Effect of Dried Mushroom Powder and *Bacillus subtilis* on Some Physiological Traits in Broiler Chicks

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Abstract

The immune response and broiler performance may be affected by physical health and challenge levels, the production of broiler chickens is a dynamically developing industry, Most feeds are based on probiotics and prebiotics as a source of diets supplements. Therefore, this study aims to explore the effect of probiotics (*Bacillus subtilis*), Prebiotics such as mushroom (*Agaricus bisporus*) or combination between them on improving humeral and cellular immunity, in addition to the health and performance of broilers. A total of 160 chicks for one day, Ross 308, were randomly assigned to four groups and designed to be four replicates. Each group is divided into 40 chickens / bird. The control group (Con) was fed a basic diet without any additives. The Mushroom group (Mush) was fed the basic diet (1% dried mushroom powder). The Bacillus subtilis group (BS) was fed a staple diet basal diet with 4×10^9 cfu/gm Bacillus subtilis (Bacillus subtilis) as a probiotic. At the same time, the compact group (Mush-BS) was fed a staple diet 1% dried mushroom powder with basal diet have 4×10^9 cfu/gm Bacillus subtilis, The results showed that the concentrations of the liver enzyme AST, ALT, and ALP for the (Mush-BS), (Mush) and (BS) groups were significantly lower ($P\leq0.05$) compared to (CON). The improvement of humeral immunity IgG titer against Newcastle Disease ND and Infectious bronchitis IB were improved significantly ($P\leq0.05$) in the (Mush-BS), (Mushroom), and (BS) groups respectively compare with the (Control) in the ninth and eighteenth age days. In conclusion: dietary mushroom with probiotics supplement may improve the affect intestinal health of chicken broiler through increase the efficacy of PH acidity levels and humeral immune response.

Key words: *Mushroom, Bacillus subtilis, Liver enzymes, Newcastle disease virus and Infectious bronchitis virus*

Introduction

Mushroom has been used as a source of food medicine [(3)].It has been used as a dietary food and medicinal supplement in china for over 2000 years ago . cultivation of *Agarius bisporus* in Europe was achieved in France on 17th century [(16)]. [(14)] showed the beneficial effect of fungi from the Basidiomycota due to contain numerous of active compound like antibiotic, glycoproteins, triterpenes and polysaccharides [(20)]. Polysaccharide has been used as immunomodulating by enhancing lymphocyte and antibody production [(7)]. In recent years, researchers have been looking for safe natural growth enhancers, such as organic acids, probiotics, prebiotics, and plant toxins.

Several studies have established the importance of probiotic as dietary supplements to improve the performance of the animals and sometime as preventative and curative [(8)]. Dietary of probiotic has become accepted to promote health for both human and animal, also, many studies suggested that using probiotic cultures in the poultry industry as a natural mean to pathogen, Control and improve performance instead of using antibiotics [(1)]. Pediococcus, Lactobacillus, Bifidobacterium and Enterococcus bacteria, as a type of microorganism, have beneficial effects on the host by enhancing nutrient absorption and immune response and reducing lipid distribution. In controlling blood and pathogens, probiotics can be used to lower cholesterol and triglycerides Esters [(5,12,19)]. The mechanism of action of prebiotics in the digestive system has been

studied in vivo and in vitro by several scientists [18,21]. Gastrointestinal bacteria compete with the host for other nutrients, induce rapid turnover of absorbable epithelial cells, increase the rate of mucus secretion by intestinal goblet cells, and cause inflammation through the immune system [6]. On the other hand, the intestinal flora assists the host by digesting easily digestible food components and producing short-chain fatty acids [10], which lowers the pH of the intestine and creates a barrier that prevents the growth of some microorganisms. Environmental conditions; bacterial pathogens in the intestine [17]. Likewise, the production of acetate from non-hydrolyzed oligosaccharides, short-chain polysaccharides fatty acids, propionates, and butyrates will increase the proliferation of intestinal epithelial cells, thus increasing the weight of intestinal tissue and altering the general shape of the intestinal mucosa, when broiler chickens are fed with 0.2% yeast cell wall feeding, villi length increases, and this increase in villi length is thought to increase the intestinal absorption surface [15].

This study will be aimed to investigate the effect of supplementation mushroom with or without probiotic on broiler chicken by estimation of: Serum enzymes activities: (serum alkaline phosphatase ALP, alanine aminotransferase ALT and aspartate aminotransferase AST). and immune humeral immunity (IgG antibody titer against avian Infectious bronchitis and Newcastle disease (ND) virus before and after vaccination).

Materials and Methods

This study was carried out in the physiological laboratory in the Veterinary Medicine collage , Kerbala University, from 4/1/2020 to 7/2/2020. Chicks were taken from commercial hatchery of Karbala governerate (hatchery Al-Rahma). 160 with 1-day-old broiler chicks (Ross 308) were randomly involved to four groups (forty birds/per group) with 4 replicates and experimental period were extend to 5 weeks. Birds in the first group control (CON) was fed basal diets without supplements(Table 3-2). The second group was fed the basal diets with 1% dried mushroom powder(MSH). The third group were fed the basal diets with 4×109 cfu/gm Bacillus subtilis(BS). The last was fed the basal diets with 4×109 cfu/gm Bacillus subtilis and 1% dried mushroom(MSH+BS). (table 3-1) showed an experimental design. Feed and water was given ad libitum. All broiler chicks were received starter diet in (1-10 days) and grower diet in (11-35 days). The starter and finisher diet of the experiment were prepared as powder from according to mash and were met the NRC requirements (NRC, 1994). Table(1) showing diets and nutrients composition of starter of time show diets.

Table 1: experimental design of current study

One hundred sixty 1-day-old broilers (Ross 308)			
CON. group	**MSH group**	**BS group**	**MSH-BS group**
40 chicks	40 chicks	40 chicks	40 chicks
4 replicates	4 replicates	4 replicates	4 replicates
(10 birds/ replicate)	(10 birds/ replicate)	(10 birds/ replicate)	(10 birds/ replicate)
Chicks were fed basal diet without supplement	Chicks were fed basal diet with 1% dried mushroom powder	Chicks were fed basal diet with 4×109 cfu/gm Bacillus subtilis	Chicks were fed basal diet with 1% dried mushroom powder and Bacillus subtilis

Because of drinking water vaccination is very common and useful procedure in commercial poultry, therefore the vaccination program was accomplished via drinking water. All vaccines programs that using in vaccination program were manufactured by Volvac® (Boehringer Ingelheim- HQ Germany). All broilers chicks were vaccinated as in (table 2).

Table 2: vaccinated program of current study

Age of chicks	Disease	Type of vaccine	Administration rout
9	Newcastle and Infectious bronchitis	Volvac® ND-IB MLV (Boehringer Ingelheim- HQ Germany)	Via Drinking water
18	Newcastle and Infectious bronchitis	Volvac® ND-IB MLV (Boehringer Ingelheim- HQ Germany)	Via Drinking water

All blood samples were collected at day 35 of age from two birds from each replicate randomly were obtained from the wing vein in a test tube without anticoagulant. The tubes were allowed to clot at room temperature and centrifuged for 10 minute/ 3000 rpm. Serum was collected and stored in freeze (-20) for physiological analysis. serum Blood were used to determine by ELISA antibody titer against ND disease vaccine according to man facture company(NEOGEN/ USA)

liver enzymes and ALP were examined by using specific Cormay Kits produced by PZ CORMAY S.A. company. Humeral immune response was determined by ProFLOK Kit produced by SYNBIOTIC USA company.

Results and Discussion

The concentrations of AST, ALT, and ALP in all groups were significantly different (P≤0.05). Compared to the (CON), (MSH) and (BS) groups (Table 4-2), the (MSH-BS) group had significantly lower AST, ALT, and ALP concentrations (P <0.05) table 3, our results were found a decrease of liver enzymes in the combination group as 14.4 ± 1.24, 13.8 ± .735 and 219.6 ± 34.49, in the AST, AST and ALP, respectively.

Table 3: liver enzymes function in the current study

Treatment Parameters	T1 con	T2 MSH	T3 Pro	T4 Msh+pro
AST	24.4 ±1.80 A	23.4± .748 AB	20.4 ±1.56 AB	14.4 ± 1.24 B
ALT	21 ±1.48 A	16.4±1.03 BC	17.8± .917 B	13.8± .735 C
ALP	227.2 ±19.02 A	228.8±37.28 A	214.2±16.32 B	219.6 ± 34.49 B

The present study shows that compared with the control group, the AST, ALT, and ALP of the (Mush-BS), (BS) and (Mush) groups were significantly reduced (Table 4-2). In our research results, probiotic and probiotic supplements caused a decrease in serum AST, ALT, and ALP from broiler chickens, which may be due to their anti-free radical and antioxidant properties; they have very good antioxidant effects and can inhibit cell membrane lipids. Peroxide. Our results are consistent with findings [19,4], as the latter indicates that the decrease in ALT and AST activities in chickens fed probiotics may be due to their major role in reducing liver damage.

AST and ALT play an important role in amino acid metabolism, as the key aminotransferases for assessing healthy liver in fish [11]. AST and ALT are used to assess the stress response of fish, and their gradually increasing levels reflect the abnormal liver function. Mushroom diet has been reported to have a protective effect on substances that cause liver cell damage [13,2].

The figure results in the (figure 1) of the humeral immunity response were showed significantly differences ($p \leq 0.05$). The improvement of humeral immunity IgG titer against Newcastle Disease ND and Infectious bronchitis IB were improved significantly ($P \leq 0.05$) in the (Mush-BS), (Mushroom), and (BS) groups respectively compare with the (Control) in the ninth age days.

On the other hand, The figure results (figure 2) of the humeral immunity response were showed significantly differences ($p \leq 0.05$). The improvement of humeral immunity IgG titer against Newcastle Disease ND nd Infectious bronchitis IB were improved significantly ($P \leq 0.05$) in the (Mush-BS), (Mushroom), and (BS) groups respectively compare with the (Control) in the eighteenth age days.

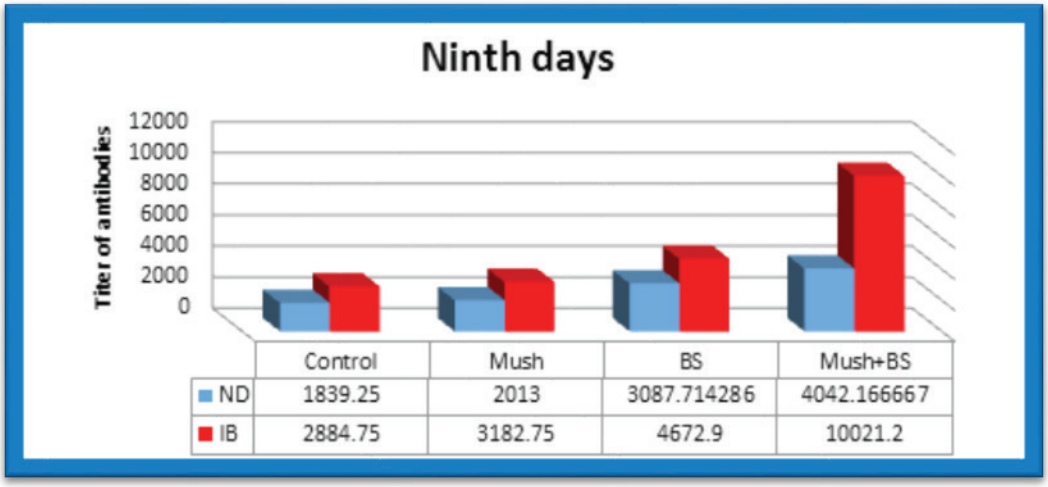

Figure 1: Antibody titers against Two viral disease vaccine measured by ELISA for different groups in the ninth age days.

The improvement of humeral immunity IgG titer against Newcastle Disease ND and Infectious bronchitis IB were improved significantly ($P \leq 0.05$) in the Mush-BS (3801.16) for Newcastle disease virus vaccination and (9880.2) for Infectious bronchitis virus vaccination , (Mushroom), and (BS) groups respectively compare with the (Control) with mean 1698.25 and 2643.75 for Newcastle and infectious bronchitis, respectively in the Eighteeth age days.

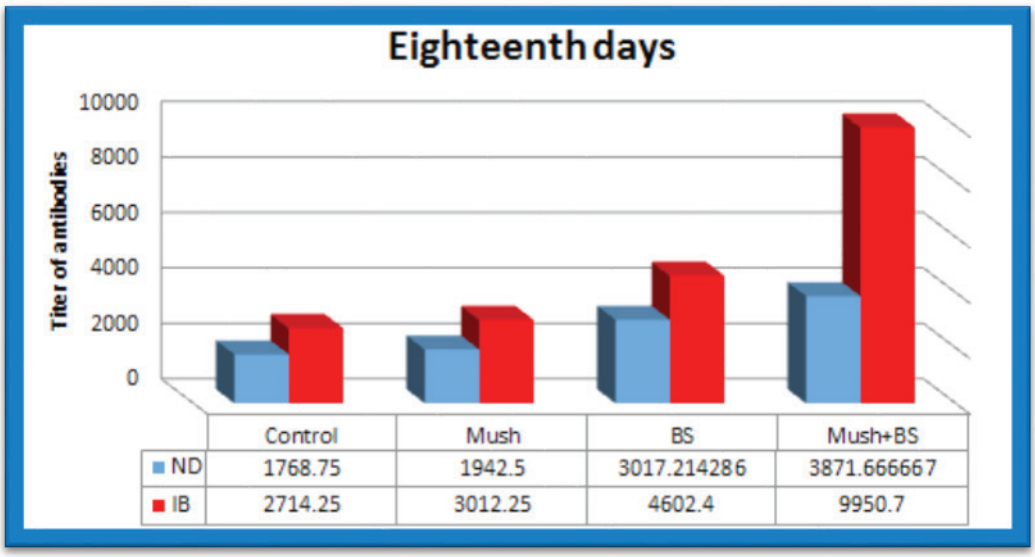

Figure 2: Antibody titers against Two viral disease vaccine measured by ELISA for different groups in the eighteenth age days.

However, all the chicks of this study recorded significantly ($P \leq 0.05$) increment in antibody titers against Newcastle Disease vaccine with progress the age, This increment in Ab appear obviously in the treatment groups as compared with the control at (9 and 18) days of age. This results was disagreement with [9] who was concluded that adding 1% of mushroom waste to the diet can enhance some immune parameters of chickens to a certain extent, but the effect on Newcastle disease virus is weak.

In conclusion : AST, ALT and ALP were decreased in fourth group compared with the other treatment groups, it is important to add a mixture of probiotics and prebiotics to their staple diet can improve the immune response by enhancing both cellular and Humeral immunity.

Conclusion

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Physiology and all experiments were carried out in accordance with approved guidelines.

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