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Effect of Adding Mushroom (*Agaricusbisporus*) Agricultural by-products with or without Enzymes in Rations on Some Immunological and Physiological Traits of Broiler Chicks

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The study was conducted in poultry farm belong to the Department of Animal Production, College of Agriculture, University of Kufa through the period 1-10-2016 to 5-11-2016. The objective was to investigate the effect of using the *Agaricusbisporus* agricultural by-products with enzymes mixtures (LABAZYME) on some of reproduction and economical traits in broiler. A total of 150 one day age, unsexed chicks (Ross-308) with average body weight about 47.7g and closed house system was used through the rearing period divided in pens with 3m² of area for each pen. Chicks were distributed randomly in five treatments (30 chicks for each treatment, 10 chicks for each replicate). Treatments were distributed randomly on pens until 5th week of age. T1: (control) standard diet with 0% of *Agaricusbisporus*, T2: 3% of *Agaricusbisporus* without enzymes, T3: 3% of *Agaricusbisporus* with enzymes, T4: 9% of *Agaricusbisporus* without enzymes and T5: 3% of *Agaricusbisporus* with enzymes. Results indicated a significant enhancement ($P < 0.05$) in antigens against Newcastle and Gumboro diseases, hemoglobin rate, PCV, red blood cells and white blood cells in the chicks that fed on diets contained 9% of *Agaricusbisporus* with enzymes.

Keywords: Broiler, Mushroom, *Agaricusbisporus*, Enzymes.

INTRODUCTION

The third world countries are suffer from acute deficiency in animal feed stuff because the high competence with human and poultry in grain consumption such as Maize (AL-Mashhadani, 2016). Maize is used in multi feed industrials such as starch and dextrin (Al-Kassar and Abdul-Abbas, 2009). The direction of European countries is to produce the biofuel (ethanol) from Maize to use it as automobiles fuel which lead to increase the cost of Maize which exported to the consume countries (Abdulrahman et al., 2017) Therefore, the price of this crop increased significantly and the export processes became not stable. There are main importance of Maize in poultry diets because it contributes about 75% of

diets components and the difficulties of Maize achieve with enough quantities make the farmers thinking seriously to use alternatives feed stuff in poultry diets instead of Maize especially when we know that the feed is contributes about 60-70% of production costs in poultry (Jardan, 2015; Hammod, 2016). In Iraq, there are many industrial byproducts which not exploited as alternatives instead of expensive imported diets to decrease the feed cost (Al-Kassar and Al-Hameed, 2007).

One of the byproducts is white bottom mushroom which found in culture centers in Al-Najaf province such as media fermented according solid state fermentation (Alkaisi et al., 2016) and this make as thinking to use it poultry feeding because the good effects on health and

increase digestibility, therefore the major aim of this study is to determine the replacement effect of mushroom by-product instead of Maize in broiler diets and investigate the effect on immune system and physiological traits.

MATERIALS AND METHODS

The study was conducted in poultry farm belong to the department of Animal Production, College of Agriculture, University of Kufa through the period 1-10-2016 to 5-11-2016 to investigate the effect of using the *Agaricusbisporus* agricultural by products with enzymes mixtures (LABAZYME) which contains protease enzyme (2.750 csu), amylase (5.500 slu) and cellulase (27.5 fpui) which added with 0.1% to diet and some of physiological traits were measured. on some of reproduction and economical traits in broiler. Experimental diets:

Chicks were fed in two periods: starts period which represented to first three weeks of age and finisher period which represented to 5th week of age. Treatments were distributed randomly on pens until 5th week of age. T1: (control) standard diet with 0% of *Agaricusbisporus*, T2: 3% of

Agaricusbisporus without enzymes, T3: 3% of *Agaricusbisporus* with t enzymes, T4: 9% of *Agaricusbisporus* without enzymes and T5: 3% of *Agaricusbisporus* with enzymes. Results a significant enhance ($P<0.05$) in antigens against Newcastle and Gumboro diseases, hemoglobin rate, PCV, red blood cells and white blood cells in the chicks that fed on diets contained 9% of *Agaricusbisporus* with enzymes.

Management:

150 of one day age, unsexed chicks (Ross 308) with average body weight about 47.7 gm and closed house system was used through the rearing period divided in pens with 3m² of area for each pen. Chicks were distributed randomly in five treatments (30 chicks for each treatment, 10 chicks for each replicate). Air freshener and air coolers were used to obtain the optimal heat temperature and sawdust was used about 7cm of thickness while drinking water and feed supplied *add-libitum*. Birds were fed the experimental diets from 1-35 days of age tables 1 and 2.

Table1: Percentage inclusion and calculated composition of starter diets.

Ingredients	T1	T2	T3	T4	T5
Corn	60	56	56	49	49
Mushroom agricultural by-products	0	3	3	9	9
Soybean meal (44% CP)	35.5	35.5	35.5	35	35
Oil sunflower	1	2	2	3.5	3.5
Premix ¹	2.5	2.5	2.5	2.5	2.5
Limestone	0.7	0.7	0.7	0.7	0.7
Salt	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100
Calculated composition					
ME(kcal/kg)	2982	2980	2980	2953	2953
CP(%)	22.44	22.50	22.50	22.49	22.49
CP/ ME	132.9	132.4	132.4	131.3	131.3

Use of Premix Jordanian Origin Type Provimi 3110 Contains: 2750 kcal/ kg Representative energy, 10% raw protein, 1.1% fat, 21% calcium, 11.0% phosphorus, 6.5% methionine, 6.5% methionine + Lysine, 4.8% Sodium, 5.4% Chloride, 575000 IU Vitamin A, 201250 IU Vitamin D3, 1380 mg Vitamin E, 138 mg Vitamin K3, 138 mg Vitamin B1, 345 mg Vitamin B, 1840 mg Vitamin B3, 552 mg Vitamin 5 B, 184 mg B vitamins, 46 mg vitamin B9, 1000 micrograms B12, 6900 micrograms peyutin, 14,000 mg choline chloride, 460 mg copper, 2760 mg iron, 3680 mg manganese, 3680 mg zinc, 50 mg iodine, 9.2 mg selenium, 30000 m Vitez mine, 250 mg antioxidants, 250 mg lincomycin, 2400 mg selenomycin) / kg.

Table2 Percentage inclusion and calculated composition of finisher diets.

Ingredients	T1	T2	T3	T4	T5
Corn	60	58	58	56	56
Mushroom agricultural by-products	0	3	3	9	9
Wheat	9	7	7	2	2
Soybean meal (44% CP)	26	26	26	26	26
Oil sunflower	1.5	2.5	2.5	3.5	3.5
Premix ¹	2.5	2.5	2.5	2.5	2.5
Limestone	0.7	0.7	0.7	0.7	0.7
Salt	0.3	0.3	0.3	0.3	0.3
Total	100	100	100	100	100
Calculated composition					
ME(kcal/kg)	3096	3096	3096	3041	3041
CP(%)	19.05	19.03	19.03	19.05	19.05
CP/ ME	162.5	162.7	162.7	159.6	159.6

(1)Use of Premix Jordanian Origin Type Provimi 3110 Contains: 2750 kcal/ kg Representative energy, 10% raw protein, 1.1% fat, 21% calcium, 11.0% phosphorus, 6.5% methionine, 6.5% methionine + Lysine, 4.8% Sodium, 5.4% Chloride, 575000 IU Vitamin A, 201250 IU Vitamin D3, 1380 mg Vitamin E, 138 mg Vitamin K3, 138 mg Vitamin B1, 345 mg Vitamin B, 1840 mg Vitamin B3, 552 mg Vitamin 5 B, 184 mg B vitamins, 46 mg vitamin B9, 1000 micrograms B12, 6900 micrograms peyutin, 14,000 mg choline chloride, 460 mg copper, 2760 mg iron, 3680 mg manganese, 3680 mg zinc, 50 mg iodine, 9.2 mg selenium, 30000 m Vitez mine, 250 mg antioxidants, 250 mg lincomycin, 2400 mg selenomycin) / kg.

Chemical analyses:

The samples of byproduct were taken and grinded by electrical grinder to analyze and estimate the crude protein, crude fiber, ash, fat and organic matter in the central laboratory (College of Agriculture, University of Baghdad) while the calcium, phosphorus and potassium were estimated in the department of Animal Production, College of Agriculture, University of Kufa. The carbohydrate were estimated theoretically.

The traits: Blood samples were collected in the end of 5th week of age from four chicks from each treatments (two males and two females) randomly by using test tubes free of anticoagulant (K-EDTA) to make the cellular tests. Another blood samples were collected by test tubes contain anticoagulant to separate the blood serum with 3000 rpm centrifuged for 15 minutes and estimate the antigens against Newcastle and Gumboro diseases. The serum collected by micropipettes and storage in freezer (-20 °C).

Heparinized microhematocrit capillary tubes were used to measure the packed cells volume (Archer, 1972) while the hemoglobin rate was estimated by transforming it to a complex called cyanomethemoglobin using Drabkin's reagent and

spectrophotometer (Varley et al., 1980). Red blood cells and white blood cells were estimated according to Natt and Herrick (1952).

ELISA test: Antigens against Newcastle and Gumboro diseases were estimated by using enzymes linked immune sorbent assay (ELISA) in the veterinary hospital located in Kufa city, Najaf province, Iraq.

Statistical analysis:

Data were analysis by SAS (2012) computer program using completely randomized design (C.R.D.) and the significance between means were tested by Duncan multiple range test (Duncan, 1955) with 0.05 of significance level.

RESULTS

Results represented in (Table 3) showed the chemical analyses of *Agaricusbisporus* for dry matter, organic matter, crude protein, crude fibers, ether extract, ash, calcium, phosphorus and potassium while the metabolic energy was estimated according to Janssen (1989). Results represented in (Table 4) showed that the antigens against Newcastle and Gumboro diseases were increased significantly ($P \leq 0.05$) in chicks that fed on T5.

Results in Table 5 showed significant increase

in PCV in chicks blood that fed on 3 and 9% of *Agaricusbisporus* compared with the other groups while the hemoglobin rate was increase significantly in T3 and T5 compared with the other groups. Red blood cells increased significantly in

chicks that fed on T5 while the white blood cells increased in chicks that fed on all treatment that contain *Agaricusbisporus* with or without enzymes.

Table 3. Approximate gross composition of Mushroom Agricultural by-products (%).

Dry matter	90.7
Organic matter	94.87
Crude protein	14.19
Crude fiber	6.23
Ether extract	0.15
Ash	4.15
Nitrogen free extract	75.28
Calcium	6.183
Phosphor	0.019
Potassium	1.075
Metabolism energy (Kcal/kg)	1471

Table 4 Mean antibody titer against Gumboro disease and Newcastle disease of broiler chicks fed experimental diets during 35 days.

Treatments	IBD antibody titer	ND antibody titer
T 1: Control	2686±497ab	923±382c
T 2: 3% white mushrooms Agricultural by-products without enzyme	5456±622a	2467±390ab
T 3: 3% white mushrooms Agricultural by-products with Enzyme	1538±119b	1296±88bc
T 4: 9% white mushrooms Agricultural by-products without enzyme	1315±60b	1675±227abc
T 5: 9% white mushrooms Agricultural by-products with the enzyme	5679±437a	2725±72a
Significant	*	*

* Different letters vertically indicate significant difference (P<0.05).

Table 5: Haematological indices of broiler chicks fed experimental diets during 35 days.

Treatments	WBC ($\times 10^3$ / μ l)	RBC ($\times 10^6$ / μ l)	Hemoglobin (mg/100 ml)	packed cell volume%
T 1: Control	7.95 \pm 0.43c	2.55 \pm 0.29b	11.75 \pm 3.20b	23.0 \pm 1.5c
T 2: 3% white mushrooms Agricultural by-products without enzyme	10.55 \pm 0.31a	2.96 \pm 0.13b	10.30 \pm 0.34b	25.0 \pm 0.57bc
T 3: 3% white mushrooms Agricultural by-products with Enzyme	9.00 \pm 0.11bc	2.89 \pm 0.05b	15.15 \pm 0.49a	27.5 \pm 0.28a
T 4: 9% white mushrooms Agricultural by-products without enzyme	10.30 \pm 0.69ab	2.87 \pm 0.30b	12.85 \pm 0.20ab	23.0 \pm 3.46c
T 5: 9% white mushrooms Agricultural by-products with the enzyme	11.3 \pm 0.57 A	3.90 \pm 0.64a	15.5 \pm 1.03a	28.0 \pm 1.15a
Significant	*	*	*	*

*different letters vertically indicate significant difference (P<0.05).

DISCUSSION

Results indicated that the fungi addition enhanced the immune system activity in chicks which may be resulted from the absorbent action of these fungi to some of toxins that found in diets and increased the harmful effects that reflected positively on bird immunity. Fungi and its secretions may be act as immune stimulators when it be inside the bird body and help for antibodies production which lead to activate the Fabricius gland (Hammod, 2012). In addition, the enhancement of chicks health reflected positively on the activity of immune system, the results is similar with those of Alssodini (2005) who referred that the enhancement of antigens against the Newcastle and Gumboro diseases when he used a fermented wheat bran with *Aspergillusniger* while the results differ with the results of AL-Mashhadani (2004) who referred that no significant effect of fungi addition on PCV, RBC and WBC when he used 4 and 8% of *Pleurotusostreatus* in broiler diets.

CONCLUSION

Depending on the results of this study, we can make a conclusion that the using of *Agaricusbisporus* at 9% with enzymes in broiler diets lead to improving some of blood parameters such as PCV, hemoglobin, white and red blood cells and also enhance the antigens content against diseases, therefore we recommend to make more studies about using mushroom by-products to determine the optimal rate which can be added to broiler diets.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Aqeel designed and performed the experiments and also wrote the manuscript. Aqeel, zeaid, and Ali performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. Ali and Aqeel designed experiments and reviewed the manuscript. All authors read and approved the final version.

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