Pakistan J. Zool., vol. 52(3), pp 1095-1100, 2020. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20190519110533

Effects of Immune Modulators on the Immune Status of Broiler Chickens

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ABSTRACT

The aim of this study was to examine the influence of immune modulators on the immune competence of broiler chickens. In three experiments (n=240 chicks in each), the immune modulators vitamin C (500 mg/l), vitamin E (200 mg/l), dietary nucleotides (100 mg/l) and DNA (100 mg/l) extracted from chicken liver were separately offered on days 1-21, 1-42 and 22-42 in experiments 1, 2 and 3, respectively. Under standard broiler management conditions, birds were divided into five treatment groups of 48 with four replicates. Commercial pre-starter feed, starter feed and finisher feed were offered on days 1-12, 13-25 and 26-42, respectively. Birds were vaccinated against Newcastle disease virus and infectious bursal disease. Relative lymphoid organ weights were recorded on day 42. Hemagglutination inhibition (HI) assay was performed against Newcastle disease virus on a weekly basis, whilst a hemagglutination (HA) assay was performed to determine immunity against total sheep red blood cell (SRBC) , mercaptoethanol-2 resistant (IgG) and mercaptoethanol-2 sensitive (IgM) antibodies on weeks 3, 4, 5 and 6. Lymphoid organ weight showed non-significant difference ($P>0.05$), with numerically higher weight in immune modulator groups. The vitamin E supplemented group had highest HI antibody titers (7.22±0.25, 7.36±0.18 and 6.55±0.38). In experiments 1 and 2, supplementation with immune modulators had significant ($P<0.05$) effects on total SRBC, IgG and IgM titers. In conclusion, vitamin E showed better immuno-modulatory effect followed by vitamin C, nucleotides and DNA, respectively. Supplementation of immuno-modulators at early age (1 to 21 days) showed more promising effect on immune performance of broiler chickens.

Article Information
Received 19 May 2019
Revised 11 July 2019
Accepted 17 August 2019
Available online 10 March 2020

Authors' Contribution
ISS, MAB, NR and MZM executed the experimental work. TMA MMT, MR, AS and AU analyzed the data and wrote the manuscript.

Key words
Immune modulators, Hemagglutination inhibition, Lymphoid organ, Broiler chicken.

INTRODUCTION

Pakistan poultry industry has been flourishing continuously for the last forty years, and it has currently attained the status of second largest industry of the country. Intensive poultry farming in Pakistan is susceptible to abrupt disease outbreaks that bring heavy financial losses due to extraordinary mortality. To overcome these losses many farmers and feed producers are implementing certain prophylactic and therapeutic measures, including use of antibiotics as a growth promoting feed additive to get better feed efficiency and to reduce mortalities at their farms. These practices resulted in antibiotic resistance in poultry and are a constant threat to the consumers (Hancock and Sahl, 2006).World Health Organization (WHO) reports have intimated that antibiotics may further lose their effectiveness as a feed supplement beyond 2020, because of the rapid emergence of drug resistance (Hamill *et al*., 2008; Dhama *et al*., 2008).

Taking into account the scenario of supplementing antibiotics in broiler diets and the ban imposed on their use as feed additives by certain governments, it is a dire need of the day to discover unconventional methods for better production and disease resistance in broiler chickens. Among many possible substitutes, use of immune modulators might be a realistic tool to maintain a strong and well-functional immune system. The peculiar function of immunomodulators is to regulate and strengthen the immune system against any threat posed by pathogens and environment (Mahima *et al.,* 2013). There are variety of compounds, herbs and elements which have protective characteristics to regulate the immune system (Dhama *et al.*, 2015; Rafeeq *et al*., 2017). Recently many immunomodulators have been practiced as feed additives in broiler chicken production with positive influences on immune regulation (Kidd, 2004; Mahima *et al*., 2013). Studies on the relationship between poultry feed and immunity need priority research to introduce the alternatives and replacements of antibiotics. Use of different potential immune modulators, such as vitamin C, vitamin E and dietary nucleotides not only provide better growth performance but also have the capabilities to decrease oxidative stress on broiler chickens under certain physiological and environmental stress. The

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0030-9923/2020/0003-1095 $ 9.00/0

aim of immunomodulation is to improve the production efficiency of chickens, enhance resistance of the body against pathogens and to reduce production of excessive free radicals that might create oxidative stress in broiler chickens. In this respect, the present study was designed to assess the influence of vitamin C, vitamin E and nucleotides on humoral and cellular immunity of broiler chickens.

MATERIALS AND METHODS

Immune modulators can improve the immunity of broiler chickens against bacterial and viral diseases. For this purpose, vitamin C (500 mg/l), vitamin E (200 mg/l), dietary nucleotides (100 mg/l) from *Saccharomyces cerevisia*e and DNA (100 mg/l) extracted from chicken liver (2-isopropanol precipitation), were offered through drinking water on days 1-21, 1-42 and 22-42 in experiments 1, 2 and 3, respectively. In these three experiments, Hubbard day old broiler chicks (n=240 in each) were randomly divided into five treatment groups of 48 chicks, with four replicates of 12 chicks each. The birds were reared following standard management requirements. In each experiment, *ad-libitum* drinking water with daily controlled commercial feed was offered. Pre-starter feed, starter feed and finisher feed were offered on days 1-12, 13-25 and 26-42, respectively. Chicks were vaccinated against Newcastle disease virus (NDV) on days 7 and 21 and against infectious bursal disease virus on days 14 and 28.

Table I.- Effect of supplementing immune modulators on the relative weight of lymphoid organs of broiler chickens at day 42 (mean±SE).

Treatment	Bursa	Thymus	Spleen
Experiment 1			
Control	0.10±0.020	0.22±0.02	0.14±0.01
Vitamin C	0.11±0.03	0.23±0.05	0.21±0.04
Vitamin E	0.16±0.02	0.27±0.03	0.22±0.05
Nucleotide	0.15±0.00	0.26±0.05	0.18±0.02
DNA	0.10±0.03	0.23±0.04	0.16±0.02
Experiment 2			
Control	0.08±0.01	0.18±0.02	0.21±0.01
Vitamin C	0.08±0.02	0.25±0.02	0.21±0.02
Vitamin E	0.12±0.03	0.28±0.05	0.22±0.03
Nucleotide	0.10±0.02	0.19±0.03	0.22±0.03
DNA	0.12±0.02	0.20±0.03	0.21±0.03
Experiment 3			
Control	0.08±0.01	0.17±0.02	0.21±0.01
Vitamin C	0.08±0.02	0.25±0.02	0.21±0.02
Vitamin E	0.12±0.03	0.28±0.05	0.22±0.03
Nucleotide	0.10±0.02	0.19±0.03	0.22±0.03
DNA	0.12±0.02	0.20±0.03	0.21±0.03

Table II.- Effect of supplementing immune modulators on haemagglutination inhibition (HI) titers of (reciprocal of log2) of broiler chickens (mean±SE).

Treatment	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Experiment 1							
Control	5.58±0.26	5.06±0.30	6.20±0.31	6.48±0.19^{b}	6.85±0.23^{b}	6.10±0.23^{b}	6.06±0.35^{b}
Vitamin C	5.60±0.18	5.21±0.25	6.24±0.16	7.45±0.33^{a}	7.59±0.26^{a}	6.48±0.19^{b}	6.36±0.18^{b}
Vitamin E	5.43±0.33	5.28±0.38	6.57±0.32	7.82±0.35^{a}	7.74±0.16^{a}	7.48±0.19^{a}	7.22±0.25^{a}
Nucleotide	5.28±0.38	5.21±0.25	6.57±0.32	7.48±0.19^{a}	7.61±0.18^{a}	6.72±0.25^{b}	6.48±0.19^{b}
DNA	5.06±0.30	5.16±0.37	6.44±0.33	7.34±0.27^{a}	7.59±0.26^{a}	6.33±0.26^{b}	6.33±0.26^{b}
Experiment 2							
Control	5.35±0.18	5.09±0.23	6.06±0.35	6.10±0.23^{b}	6.48±0.19^{b}	5.69±0.31^{b}	5.71±0.25^{c}
Vitamin C	5.48±0.19	5.09±0.23	6.24±0.16	7.10±0.23^{a}	7.34±0.26^{a}	6.85±0.23^{a}	6.61±0.18^{b}
Vitamin E	5.60±0.18	5.18±0.31	6.36±0.18	7.36±0.18^{a}	7.61±0.18^{a}	7.36±0.18^{a}	7.36±0.18^{a}
Nucleotide	5.23±0.16	4.97±0.23	6.07±0.30	6.96±0.27^{a}	7.24±0.16^{a}	6.72±0.25^{a}	6.59±0.26^{b}
DNA	5.71±0.25	5.12±0.13	6.10±0.23	6.85±0.23^{a}	7.10±0.23^{a}	6.57±0.32^{a}	6.21±0.25^{bc}
Experiment 3							
Control	5.09±0.23	4.70±0.25	5.21±0.25	5.23±0.16	6.07±0.30	6.36±0.18	5.48±0.19^{b}
Vitamin C	5.40±0.38	4.64±0.41	5.23±0.16	5.58±0.26	6.33±0.26	6.61±0.18	6.21±0.25^{ab}
Vitamin E	5.43±0.33	4.45±0.27	5.35±0.18	5.48±0.19	6.57±0.32	6.61±0.18	6.55±0.38^{a}
Nucleotide	5.33±0.26	4.35±0.18	5.12±0.13	5.21±0.25	6.24±0.16	6.48±0.19	6.18±0.37^{ab}
DNA	5.16±0.37	4.70±0.25	5.12±0.13	5.09±0.23	6.10±0.23	6.10±0.23	6.21±0.25^{ab}

$^{a, b, c,}$ values within a column followed by different superscripts denotes significantly different variations (P<0.05).

At the age of 42 days, four birds from each treatment group were humanely killed and the weights of lymphoid organs (bursa, spleen and thymus) were measured. Blood serum was obtained from eight birds per treatment for hemagglutination inhibition (HI) assay. The HI test was done on a weekly basis to determine the antibody titer against NDV. Moreover, anti- sheep red blood cell (SRBC) antibody production for total, 0.01 M mercaptoethanol-2 (ME) sensitive (IgM) and mercaptoethanol-2 resistant (IgG) responses were evaluated on weeks 3, 4, 5 and 6 (Wu *et al*., 2015). The mercaptoethanol-2 sensitive (IgM) response was calculated with help of the following formula:

IgM response = Total anti-SRBC antibody response - IgG antibody response

Statistical analysis

The data collected were summarized using MS excel, and subjected to statistical analysis using SPSS 20 (for windows). Means with significant difference ($P<0.05$) were equated with Duncan's Multiple Range (DMR) test.

RESULTS

The relative weights of lymphoid organs from experiments 1, 2 and 3 are presented in Table I. The weight of bursa, thymus and spleen showed no significant difference ($P>0.05$) among treatments. However, numerically higher weight was recorded in immune modulator supplemented groups. The HI antibody titers against NDV for experiments 1, 2 and 3 are presented in Table II. There was no significant difference ($P>0.05$) amongst treatment groups during weeks 0, 1 and 2 of each experiment. A significant effect ($P<0.05$) of treatments on antibody titer was observed on week 3 of experiments 1 and 2. At week 6 of experiments 1, 2 and 3 antibody titer also showed significant differences ($P<0.05$) among treatments. The highest titer was observed for vitamin E treatment, whilst the lowest titer was in the control. The weekly SRBC antibody titers for experiments 1, 2 and 3 are given in Table III. Treatments showed significant differences ($P<0.05$) in the SRBC titers at weeks 3, 4, 5 and 6 in experiments 1 and 2. Vitamin E supplementation groups showed highest SRBC titers at day 42 of the trial. The anti-SRBC antibody response for total, ME sensitive (IgM) and resistant (IgG) from experiments 1, 2 and 3 are given in Tables IV and V, respectively. Supplementation with vitamin E resulted in a significantly higher IgM antibody titer in comparison to other groups at week 6 of experiments 1 and 2. ME sensitive (IgM) titer was also significantly greater ($P<0.05$) in vitamin E, vitamin C, nucleotide and DNA treated groups as compared to control. A similar trend was observed for the resistant (IgG) antibody titers.

Table III.- Effect of supplementing immune modulators on anti-sheep red blood cell (SRBC) titers (reciprocal of log2) of broiler chickens (mean±SE).

Treatment	Week 3	Week 4	Week 5	Week 6
Experiment 1				
Control	4.95±0.27^{d}	4.35±0.18^{c}	5.33±0.26^{c}	4.58±0.26^{c}
Vitamin C	5.73±0.16^{bc}	5.48±0.19^{b}	6.10±0.23^{b}	5.71±0.25^{b}
Vitamin E	6.72±0.25^{a}	6.36±0.18^{a}	6.98±0.19^{a}	6.48±0.19^{a}
Nucleotide	6.36±0.18^{ab}	6.12±0.13^{a}	6.74±0.16^{ab}	6.36±0.18^{ab}
DNA	5.18±0.31^{cd}	4.95±0.27^{b}	5.33±0.26^{c}	4.95±0.27^{c}
Experiment 2				
Control	4.58±0.26^{d}	4.20±0.25^{d}	4.84±0.23^{d}	4.31±0.26^{d}
Vitamin C	5.60±0.18b^{c}	5.35±0.18^{bc}	5.98±0.19^{bc}	5.48±0.19^{bc}
Vitamin E	6.59±0.26^{a}	6.10±0.23^{a}	6.85±0.23^{a}	6.36±0.18^{a}
Nucleotide	6.21±0.25^{ab}	5.71±0.25^{ab}	6.44±0.33^{ab}	5.98±0.19^{ab}
DNA	5.46±0.27c	4.97±0.19^{c}	5.71±0.25^{cd}	4.95±0.27^{cd}
Experiment 3				
Control	4.73±0.16	4.20±0.25	5.19±0.31	4.44±0.27
Vitamin C	4.58±0.26	4.47±0.19	5.16±0.37	4.58±0.26
Vitamin E	4.81±0.30	4.73±0.16	5.35±0.18	4.97±0.19
Nucleotide	4.95±0.27	4.58±0.26	5.21±0.25	4.58±0.26
DNA	4.95±0.27	4.47±0.19	5.09±0.23	4.60±0.18

$^{a, b, c,}$ values within a column followed by different superscripts denotes significantly different variations ($P<0.05$).

Table IV.- Effect of supplementing immune modulators on mercepto-ethanol- 2 (ME) sensitive IgM antibody titers (reciprocal of log2) of broiler chickens (mean±SE).

Treatment	Week 3	Week 4	Week 5	Week 6
Experiment 1				
Control	3.46±0.19^{c}	2.81±0.23^{b}	3.59±0.18^{c}	1.29±0.18^{c}
Vitamin C	4.08±0.23^{b}	3.46±0.19^{b}	4.08±0.23^{bc}	1.41±0.19^{bc}
Vitamin E	4.86±0.13^{a}	4.35±0.18^{a}	5.23±0.16^{a}	2.10±0.31^{a}
Nucleotide	4.73±0.16^{a}	4.43±0.27^{a}	4.56±0.26^{b}	2.03±0.23^{bc}
DNA	3.69±0.25^{bc}	3.02±0.30^{b}	3.46±0.19^{c}	1.44±0.32^{bc}
Experiment 2				
Control	2.91±0.27^{c}	2.58±0.18^{c}	3.34±0.18^{c}	1.19±0.16^{c}
Vitamin C	3.83±0.23^{ab}	3.46±0.19^{b}	3.83±0.23^{bc}	1.54±0.18^{bc}
Vitamin E	4.47±0.19^{a}	3.59±0.18^{ab}	4.86±0.13^{a}	2.21±0.16^{a}
Nucleotide	4.05±0.30^{a}	4.20±0.25^{a}	4.35±0.18^{ab}	1.93±0.19^{ab}
DNA	3.34±0.18^{bc}	3.02±0.30^{bc}	3.83±0.23^{bc}	1.41±0.19^{bc}
Experiment 3				
Control	3.34±0.18	2.45±0.32	3.69±0.25	1.68±0.16
Vitamin C	3.18±0.25	2.96±0.19	3.94±0.27	1.62±0.25
Vitamin E	3.46±0.19	3.06±0.19	3.69±0.25	2.03±0.23
Nucleotide	3.41±0.27	3.22±0.16	3.72±0.16	1.92±0.19
DNA	3.04±0.45	2.81±0.33	3.29±0.26	1.77±0.23

$^{a, b, c,}$ values within a column followed by different superscripts denotes significantly different variations ($P<0.05$).

Table V.- Effect of supplementing immune modulators on merceptoethanol-2 (ME) resistant IgG antibody titers (reciprocal of log_2) of broiler chickens (mean±SE).

Treatment	Week 3	Week 4	Week 5	Week 6
Experiment 1				
Control	1.36±0.27	1.36±0.27	1.57±0.31	3.13±0.31^{b}
Vitamin C	1.49±0.26	1.86±0.27	1.80±0.33	4.20±0.25^{ab}
Vitamin E	1.68±0.35	1.93±0.19	2.21±0.32	4.29±0.32^{a}
Nucleotide	1.49±0.26	1.44±0.32	1.41±0.19	3.94±0.44^{ab}
DNA	1.41±0.19	1.71±0.30	1.71±0.30	3.13±0.38^{ab}
Experiment 2				
Control	1.41±0.38	1.49±0.26^{b}	1.41±0.19	2.91±0.35^{b}
Vitamin C	1.62±0.25	1.77±0.23^{bc}	1.96±0.30	3.80±0.30^{ab}
Vitamin E	1.93±0.35	2.33±0.33^{b}	1.86±0.27	4.11±0.13^{a}
Nucleotide	1.93±0.35	1.41±0.19^{b}	1.80±0.44	3.92±0.33^{ab}
DNA	1.96±0.30	1.71±0.30^{bc}	1.62±0.40	3.39±0.33^{ab}
Experiment 3				
Control	1.30±0.18	1.54±0.18	1.41±0.19	2.58±0.31
Vitamin C	1.30±0.18	1.41±0.19	1.19±0.16	2.63±0.40
Vitamin E	1.42±0.19	1.49±0.26	1.49±0.26	2.81±0.23
Nucleotide	1.36±0.27	1.30±0.18	1.41±0.19	2.39±0.42
DNA	1.19±0.25	1.36±0.27	1.62±0.25	2.58±0.31

$^{a, b, c,}$ values within a column followed by different superscripts denotes significantly different variations ($P<0.05$).

DISCUSSION

Supplementing feeds with vitamin E, vitamin C, nucleotides and other renowned immune modulators can hinder the adverse consequences of compounds or elements created by some of the feed ingredients. It can also reduce the stresses produced due to heavy stocking densities of chicks during their rearing periods, transportation or any other environmental factor. This supplementation may be beneficial not only for their growth performance and improving feed efficiency but also can develop their physiological and immunological status by reducing hostile anti-oxidant effects of broiler diets (Lohakare *et al*., 2005; Falk *et al*., 2018). The findings of this study are in agreement with those of Iqbal *et al.* (2001) and Bergman *et al.* (2004) who reported that use of vitamins E and C and selenium showed such optimistic characteristics in fast growing chicks that can noticeably reduce susceptibility to lipid peroxidation in proliferating tissues. This is an effective nutritional tool to deal with several commercial stresses in poultry production that enhances their immune status. The results of current study are also in agreement with other researchers like Zhai *et al.* (2011) and Zhang *et al.* (2015) who reported vitamin E and C as important antioxidants that protect the body from various microbes including bacteria, viruses and parasites. Min *et al.* (2018) and Mohamed *et al.* (2019) also supported an immunomodulatory effect of vitamin E on T cells that may have complimentary effects on the immune function and health of broiler chickens. Habibian *et al.* (2014) reported enhancement of primary and secondary antibody responses while using vitamin E as a feed supplement that also strengthens the findings of our trial. The study of Hossein *et al.* (2018) showed that selenium, a renowned anti-oxidant, like vitamins E and C could improve the immune system through increase in GPx activity. Our studies are in partial agreement with the findings of Bhatti *et al.* (2016), who reported that geometric mean HI antibody titers against NDV remained maximum in groups offered vitamin C as compared to vitamin E, whilst our results showed that vitamin E performed better than vitamin C. Rehman *et al.* (2017) reported that antibody titers against infectious bursal disease was significantly higher in vitamin E supplemented birds compared to the other treatments. The results of present study also in agreement with those of Lin and Chang (2006) who reported that moderate supplementation of vitamin E enhanced immune response for SRBC. Tras *et al.* (2001) reported that vitamin C administration to diets may be useful for broiler breeds due to the observed increased IgG level that also validates the findings of our trial. Habibian *et al.* (2014) and Hossein *et al.* (2018) were of the view that vitamin E and selenium had synergistic effects on anti-SRBC titers and dietary vitamin E significantly increased the antibody response to SRBC. Supplementation with vitamin C promotes the growth and feed efficacy and increases the size of intestinal villi and immunity of heat-stressed broiler chickens (Jahejo *et al*., 2019). However, outcomes of a study by Alizadeh *et al.* (2016) did not validate the findings of our study for improvement in humoral immunity, as they observed that the diet supplemented with yeast cell wall did not increase serum immunoglobulin IgA levels compared with the antibiotic supplemented group. Furthermore, they reported that IgG and IgM levels were not influenced by dietary supplementation with yeast cell wall.

In our study, comparatively heavier lymphoid organ weights were recorded in immune modulator treated groups. Such results were also reported by Wang *et al.* (2013) and Cheng *et al.* (2017) observed that supplementation with vitamin E results in prompt development of lymphoid organs and heavier relative weight of these organs in broiler chicks. Ozpinar *et al.* (2010) reported that an increase in antibody levels in vitamin C supplemented group may be due to rapid differentiation of lymphoid tissues from additional action of the hexose monophosphate pathway that enhances the circulating antibody.

Alizadeh *et al.* (2016) found that supplementing broiler diets with yeast cell wall improved the systemic

innate immune reactions of broiler chickens, and this is in line with the findings of our trial. Yalçin (2013) also reported increased antibody titers to SRBC. Ozpinar *et al.* (2010) suggested that supplementation with Bio-MOS®, vitamin E or vitamin C may not improve the immune response in healthy broilers. Similarly, Hess *et al.* (2012) reported that supplementation with yeast extract and prebiotic in the early age of rearing broiler chickens did not alter the humoral immune response for NDV or infectious bursal disease titers, which contradicts the results of our trial. Differences in these results might be due to variations in species or strain of birds, climate and/or source of the ingredients or supplements used in the studies.

CONCLUSION

In conclusion, it is suggested that vitamin E supplementation as an immune modulator exhibited better immune response followed by vitamin C, nucleotides and DNA. Furthermore, use of immuno-modulators at early age (1 to 21 days) seemed to reveal improved immune response in broiler chickens.

ACKNOWLEDGMENTS

We are highly obliged to Dr. Tariq Javaid, Chief Executive, MediExcel Pharmaceuticals Islamabad, Pakistan for providing technical assistance and provision of materials to conduct these experiments at CASVAB, University of Balochistan.

Statement of conflict of interest

The authors declare no conflict of interest.

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