Effect of Herbal Preparations on Immune Response in Broilers immunized with Gumboro Disease Vaccine

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ABSTRACT

This study aimed to know the effect of three commercial products, namely, Sumerfeed, Oregofarm and garlic, which are routinely used in poultry farms, on enhancing immunity. Seventy-five one-day-old broiler chicks were used and divided into five equal groups. Three groups were treated with Sumerfeed, Oregofarm, and garlic, respectively and vaccinated against Infectious Bursal Disease (IBD). The fourth group was left as a positive control group without any treatment but vaccinated against IBD, and the fifth group was left as a negative control that was neither treated nor vaccinated. Antibodies against IBD, in addition to $CD4^+$ and $CD8^+$ T-cell levels, were estimated using commercial ELISA kits. The results of the study showed a high efficiency with Sumerfeed and Oregofarm, followed by garlic in enhancing immune response against the IBD vaccine. This study showed increasing of specific antibodies against IBD with increasing levels of $CD4^+$ and $CD8^+$ cells.

Keywords: Immune enhancers, immune response, IBD vaccine, $CD4^+$, $CD8^+$

INTRODUCTION

Immune enhancers, when used, improve the immune responses and success of vaccination programs against many endemic viral diseases (Yu *et al*., 2021). Balanced feed additives are very important and can improve nutrition, cover all requirements of normal physiological processes and influence the immune system of the birds indirectly and improve immune responses (Seidavi *et al*., 2023). There is a wide range of feed additives and immune enhancers used in poultry, such as essential amino acids, Vit E, Se, Zn, and others (Kallam and Sejian, 2021). Oregano oil is reported to enhance immune response and growth performance apart from its antioxidant activity in broilers (Adaszynska-Skwirzynska and Szczerbinska, 2019; Bauer *et al*., 2019). Allicin has been found to increase antibody levels, lymphocyte proliferation, antibacterial, anti-parasitic and antioxidant activities (Abd El-Ghany, 2024). Infectious bursal disease (IBD) is one of the most highly contagious viral diseases of young chickens. IBD can affect young chicks and cause severe immunosuppression, which can last for a long time (Eterradossi and Saif, 2019), and vaccination is the better method to control the disease. Humoral and cell-mediated immunity (CMI) is very important in the protection of the chicks. In this study, the effect of immune enhancers on IBD vaccination has been assessed.

MATERIALS AND METHODS

Chicks: Seventy-five one-day-old Ross 308 broiler chicks were used in the study, and all chicks were raised in animal houses in the College of Veterinary Medicine, University of Mosul, Iraq. Under strict temperature, light, and air-purification conditions, chicks were fed concentrated pellet feed supplied by Erbil feed No. 1 until 20 days of age, then switched to pellet No. 2 until the end of the experiment, with ad libitum access to drinking water.

Commercial Immune Enhancer Preparations: *Sumerfeed (Ekotopia Company/Lawa Factory, Sulaimanyah, Iraq)*: It contains a wide range of vitamins, amino acids, and minerals, including zinc, magnesium, and selenium. The preparation was used as per the manufacturer's recommendations at a rate of 3ml/L for six consecutive days after the prime vaccination dose and also for six consecutive days after the booster dose.

Oregofarm Farmavet International, Turkey): It contains Oregano and a wide range of other chewing gum additives, which are used as per the manufacturer's recommendations at a rate of 0.5 ml /L for six consecutive days after the prime vaccination dose and also for six consecutive days after the booster dose.

Garlic (Anvet Asintech / Vietnam): It contains ALLICIN 200 and is used as per the manufacturer's recommendations at a rate of 0.5 ml /L for six consecutive days after the prime vaccination and also for six consecutive days after the first booster dose.

Experiment Design: Seventy-five chicks were divided into five equal groups (N=15). The members of each group were placed in a separate room with appropriate temperature and ventilation. The five groups were treated as follows. The first (A) group received Sumerfeed. The second (B) group received Oregofarm. The third (C) group received Garlic. The fourth (D) group was used as a positive control group and received only the vaccine. The fifth (E) group was used as a negative control and neither vaccinated nor received any commercial product. Each of the first, second, third, and fourth groups was vaccinated with Nobilis Gumboro D78, which is a live vaccine against IBD (MSD Animal Health), through drinking water as primary vaccination at the age of five days. Then the first group was treated with sumerfeed, the second group treated with oregofarm, while the third group was treated with garlic from the age of 6 days until the age of 12 days. The booster vaccine was given in drinking water at 14 days, and the groups were treated with commercial products as above for the second time from the age of 14 days until the age of 20 days. On 14, 21 and 33 days post-vaccination, 5 chicks from each group were slaughtered, and blood was collected from the jugular vein on a gel tube, serum was separated, and collected sera were stored in deep freezer until used to measure antibodies against IBD, as well as the levels of $CD4^+$ and $CD8^+$ T-cells, using ELISA kits (ELK biotechnology, USA)

Statistical Analysis: The GraphPad Prism 8 statistical program was used to analyze the results. Two-way ANOVA was used to compare the data means of groups, and all results were given as means ± standard errors. Graphs and significant values were drawn using the same program.

RESULTS

Antibodies against IBD: The results showed identity on both 14 and 21 days of the experiment, indicating a significant increase in the level of antibodies against IBD in each of the three groups treated with Sumerfeed, Oregofarm and garlic, compared to the positive and negative control groups. While comparing the treated groups, the results showed a significant increase in the two groups treated with Sumerfeed and Oregofarm than the group treated with garlic, which was not significantly different from the positive control group. Results on day 33 showed a significant increase in the level of antibodies against IBD among all treated groups, including the positive control group, compared to the negative control group. When comparing the groups among themselves, the results showed that there was no significance between all treated groups, but the group treated with garlic only did not give a significant increase when compared to the positive control group (Table I and Figure 1).

Table I: Antibody Levels against IBD in Groups Treated with Sumerfeed, Oregofarm, and Garlic

Groups	Days					
	14		21		33	
Sumerfeed	807.3±0.0	C, D	838.3±8.8	C, D	1308±0.0	C, D, E
Oregoform	799.0±5.7	C, D	837.0±44	C, D	1301±60.3	C, D, E
Garlic	610.0±6.8	A, E	716.7±9.8	A, E	1103±60.5	A, C, D, E

Control positive	619.0±0.0	A	652.0±0.0	A	1013±0.0	A
Control negative	317.0±3.7	B	354.0±4.4	B	333.0±3.5	B

Capital letters (Groups) mean the presence of significant differences between groups at P < 0.05

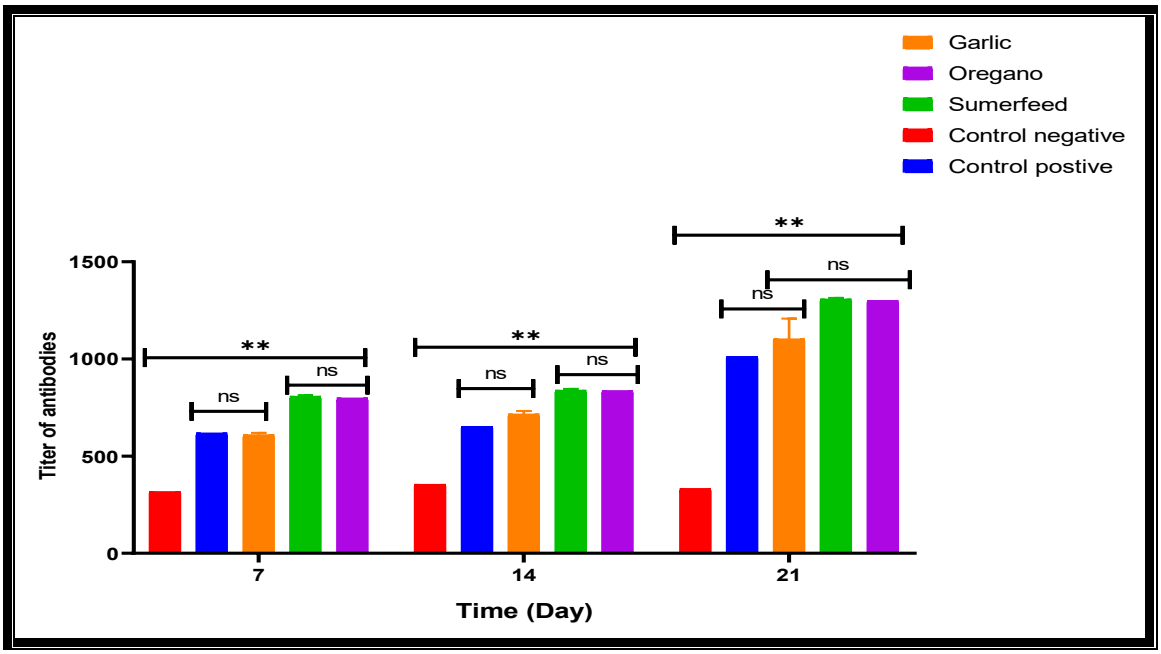

Figure 1: Antibody Levels against IBD in Groups Treated with Sumerfeed, Oregofarm, and Garlic

Concentration of CD4⁺ T-cells: The results showed identity on both 14 and 21 days of the experiment, with a significant increase in the level of CD4⁺ T-cells in all treated groups, with each of the groups treated with sumerfeed, oregofarm, and garlic, respectively. There was a significant increase in the two groups treated with sumerfeed and oregofarm compared to the group treated with garlic, which also significantly decreased when compared to the positive control group. The results on day 33 showed a significant increase in the level of CD4⁺ T-cells between all treated groups and the positive control group compared to the negative control group. When comparing the groups among themselves, there was no significant difference between the treated groups. However, the group treated with garlic only did not show a significant increase when compared to the positive control group (Table II). There was a significant increase in the group treated with sumerfeed compared to the groups treated with oregofarm and garlic.

Table II: CD4⁺ T-cell levels in groups treated with Sumerfeed, Oregoform, and Garlic

Groups	Days					
	14		21		33	
Sumerfeed	8.500±0.10	C	8.800±0.10	C	9.033±0.15	C
Oregoform	8.267±0.00	D, C	8.600±0.00	D, C	8.900±0.17	D, D
Garlic	7.026±0.10	E	7.140±0.10	E	7.114±0.15	E
Control positive	7.397±0.00	A	7.208±0.00	A	7.173±0.00	A
Control negative	4.435± 0.05	B	4.098±0.00	B	4.671±0.08	B

Capital letters mean the presence of significant differences between groups at P < 0.05

Concentration of CD8$^+$ T-cells: The results on days 14, 21, and 33 showed a significant increase in the level of CD8$^+$T-cells in all the treated groups and the positive control group compared to the negative control group. A significantly higher increase was observed in sumerfeed and oregofarm fed groups than that treated with garlic, which was also significantly lower than the positive control (Table III).

Table III: CD8$^+$ T-cell Levels in Groups Treated with Sumerfeed, Oregofarm and Garlic

Groups	Days					
	14		21		33	
Sumerfeed	6.007±0.00	D	7.070±0.10	D	6.800±0.20	D
Oregoform	6.181±0.00	E, D	6.568±0.00	E, D	6.700±0.00	E, D
Garlic	4.326±0.00	F	5.200±0.10	F	5.400±0.10	F
Control positive	5.451±0.00	A	5.259±0.00	A	4.447±0.00	A
Control negative	3.345±0.09	B	3.124±0.00	B	3.380±0.00	B

Different capital letters mean the presence of significant differences between groups at P < 0.05

DISCUSSION

Immune enhancers are important in the poultry industry to improve the ability of the immune system. This study has proved the immune-enhancing activity of the three herbal products to different degrees. Sumerfeed, which contains many vitamins, amino acids, and minerals, is believed to improve the immune system. Giving this, along with IBD vaccine resulted in significant elevation of antibody titers against IBD and CD4$^+$ and CD8$^+$T-cell levels when compared to other preparations used in this study. Activation of both CD4 and CD8 T cells observed in this study agreed with the findings of Hojyo and Fukada (2016). Earlier researches have also found that adding Zinc as a supplement enhanced the immune system in addition to its effects on many mechanisms in broilers (Hidayat *et al*., 2020; Sizova *et al*., 2023; Agashe *et al*., 2024). Selenium has also been reported to increase the antibody titers in broilers (Habibian *et al*., 2014; Bakhshalinejad *et al*., 2018), which was found in this study too.

Arginine plays a key role in the synthesis and induction of nitric oxide, which is a key immune mechanism mediator (Wu, 2013). Presence of arginine in sumerfeed improved the humoral immune response as cited by Junior *et al*. (2019). *Oregano vulgare* oil has a stimulating effect on the immune system (Parvizi *et al*., 2020; Aly *et al*., 2022; Maarek *et al*., 2023). Since Oregofarm contains *Oregano vulgare* oil, it also stimulated the immune response post IBD immunization, though not to the extent of sumerfeed. The high concentration of essential amino acids, Vitamin E, Se, Zn and others found in sumerfeed might have given a better response (Junior *et al*., 2019). Allicin could stimulate immunity by inducing mature dendritic cells in the early stage of infection, leading to increased levels of pro-inflammatory cytokines induced by proliferative CD4$^+$ T-cells and macrophages (Feng *et al*., 2012). Though garlic supplementation improved the immune levels, the effects were comparatively lower than those of Sumerfeed and Oregofarm (Chang *et al*., 2021; Ismail *et al*., 2021).

CONCLUSION

Sumerfeed induced a better immune response in broilers in response to IBD vaccination followed by Oregoform and Garlic.

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