was (0.250, 0.5, 0.750) gm per 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 treatment (T4) to which the synergistic mixture was added 1.5 gm per kg for the starter diet and 0.5 gm per kg for the growth ration, for the total bacterial content, beneficial bacteria (Lactobacilli) and less content of harmful bacteria (E.coli) as well. Significant ($p<0.05$) improvement was found in carcass weight, dressing percentage, and breast segments. In addition to the chemical properties of meat. In conclusion, it can be adopted to improve the microbial content and chemical characteristics of the meat, which will reflect positively on the productive characteristics of broilers subjected to heat stress.

Keywords: beta-glucan, oyster mushroom, microbial content, chemical properties, heat stress, synergistic mixture.

Introduction

[19], heat stress has been a major limiting factor for the poultry industry in tropical and subtropical regions for decades. When temperatures exceed comfort levels in poultry fields, birds attempt to reduce metabolic heat production by reducing feed consumption, which is reflected in growth and profitability [22]; [33] Broilers are more susceptible to heat stress due to their faster growth and associated increased metabolic heat production [13]. One of the strategies used to eliminate the effects of heat stress (H.S) is the use of Probiotic, Prebiotic (Synbiotic), an increase in growth and performance, a decrease in FCR, and it works to enhance beneficial bacteria, which is positively reflected in improving gut function and disease resistance [28].

[37] and [23] that heat stress significantly affects gut bacteria, and the use of probiotics has many beneficial properties for improving immunity, gut structure, and gut barrier function in broiler chickens. Based on global trends towards the use of natural alternatives, there are many studies that used natural alternatives to enhance the microbial content of the intestine in normal conditions. Including the use of some plant additives such as the addition of P.

oleracea seeds or leaf powder has a significant effect in reducing all types of bacteria (total bacteria, lactobacilli and Escherichia coli) [29]. Ground okra seeds OSP reduced harmful bacteria (E.coli) and had the ability to enhance the productive performance of broilers [1].

The oyster mushroom is one of the fungi of the Basidi family and For the properties of oyster mushrooms and their importance in getting rid of cellulosic materials and containing nutrients such as carbohydrates, protein, ash, calcium, magnesium, crude fiber, and fats [24]. And biologically active compounds in the form of flavonoids, antioxidants, anthocyanins, and saponins reduce the growth of pathogenic bacteria and increase beneficial bacteria, when added to poultry diets [35].

[31] ,confirmed that the aqueous extracts of the oyster mushroom Pleurotus ostreatus (Oyster) and its isolated proteins have an important effect on production and health promotion as an immunomodulator and antioxidant in broiler chickens. [15], noted that feeding poultry on oyster mushroom residues at the level of 1% has positive effects on improving performance, the humoral immune response to disease vaccines, and contributes to delaying spoilage of poultry meat. In addition to the possibility of adding beta-glucanase to broiler diets as a prebiotic. [16] , Beta-glucans are chained D-glucose polymers، of high molecular weight، found in the cell walls of many yeasts and grains showed that beta-glucan can be a useful and good feed additive, especially for feeds that contain a high percentage of grains. Being a complex polysaccharide with biologically active properties, it plays an important role in modulating immunity. Among its most important natural sources are yeast, oats, barley, mushrooms, and algae. Yeast-derived glucans can aid in host defense against infections [32].

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 (35) a day. In this research, 300 unsexed Ross 308 broiler chicks were used in this research, with an average starting weight of 42 g., Birds were distributed randomly into four treatments, and each treatment was divided into three replications (25 birds in each replicate). The chicks were reared on the floor and sawdust was used as a mattress to cover the floor. The hall was divided in the form of 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). The synergistic mixture was added according to the quantities shown in (Table 2). The birds were exposed to heat stress from the age of one day, by exposing all the birds to heat stress for 8 hours a day by raising the room temperature (Table 3).

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.

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

- The spindle of oyster mushrooms growing on the carrier was added with the same amount of beta-glucan to the ration Oyster fungus was added with the same amount of beta-glucan to the diet. It was mixed well, then incubated with polyethylene bags at a temperature ranging from (28-30) 0 and a humidity ranging from (40-50)% for a period of (7-10) A day u

ntil the mycelium grows on all the amount of feed to which the mycelium is added, which is characterized by its strong smell, then it is mixed from the amount required to feed the birds. [25].

Studied traits:

net ratio:

The percentage of filtration was calculated based on the equation reported by [5].

Relative weights of carcass pieces:

The relative weights of the segments were calculated according to what was reported by [6].

Meat chemical properties:

Moisture content, crude protein, crude fat and ash were estimated according to the method described by [8].

Microbial content in the jejunum:

Estimation of the total number of bacteria

Estimation of the number of coli bacteria (E.coli)

Estimation of Lactobacilli numbers

The numbers of bacteria in the jejunum were estimated from the small intestine according to the Pour Plate Method mentioned by [17].

statistical analysis

Using the complete random design (CRD), using the (SPSS, 2019) program in the statistical analysis and using the following mathematical model ($yij = \mu + ti + eij$). [30]

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

the components (%)	**starter feed days (21-1)**	**The ultimate feed 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% methionine, methionine + cysteine 0.42%, lysine 4.12%, tryptophan 0.42%, threonine 1.70%, sodium 2.50%, chlorine 4.20%, copper 200 mg / kg, manganese 1,600 mg / kg, zinc 2,000 mg / kg, iodine 20.00 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 [21].**

Table (2) Quantities added from the synergistic mixture to broiler diets (g)

Amount of addition (g)		
transactions	the starter mixture	growth mixture
T1 (control treatment)	standard mixture	
T2	0.5	0.25
T3	1	0.5
T4	1.5	0.75

Table (3) The approved periodic temperatures during the experiment period

age / day	**1-7**	**8-21**	**22-35**
temperature	**38-40^0**	**34-36^0**	**27-29^0**

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

Results and discussion

Cleaned carcass weight, net percentage and relative weights of main cuts:

The results in Table (4) show that there is a significant (P<0.05) superiority in the average live weight, weight of cleaned carcasses, and percentage of purification at the age of 35 days in all treatments to which the synergistic mixture was added at different levels compared to the control treatment. It was found from the table that there was a significant superiority (p < 0.05) for the fourth treatment in the average final live body weight, which recorded (1710.96) gm, compared with the third and second treatments and control (T1), which each recorded (1633.37, 1566.06, 1535.15) gm. Respectively, the fourth treatment recorded the highest average weight of the cleaned carcass and was significantly superior to all treatments, amounting to 1243.95 g, compared to the control treatment, in which the average weight of the cleaned carcass was 1076.60 g. For the fourth treatment, which did not differ significantly from the third treatment, in which the net ratio reached 72.71 and 71.95%, respectively, compared with the control treatment (T0), which recorded the lowest rate of 70.14%. The percentage of (32.12)% did not differ significantly from the third treatment, which amounted to (31.74%), but it differed significantly from the first treatment (control), which amounted to (30.58%).The data obtained from Table (4) indicate that there are no significant differences between coefficients in th weight Relative to the cut of the thighs. Based on the above data and in line with what was shown by [5], the characteristics of the carcass, including the percentage of dressings, the weight of the carcass, and the relative weight of the main parts, the breast and thighs, are important economic indicators for the poultry industry, and these characteristics are directly affected by the live weight before slaughter. On the other hand, this improvement may be attributed to the role of mushrooms because they contain biologically active compounds that balance the intestinal microflora content, which improves digestion and health [9] .It was observed [12], that there is a significant difference (P.0<05) when using 1% of the residues of oyster mushroom cultivation led to a significant increase in body weight (BW), weight gain (WG), and feed intake (FI).) In addition to improving gut health in poultry, which was confirmed by [16]. Which was consistent with the findings of [10] , which confirmed that there was a significant increase in weight as a result of its impact on gut health and was reflected in the characteristics of production, and it also improved the quality of produced meat and breast cuts. In addition to the role of beta-glucan as a safe food that regulates

intestinal expression, thus it can be considered as a growth stimulator in broilers [27].

Table (4) The effect of adding the synergistic mixture of *Pleurotus ostreatus* and beta-glucan to the diet of broilers subjected to heat stress at different age stages on Qualitative characteristics of carcasses (mean ± standard error)

Transactions	live weight gr	Cleaned carcass weight	% net	the chest %	thighs %
T1	±1535.15d 3.77	±1076.60^{d} 4.18	70.13^{b}± 0.20	30.58^{b}± 0.33	±30.80 0.51
T2	±1566.06c 2.69	±1104.41^{c} 7.52	70.52 ^{b} ± 0.51	30.88^{b}± 0.55	.04± 31 0.70
T3	±1633.37b 3.81	±1175.17^{b} 7.55	71.95^{a}± 0.40	31.74^{ab} ± 0.26	31.58± 0.59
T4	±1710.96a 3.46	±1243.95^{a} 3.47	72.71^{a}± 0.26	±32.45^{a} 0.47	±32.49 0.29
Effect	*	*	*	*	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 ***Transactions (T1) were the control treatment without addition, while the second treatment (T2) was The addition of the tarzi mixture of oyster mushrooms and beta-glucan was 0.5 g per kg of starter feed and 0.25 g per kg of final feed for both oyster mushrooms and beta-glucan. As for the third treatment (T3), the amount was 1 g per kg of starter feed and 0.5 g of each kg of final feed for both oyster mushrooms and beta-glucans and the fourth treatment (T4) the mixture was added at the rate of 1.5 g per kg of starter feed and 0.75 g per kg of final feed for both oyster mushrooms and beta-glucans.**

Chemical properties of meat for breast and thigh cuts

Table (5) indicates that there is a significant effect (P<0.05) in the average percentage of the chemical content of breast cut meat. The percentage of protein in the breast segments of the fourth treatment was (21.78)%, in which the synergistic mixture of oyster mushrooms was added to its diet with the same amount of beta-glucan at a rate of 1.5 g/kg for the starter and 0.75 g/kg for the final one, compared with the control treatment (T1), which It recorded (19.02)% protein. The percentage of fat for the fourth and third treatment, which did not differ significantly, was (2.05, 1.95)%, respectively, but they differed significantly from the first treatment (control), as the percentage of its fat content was (1.65)%. The results of the table also indicated a significant difference for the percentage of moisture and ash for the fourth treatment. Which amounted to (71.55, 1.48)%, respectively, compared with the control treatment, which recorded (68.51, 1.21)%, respectively, for moisture and ash.As shown in Table (5), there is a significant effect (P<0.05) in the average chemical content of definitive meat. The thigh, compared with the control coefficient, where the fourth treatment recorded a significant difference in protein percentage, reaching 19%, compared with the control treatment, which had a protein percentage of 17.47%. The table also indicated a significant difference in the content of the thigh segments for the fourth and third treatments, which did not differ significantly between them, which

recorded a percentage of (9.68, 9.60)% compared to the control treatment. It is clear from the table that it recorded a percentage of (8.30%). It was significantly superior to the control treatment, as it recorded (69.85, 1.19)%, respectively, compared with the control treatment, which had a percentage of moisture and ash (64.26, 1.07), respectively. From the above results, we conclude that there is a significant difference in the protein and fat content of the carcasses subjected to heat stress, which is consistent with what was mentioned by [5], who mentioned that there is an effect of the temperature of the breeding pens on the nutrition and the percentage of fatty acids involved in the formation of body fat for birds. Which was confirmed by [26], that both broiler breeders experience deterioration in production traits when they are affected by chronic heat stress.

One study showed a 16.4% decrease in chicken feed consumption, which resulted in a 32.6% decrease in weight gain, and a 25.6% decrease in feed efficiency. Exposure to high temperatures during the growth stage of broiler chickens weakened the characteristics of broiler meat and lost its quality [20]; [36]. And that these significant differences in the chemical properties of the treatments to which the synergistic mixture is added. It is consistent with the data noted by [14] .that there is an improvement in the quality of meat as a chemical substance due to the increase in protein in muscle formation. In line with what was mentioned [18], it was found that mushroom powder (1.2%) and 0.3% probiotic (Saccharomyces Cerevisiae) in the diet of broiler chickens led to an improvement in meat quality, which was confirmed by [11], the addition of the oyster mushroom *Pleurotus ostreatus* has a significant effect on the quality characteristics of chicken breast meat, and it also showed that it had an effect ($P < 0.05$) on the crude protein and the ability of WHC (water holding capacity) in the meat. The inclusion of OMW (oyster mushroom droppings) at 0.4% generally improved the health of the birds, taking into account the effect on the measured blood parameter. It also supports the growth of broilers [2]. It is worth noting that the increase in feed consumption and weight and the improvement in meat quality is the result of the bioactive content of flavonoids, antioxidants and antibacterial compounds that balance the gut micro flora, thus improving digestion [3].

Table (5) the effect of adding a synergistic mixture of oyster mushroom *Pleurotus ostreatus* and beta-glucan at different levels to broiler diets on the chemical characteristics of cutlets (breast-thigh) at the age of 35 days (mean ± standard error).

Transactions	chest piece				thigh piece			
	protein	fat	Moisture	Ash	protein	fat	Moisture	Ash
T1	19.02^{c}± 0.02	±1.65^{b} 0.05	68.51^{b}± 0.90	1.21^{c}± 0.50	±17.47^{c} 0.28	8.30^{b} ± 0.40	64.26^{c}± 1.51	1.07^{b}± 0.03
T2	±19.08^{c} 0.12	1.70^{b}± 0.02	68.67^{b}± 0.91	±1.32^{b} 0.03	18.28^{b}± 0.28	8.70^{b}± 0.12	65.91^{bc}± 0.32	1.10^{ab}± 0.02
T3	21.03^{b}± 0.04	±1.95^{a} 0.06	±70.77^{ab} 0.59	±1.44^{a} 0.02	±18.92^{ab} 0.04	9.60^{a}± 0.12	68.04^{ab}± 0.26	±1.23^{a} 0.09
T4	±21.78^{a} 0.25	±2.05^{a} 0.04	±71.55^{a} 0.33	1.48^{a}± 0.01	±19.00^{a} 0.03	±9.68^{a} 0.33	69.85^{a}± 0.49	1.19^{ab}± 0.02
Effect	*	*	*	*	*	*	*	*

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 ***Transactions (T1) were the control treatment without addition, while the second treatment (T2) was The addition of the tarzi mixture of oyster mushrooms and beta-glucan was 0.5 g per kg of starter feed and 0.25 g per kg of final feed for both oyster mushrooms and beta-glucan. As for the third

treatment (T3), the amount was 1 g per kg of starter feed and 0.5 g of each kg of final feed for both oyster mushrooms and beta-glucans and the fourth treatment (T4) the mixture was added at the rate of 1.5 g per kg of starter feed and 0.75 g per kg of final feed for both oyster mushrooms and beta-glucans.

Indicators of bacteria numbers:

It was observed from the results of Table (6) regarding indicators of bacterial numbers in the jejunum part of the small intestine that the treatments added to the synergistic mixture of oyster mushrooms and beta-glucan excelled in total bacterial numbers by 671.76 $X10^4$ and 614.68 $X10^4$, respectively, for the fourth and third treatment, compared with the control treatment. Which recorded 461.68 $X10^4$, which did not differ significantly from the first treatment. As well as in the numbers of Lactobacilli bacteria, where the numbers of bacteria for the fourth and third treatments were 435.65 $X10^5$ and 415 $X10^5$, respectively, while the number of harmful E coli bacteria decreased in the fourth treatment to which the synergistic mixture was added by 50.12 $X10^3$ and the second treatment at a rate of 55.80 $X10^3$ compared with the control treatment, which recorded the highest presence of bacteria coli at a rate of 91.77 x 10^3, followed by the second treatment, which was 88.74 x 10^3. The presence of E coli is also an important indicator of the intestinal content of pathological and fecal bacteria, while the presence of Lacto bacilli is evidence of the role of beneficial and necessary bacteria for the process of competitive exclusion of many types of pathological bacteria.

Measuring the numbers of total bacteria, Lacto bacilli and E coli in the intestines of birds is to identify the microbial content in their digestive tract and the state of balance in the intestine [4]. It has an important role in improving the health of the host by removing toxins and increasing the absorption of nutrients completely. In addition, this type of bacteria produces lactic acid, short-chain fatty acids and other organic acids. Lactic acid bacteria also produce antimicrobials (bacteriocins) that it is anti-growth of pathogenic bacteria in the gastrointestinal tract [34]. Oyster mushroom also has the ability to synthesize silver nanoparticles (AgNPs) that inhibit the growth of pathogenic bacteria and thus showed the possibility of using it as a natural antibiotic [7]. The reason for the decrease in harmful bacteria and the increase in the number of beneficial bacteria in the jejunum region may be due to the presence of oyster mushrooms in addition to beta-glucan, where mushrooms play an important role in stimulating beneficial microorganisms because they contain saponins that have the ability to suppress pathogenic bacteria, which is reflected in the increase in bacterial growth. [10]. In addition to the role of beta-glucan, whose addition has been shown to improve the gut health of poultry in general or those suffering from pathological bacterial infections [28].

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 ***Transactions (T1) were the control treatment without addition, while the second treatment (T2) was The addition of the tarzi mixture of oyster mushrooms and beta-glucan was 0.5 g per kg of starter feed and 0.25 g per kg of final feed for both oyster mushrooms and beta-glucan. As for the third treatment (T3), the amount was 1 g per kg of starter feed and 0.5 g of each kg of final feed for both oyster mushrooms and beta-glucans and the fourth treatment (T4) the mixture was added at the rate of 1.5 g per kg of starter feed and 0.75 g per kg of final feed for both oyster mushrooms and beta-glucans.

Table (6) Effect of adding a synergistic mixture of *Pleurotus ostreatus* and beta-glucan to the ration of broiler rations subjected to heat stress on the average balance of intestinal flora in the jejunum at 35 days of age (mean ± standard error).

Transactions	*Lactobacilli X10* ^{5}	^{3}*E.coli X10*	*Total bacteria x10* ^{4}
T1	**375.73** ^{c} **± 0.25**	**91.77** ^{a} **± 0.85**	**461.68** ^{c} **± 4.05**
T2	**374.41** ^{c} **± 2.00**	**88.74** ^{b} **± 0.77**	**445.78** ^{c} **± 4.81**
T3	**415.77** ^{b} **± 0.44**	**55.80** ^{c} **± 1.22**	**614.68** ^{b} **± 16.07**
T4	**435.65** ^{a} **± 0.62**	**50.12** ^{d} **± 0.39**	**671.76** ^{a} **± 27.65**
Effect	*	*	*

conclusions

It can be concluded that the addition of the synergistic mixture of oyster mushrooms and beta-glucan at the level of 1.5 gm per kg of feed for the starter ration and 0.5 gm per kg for the final ration is the best level, in addition to that it showed a significant improvement in the weight of the cleaned carcass, the percentage of clearance, and the cut of the breast, in addition to that. To the microbial content through the increase in the lactic acid content and the decrease in the proportion of coli bacteria (E.Coli). The treatment of the experiment with the addition of the synergistic thread also witnessed a significant increase in the chemical properties of broiler meat subjected to heat stress, and no negative effect was observed on the birds.

References

[1] Abbas, R. J. (2021). Effect of Supplementing Different Levels of Okra Seed (Abelmoschus esculentus L.) Powder on Growth Performance, Carcass Characteristics, Blood Parameters and Gut Microbial Populations in Broiler Chickens. *Basrah Journal of Agricultural Sciences, 34*(2), 29-41.

[2] Adebomi, T. I., Tayo, G. O., Olumide, M., & Adeyemi, O. A. (2021). Haematological and serum biochemical parameters of broiler chicken fed varying levels of oyster mushroom (pleurotus ostreatus) waste meal. UNIOSUN JOURNAL OF AGRICULTURE AND RENEWABLE RESOURCES, 3(1).

[3] Akomah, C., Sogunle, O. M., Adeyemi, O. A., & Bamgbose, A. M. (2021). Growth performance and haematological characteristics of pullet chickens fed different feed forms supplemented with or without Oyster mushroom (Pleurotus streatus). Nigerian Journal of Animal Production, 48(5), 203-212.

[4] Al-Tamimi, Ammar Talib, Diab Saleh (2004) A comparative study of the effect of using zinc pastracene and the local probiotic as growth stimuli on the productive performance of broiler chickens. Master's thesis, College of Agriculture, University of Baghdad.

[5] Al-Fayadh, H., Naji, S., & Al-Hajo, N. (2011). Poultry Meat Technology. 2nd part. In: Higher Education Press,

University of Baghdad, 292pp.(In Arabic).

[6] Al-Fayyad, Hamdi Abdel-Aziz and Naji, Saad Abdel-Hussein (1989) Poultry Products Technology, first edition, Directorate of Higher Education Press, Baghdad, Iraq.(In Arabic)

[7] Al-Bahrani R, Jegadeesh R, Hariprasath L, Abdullah A H, Vikineswary S 2017 *Materials Letters* 186 21-25

[8] AOAC, G. (2016). Official methods of analysis of AOAC International. Rockville, MD: AOAC International, ISBN: 978-0-935584-87-5. In.

[9] Bederska-Łojewska, D., Świątkiewicz, S., & Muszyńska, B. (2017). The use of Basidiomycota mushrooms in poultry nutrition—a review. *Animal Feed Science and Technology*, *230*, 59-69.

[10] Ekunseitan, D. A., Osuntola, F. J., Odukoya, A. I., Sobayo, R. A., & Njoku, C. P.(2019). Response of two strains of broiler chickens to oyster mushroom (Pleurotus ostreatus) extract in the tropics. *Polish Journal of Natural Sciences*, *34*(1), 33-47.

[11] Ekunseitan, D. A., Owosangba, J. F., Oladele, T. O., Oluwajuyigbe, S. A., & Omotoso, O. O. (2021). Does Pleurotus ostreatus influence health status and meat quality attributes of broiler chickens?. *The Indian Journal of Animal Sciences*, *91*(9).

[12] Fard, S. H., Toghyani, M., & Tabeidian, S. A. (2014). Effect of oyster mushroom wastes on performance, immune responses and intestinal morphology of broiler chickens. *International Journal of Recycling of Organic Waste inAgriculture*, *3*(4), 141-146.

[13] Gous, R.M. Nutritional limitations on growth and development in poultry. Livest. Sci. 2010, 130, 25–32.

[14] Grashorn, M. A., & Clostermann, G. (2002). Performance and slaughter characteristics of broiler breeds for extensive production. *Archiv fur Geflugelkunde*, *66*(4), 173-181.

[15] Hassan, R. A., Shafi, M. E., Attia, K. M., & Assar, M. H. (2020). Influence of oyster mushroom waste on growth performance, immunity and intestinal morphology compared with antibiotics in broiler chickens. *Frontiers in Veterinary Science*, *7*, 333. *doi: 10.3389/fvets.2020.00333.*

[16] Heidary Vinche, M., Khanahmadi, M., Ataei, S. A., & Danafar, F. (2021). Investigation of the effects of fermented wheat bran extract containing beta-glucanase on beta-glucan of cereals used in animal feed. *Cereal Chemistry, 98*(3), 651-659.

[17] Harrigan,W.F., and McCance ,M.E.(1976).Laboratory methods in food and dairy microbiology .Academic press Inc.(London),pp 170-210

[18] Kumar R, Nazim A, Siddique RA, Rajkumar SDS, Debashis R, Ahmad F. The effect of different level of mushroom (Agaricus bisporus) and probiotics Saccharomyces cerevisiae) on sensory evaluation of broiler meat. J Entom. (2019) 9.

[19] Lin, H., Jiao, H. C., Buyse, J., & Decuypere, E. (2006). Strategies for preventing heat stress in poultry. Worlds Poultry Science, 62, 71–85.

[20] Lu, Q., Wen, J. and Zhang, H. (2007). Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. Poult. Sci., 86: 1059–1064

[21] National Research Council.(NRC) (1994). Nutrient Requirements of Poultry. 9th ed. National Academy of Science. Washington, D.C.: 176pp.

[22] Quinteiro-Filho, W.M.; Ribeiro, A.; Ferraz-de-Paula, V.; Pinheiro, M.L.; Sakai, M.; Sá, L.R.M.; Ferreira, A.J.P.; Palermo-Neto, J. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. Poult. Sci. 2010, 89, 1905–1914.

[23] Rahimi, S. H. & Khaksefidi, A. 2006. A comparison between the effects of a probiotic (Bioplus 2B) and an antibiotic (virgeniamycin) on the performance of broiler chicken under heat stress. Iran. J. Vet. Res., 7:48-56.

[24] Randive, S. D. (2012). Cultivation and study of growth of oyster mushroom on different agricultural waste substrate and its nutrient analysis. *Advances in Applied Science Research*, *3*(4), 1938-1949.

[25] Saadoun, S. A. A., & Timosz, S. H. (2017). Molecular Diagnosis of oyster mushroom Pleurotus ostreatus and screening for resistance gene of plant pathogenic fungi Osterolysin. *Al-Qadisiyah Journal of Pure Science*, *22*(3), 380-392. http://www.qu.edu.iq/journalsc/index.php/JOPS/article/view/678

[26] Sohail, M., Hume, M., Byrd, J., Nisbet, D., Ijaz, A., Sohail, A., . . . Rehman, H. (2012). Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. *Poultry Science*, *91*(9), 2235-2240.

[27] Schwartz, B., & Vetvicka, V. (2021). Review: β-glucans as Effective Antibiotic Alternatives in Poultry. *Molecules*, *26*(12), 3560. Retrieved from

[28] Shao, Y., Wang, Z., Tian, X., Guo, Y., & Zhang, H. (2016). Yeast β-d-glucans induced antimicrobial peptide expressions against Salmonella infection in broiler chickens. *International journal of biological macromolecules*, *85*, 573-584.

[29] Shirshaab, A. J., & Jassim, J. M. (2021). Effect of Adding Different Levels of Portulaca oleracea L. Seeds and Leaves Powder to The Diet on Productive and Physiological Performance of Broiler Chickens (Ross 308). *Basrah Journal of Agricultural Sciences*, *34*(1), 38-48

[30] SPSS. (2019). SPSS User's Guide Statistics. Version 26. Copyright IBM, SPSS Inc.

[31] Swennen, Q., Verhulst, P. J., Collin, A., Bordas, A., Verbeke, K., Vansant, G., ... & Buyse, J. (2007). Further investigations on the role of diet-induced thermogenesis in the regulation of feed intake in chickens: Comparison of adult cockerels of lines selected for high or low residual feed intake. Poultry science, 86(9), 1960-1971.

[32] Thomas, S., Rezoagli, E., Abidin, I. Z., Major, I., Murray, P., & Murphy, E. J. (2022). β-Glucans from Yeast—Immunomodulators from Novel Waste Resources. *Applied Sciences*, *12*(10), 5208.

[33] Ullah, M. I., Ijaz, M. U., Hussain, G., Faisal, M. N., Rasul, A., Bukhari, S. A., . . . Anwar, H. (2020). Estimation of Protein and Productive Efficiency Profile of Locally Produced Oyster Mushroom (Pleurotus Ostreatus) in Broiler.

[34] Yakhkeshi, S.; Rahimi, S. and Gharib Naseri, K. (2011). The effects of comparison OF herbalextracts, antibiotic, probiotic and organic acid on serum lipids, immune response, GIT microbial population, intestinal morphology and performance of broilers. *Journal of Medicinal Plants*, 10(37): 80-95.

[35] Zahro, L., & Agustini, R. (2013). Uji Efektivitas Antibakteri ekstrak kasar saponin jamur

tiram putih (Pleurotus ostreatus) terhadap Staphylococcus aureus dan Escherichia coli. *UNESA Journal of Chemistry*, *2*(3), 120-129.

[36] Zhang, Z.F.; Zhou, T.X.; Ao, X.; Kim, I.H. Effects of β-glucan and Bacillus Subtilis on growth performance, blood profiles, relative organ weight and meat quality in broilers fed

[37] Zulkifli, I., Abdullah, N., Azrin, N. M., & Ho, Y. (2000). Growth performance and immune response of two commercial broiler strains fed diets containing Lactobacillus cultures and oxytetracycline under heat stress conditions. *British poultry science*, *41*(5), 593-597.