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Phytomedicine (*Allium cepa* L.) effect on broiler immunity against infectious diseases

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Abstract

The extensive use of antibiotics in poultry has led to severe health concerns for human utility and accounted for multidrug resistance. The phytochemicals and probiotics are highly preferred as an alternative to antibiotics supplementation in the poultry feed. In this contribution, we supplemented commercial broiler feed with *Allium cepa* L. (Onion) powder at various concentrations. The dietary supplementation of *Allium cepa* L. exerted a significant positive effect ($p < 0.05$) on antibodies titer against Newcastle Disease (ND) virus, Infectious Bronchitis (IB), and Infectious Bursal Disease (IBD) antibodies titer in all treated groups. The serum proteins were increased significantly ($p < 0.05$) in treatment groups. The *Allium cepa* L. also revealed a significant lowering effect ($p < 0.05$) on the liver function enzymes, i.e., Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphates (ALP). We could not find any significant effect on lymphoid organs (i.e., thymus, spleen, and bursa of Fabricius) after supplementation of onion in the feed. In conclusion, *Allium cepa* L. supplementations in broiler revealed a positive effect on performance in terms of immunity and liver functionality.

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Introduction

Poultry feed is mostly comprised of antibiotics as growth promoters to increase production in terms of maximum economic benefits. In countries like Pakistan, the estimates for antibiotic growth promotors were ranged about three rupees per kg of feed. [1]. Currently, throughout the world, several billion USD agricultural products and byproducts are employed in poultry ration. In the year 2010-11, the poultry feed industry produced 4.9 million tons of feed, hence based on these statistics, the poultry feed growth promoters can be summed up to one billion PKR. Such a higher level of synthetic antibiotic employment has led to serious concerns in the medical community about multiple drug resistance and human health ailments. The persistent employment of antibiotics in poultry feed has also led to antibiotic resistance in human beings after the utilization of their products[2]. Therefore, in 2006, the EU (European Union) banned the employment of antibiotics in poultry feed due to adverse effects on human health. Therefore, medicinal plants based growth promoters, i.e., phytochemicals, are a welcome option to replace synthetic drugs and antibiotics [3].

In poultry feed, the phytochemical or phytogenic compounds are potential pharmacologically active substances that enhance the growth performance by regulating the digestive enzymes for better appetite and digestion, immune boosters, and antibacterial effects against pathogenic bacteria, resulting in better feed conversion ratio [4]. Among various phytogenic compounds employment in poultry feed and medication, the Allium spp. are highly favored for their cost-effectiveness and vast medicinal properties. Allium cepa L. (Onion) is a commonly utilized and readily available vegetable all around the globe. It is lower in calories, moderately higher fiber, and sugar constituents along with vitamins (B6, folic acid) and minerals content, i.e., calcium, magnesium, phosphorus, and potassium. Allium cepa L. is rich in flavonoids contents, and so far, 25 various types of flavanols have been characterized, especially the anthocyanins that impart purple or red color and flavonol (quercetin) responsible for yellowish color in certain varieties [5, 6].

Currently, the prevalent infectious diseases pose a significant threat to the poultry industry leading to severe financial crises and stake the world food security. In addition to various other factors, the misuse of antibiotics, immunosuppression by stress and failure of the vaccine due to compromised quality or handling, can lead to the severe immune deficiency that may result in a disease outbreak. Among various endemic diseases, Infectious Bronchitis (IB) is a contagious poultry disease caused by a coronavirus, primarily infecting the respiratory system, followed by nephritis and impact on egg quality[7]. In non-vaccinated birds, the morbidity is up to 100%, with ~ 60% mortality has been observed[8]. Likewise, the infectious bursal disease (IBD), also known as Gumboro disease, is also an infection of the bursa of Fabricius caused by IBD virus (Avibirna virus), resulting in severe immunosuppression at 3-6 weeks age. Thus, prone the birds to severe secondary infections leading to higher mortality rates[9, 10]. Besides, the Newcastle disease (ND) also a viral disease caused by Avulavirus, accompanied by severe signs of gasping, twisted head, greenish diarrhea, muscle swelling around eyes, drooping wings inappetence, depression circling, etc.[11] All these diseases are accompanied by severe economic losses and attributed to their first signs and severe immunosuppression, exposing birds to other infectious diseases. Moreover, the normal function of the liver guarantees proper metabolism, which results in balanced FCR and, ultimately, higher production.

Although the *Allium cepa* L. has many medicinal properties still a comprehensive study on their effect on the lymphoid organs and immunity to prevalent diseases is poorly understood in poultry medicine. Therefore, we conducted a study to investigate the *Allium cepa* L. dietary supplementation effect on the immune response to common infectious disease (IB, IBD, and ND) and liver functionality in commercial broiler chickens.

Materials and Methods

Experiment design

Day-old broiler chicks of same weight and size were randomly assigned to four different groups named as A, B, C and D (n=320, 80/group). The group A was kept as untreated control, whereas B, C, and D group feed were supplemented with onion powder at the rate of 8, 9 and 10 g/kg feed. The birds were reared in open-sided poultry shed, whereas standard feed and water were provided with optimum environmental conditions. The feed composition is provided in **Table 1**. The experiment continued for 42 days. All the experiments were approved by the animal research and care committee of the University of Agriculture Peshawar, and their guidelines were followed during the experimental trial.

Table 1: Ingredients and composition of the basal diet (as offered)

Ingredients (g/kg of diet)	Starter	Finisher
Maize, yellow	354.0	250.0
Soybean meal (480 g CP/Kg)	275.0	170.0
Sunflower meal 350 g CP/Kg)	110.0	110.5
Wheat	99.0	331.0
Wheat bran	-	-
Meat-bone meal	65.0	49.5
Vegetable oil	73.9	73.5
Limestone	13.5	-
Mineral-vitamins premix[1]	3.5	3.5
Sodium chloride	3.1	2.5
L-lysine	0.4	0.1
DL-Methionine	1.6	1.7
Calculated chemical composition (per Kg of diet)		
ME (MJ)	13.2	13.4
Crude Protein (g)	231.2	189.8
Calcium (g)	15.0	8.0
Available phosphorus (g)	5.0	3.9
Lysine (g)	12.0	8.5
Methionine (g)	5.6	5.2
Methionine + cystine (g)	9.3	8.4
Sodium chloride (g)	3.4	2.9

Note: Provides per kg of diet: Mn 80 mg; Zn 60 mg; Fe 60 mg; Iron 5mg; Cu 5 mg; Co 0.2 mg; I 1 mg; Se 0.15 mg; choline chloride 200 mg; vitamin A 12 000 IU; vitamin D3 2 400 IU; vitamin E 50 mg; vitamin K3 4 mg; vitamin B1 3 mg; vitamin B2 6 mg; niacin 25 mg; calcium-d- pantothenate 10 mg; vitamin B6 5 mg; vitamin B12 0.03 mg; d-biotin 0.05 mg; folic acid 1 mg

Preparation of Onion Powder

The powder was prepared from the onion bulb (*Allium cepa L.)*, and onion was cut into pieces. The onion was air-dried and put into the grinder for preparation of powder. The onion powder was given @ of 8, 9, and 10 g/kg of feed to groups B, C, and D, respectively, whereas group A was kept as control.

Allium cepa L. is divided into three main parts, i.e., top-bottom, outer scale, and onion bulb, the later one is comprised of crude protein (8.75%), crude fiber (6.05%), crude fat (14.98%), ash content (2.32%), moisture content (8.78%) and carbohydrates (65. 16%) [12].

Vaccination schedule

All the birds of each group A, B, C, and D were vaccinated against ND, IBD on day seven, and IB on the 24th day as per the guidelines of the company. The birds were administered with boosting dose of IBD on the day 18th and ND at day 21st. During the entire research period, birds were observed for mortality, and dead birds were dissected to find out the possible causes of mortality.

Lymphoid organs weight

On the final day of the experiment, the birds from all treatment groups were sacrificed, and vital lymphoid organs, i.e., Bursa of Fabricius, Thymus, and Spleen, were weighed on a digital weighing balance.

Antibodies titer determination against ND, IB, and IBD

At day 42 of treatment, three mL of blood was collected from the randomly selected ten birds in each group and centrifuged for the serum separation at 4000 RPM for 15 minutes at 4^{o}C. The serum was then separated and stored in -80^{o}C for further evaluation. The Enzyme-linked immunosorbent assay (ELISA) was performed for the detection of antibodies titer against IBD (IBDV-ELISA kit, Abbexa®, 181 Cambridge Science Park, UK), whereas hemagglutination inhibition technique was used to evaluated the antibodies titer against ND and IB by the procedure mentioned earlier[13].

Liver Function Tests

At day 42 of the experiment, ten birds were randomly selected from each group, and after blood collection (3 mL each), the serum was separated as by procedure, as mentioned above. Then the liver function tests for Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) were performed by COBAS INTEGRA® 400 plus analyzer system in the pathology lab of Khyber Medical University, Peshawar Pakistan.

Determination of serum proteins

Proteins in serum are found in higher concentrations performing different functions. Due to the protein mixture in the blood serum, it is hard to find the molecular weight of various proteins, especially the albumins and globulins. That's why their concentration unit is g/L. Serum proteins were determined through the Biuret method. The measured reaction product has a violet color. The name Biuret arises from the word bureau, which is a compound produced when urea is warmed at 180 °C. For this purpose, 5 mL of blood was collected from 10 randomly selected birds in each treatment group, and the serum was separated and utilized for protein determination.

Statistical Analysis

Data was initially recorded in MS Excel and statistically analyzed for the analysis of variance (ANOVA) by using digital software named as SAS statistical package (SAS Institute Inc., NC USA). The

data is presented in the mean ± standard deviation. The probability value < 0.05 was considered as statistically significant.

Results

Supplementation impact on Antibodies titer of IB, IBD, and ND

The highest antibodies titer after *Allium cepa L.* is divided supplementation was found in 10 g/kg feed group (2911.00±2.43) followed by 8 and 9 g/kg of feed, i.e., 1930.75±1.07 and 1717.00±1.59, respectively, as compared to control group. It was noticed that the two-fold antibodies titer was increased in the 10 g/kg treated group as compared to the untreated group. Likewise, the IB and IBD had the highest antibodies titer in 10 g/kg treated group, followed by other treated groups in a dose-dependent manner. It was found that antibodies titer in *Allium cepa L.* treated group was significantly ($p > 0.0.1$) higher as compared to control, as shown in **Table 2** and **Fig. 1**.

Lymphoid Organs and Liver function

The lymphoid organs (i.e., Spleen, Thymus, Bursa) functionality is also of prime importance, and their proper fictionality is directly proportional to the immunity of the body against antigens. We observed that after *Allium cepa L.* supplementation, the antibody titer was increased, but the average weight of lymphoid organs remained unaffected. No significant harmful effects and organ hypertrophy was observed. The average weight of lymphoid organs in various treated groups is given in table 2.

The liver health is measured by the proper functionality of enzymes named as AST, ALT, ALP, and SP. The *Allium cepa* L. also revealed a significant lowering effect ($p < 0.05$) on the liver function enzymes, i.e., Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphates (ALP) and no deleterious effects were observed in either of the treated groups liver. The results are displayed in **Fig. 2**.

Table 2: In vivo effect of *Allium cepa* L. supplementation on the immunity of broiler

Antibodies titers and Lymphoid organs weight	Treatment A	B	C	D
Newcastle disease	1550.50 ± 1.42^{c}	1930.75±1.07^{b}	1717.00 ±1.59 ^{b}	2911.00 ±2.43^{a}
Infectious Bronchitis	1422 ± 0.711^{c}	2695.00±1.047^{b}	2189.00± 1.154^{b}	3117.00±0.65^{a}
Infectious Bursal Disease	1376.00 ± 2.217^{c}	2340.00± 1.49^{b}	2553.50±2.95^{b}	3295.00±1.10^{a}
Spleen (g)	3.250 ± 0.25^{a}	3.500± 0.28^{a}	3.500 ± 0.28^{a}	4.250±0.25^{a}
Thymus(g)	4.150 ± 0.47^{a}	4.000± 0.40^{a}	4.00 ±0.40^{a}	4.300±0.28^{a}
Bursa(g)	3.2500 ± 0.28^{a}	3.650 ±0.47^{a}	3.9500 ± 0.64^{a}	4.850±0.47^{a}

Note: The Allium cepa L. was supplemented @ 0, 8, 9 and 10 g/kg of feed to group A, B, C, and D, respectively. Data is presented as Mean ± Sd.

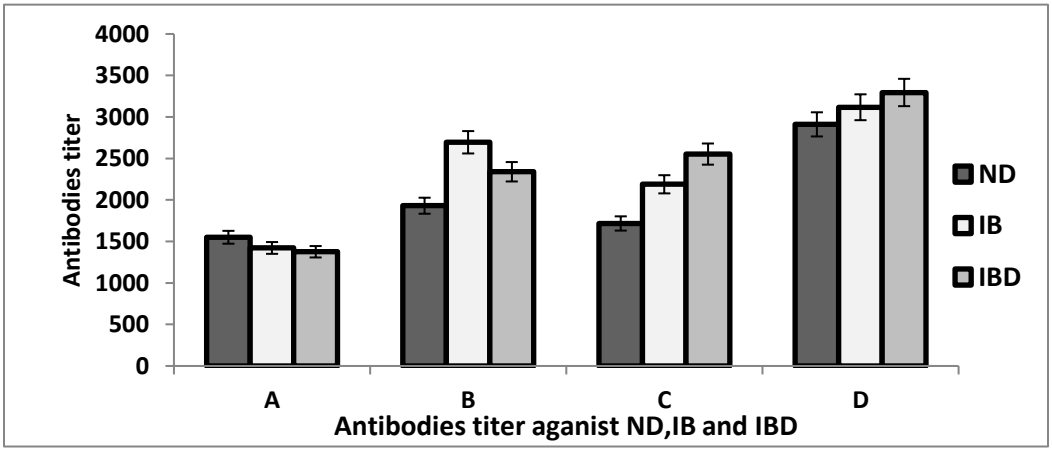
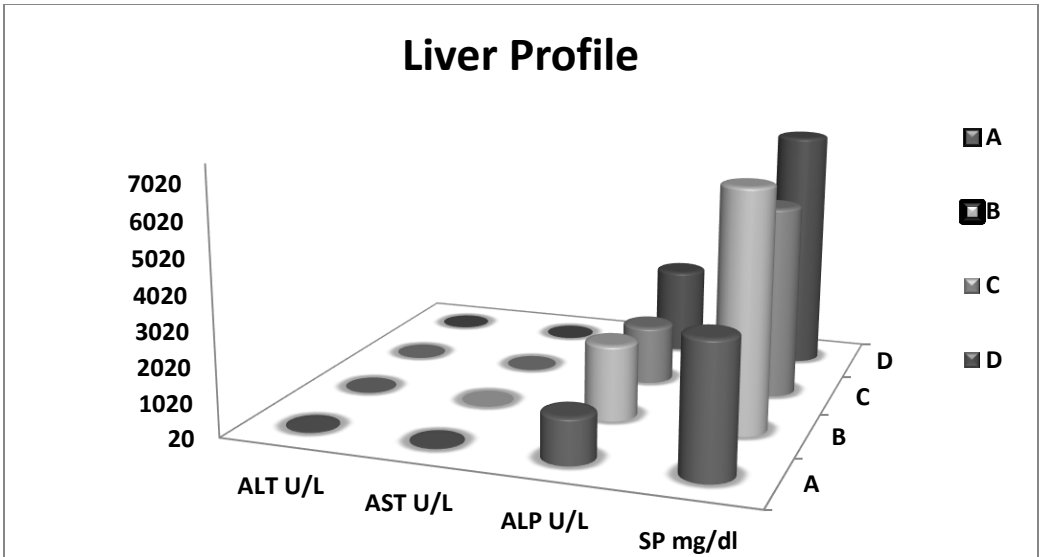


Fig. 1: Mean Antibodies titer against Newcastle Disease (ND), Infectious Bronchitis (IB) and Infectious Bursal Disease (IBD) were significantly increased ($p < 0.05$) With supplementation of *Allium cepa L.* given @ of 0, 8, 9 and 10 g/kg feed to A, B, C, and D, respectively.



	ALT U/L	AST U/L	ALP U/L	SP mg/dl
A	43.75	41.5	1327	3800
B	19.5	22	2196.25	6875
C	17.75	16.75	1709.5	5575
D	24.25	21.75	2530.25	7010

Fig. 2: Mean unit per liter ALT, AST, ALP, and Serum Proteins mg/dl of serum were significantly ($p < 0.05$) increased with supplementation of *Allium cepa* L. given @of 0, 8, 9 and 10 g/kg feed to group A, B, C, and D, respectively.

Discussion

Poultry, being a significant contributor to human food, has also had a direct impact on human health in terms of zoonosis (e.g., Avian Influenza, etc.) or drug resistance[14]. Therefore, the poultry industry has extensively been observed and instructed by regulatory bodies (i.e., FDA, EU) for healthy practices. The poultry industry is extensively employing growth promotors and antibiotics to enhance their production. It has been estimated that around 80% of animals are fed with synthetic growth promoters and antibiotics. For instance, in the year 2012, an estimate of 4500 tons of antibiotics was used in China as growth promoters[15]. Approximately 80% of domestic animals have been fed with synthetic (antibiotics) compounds for medication and as a growth promoter. Therefore, the employment of phytogenic compounds as a feed additive is a welcomed option.

In this contribution, we have used *Allium cepa* L. as a feed supplement in the commercial broiler feed to check its effect on the birds' immunity against common infectious diseases, i.e., IB, IBD, and ND. Post-vaccination of the anti-bodies titer was significantly increased in *Allium cepa* L. treated birds as compared to the control group, i.e., without any supplementation. The result showed a ~ 100 percent antibodies titer increase in the group treated with 10 g/kg of feed for all the three diseases.

Our results are in corroboration with Szigeti et al., who reported that supplementation of Allium could increase the antibody titer against the ND virus[16]. Many studies have attributed the immunity-enhancing the property of Allium spp. To the sulfur contents that not only work as antibacterial[17] and antiprotozoal[18] but also promote the phagocytic activity of macrophages and natural killer cells[19, 20]. Besides, the flavonoid's high content has been considered to boost immunity by its antioxidant effect[21]. The finding of Hanieh et al. have revealed that Allium spp. may not immediately increase the antibody titer; however, in response to secondary immunization, the antibody titer was significantly increased, suggesting their role in the long term immunity and as potential diseases ameliorants[21]. Likewise, Kyo et al. have also found that Allium spp.

Supplementation in mice models could prevent the anti-SRBC antibody titer reduction[22].

The *Allium cepa* L. supplementation at an optimum level less than 30g/kg in feed is considered safe [21], and in our findings, we found no detrimental effect on the vital lymphoid organs and liver functionality enzymes concertation. Moreover, the herbal medicine Phyto-origin and cost-effectiveness, encourage poultry nutritionists to employ them as an additive in the ration and avoid synthetic drugs.

Conclusion

In summary, we report the *Allium cepa* L. medicinal effects on broiler immunity against IB, IBD, and ND. In addition, liver functionality was also enhanced in terms of improved AST, ALT, and ALP. The *Allium cepa* L. addition in feed on broilers can be an alternative to traditional medicine used in broiler feed. Moreover, it enhances the immunity against most prevalent poultry disease in a cost-effective and facile manner.

Conflict of interest

The authors declare no conflict of interest.

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