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EFFECTS OF IMMUNE MODULATORS ON GROWTH AND ECONOMIC PERFORMANCE OF BROILER CHICKENS

I. S. Sheikh[1], T. M. Asmat[1*], M. A. Bajwa[1], M. Z. Mustafa[1], N. Rashid[1], M. Rafeeq[1], M. M. T. Kiani[1], H. M. Ali[2] and A. Raziq[3]

[1] Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan
[2] University College of Veterinary and Animal Sciences, Islamia University of Bahawalpur.
[3] Department of Statistics, University of Balochistan, Quetta, Pakistan.
[*]Correspondence author's email: tauseefcasvab@gmail.com

ABSTRACT

Immune modulators have been found beneficial for growth performance, physiological status, and immune function in broilers. Imbalance between pro-oxidants and anti-oxidants creates oxidative stress in fast growing broiler birds. Immune modulators are capable of reducing oxidative stress produced by excess production of ROS/RNS in rapidly growing body. The current project was designed to study the effects of supplementing different immune modulators on growth performance indices in broiler chicken in 3 separate experiments (n=240 chicks in each experiment). Immune modulators viz. vitamin C (500mg/L), vitamin E (200mg/L), nucleotides mixture (100mg/L) and DNA extract (100mg/L) were provided through drinking water. In the experiment I, immune modulators were offered only from day 1 to 21. In experiment II, immune modulators were supplemented for complete rearing period i.e. from day 1 to 42; whereas in the experiment III, immune modulators were only provided from day 22 to 42. Supplementation of immunomodulators revealed significantly ($P<0.05$) higher live body weight gain and better feed conversion ratio as compared to control group. Over all nucleotides performed more efficiently compared to other used immunomodulators in all three experiments with better feed conversion ratio. It was thus concluded from the study that the provision of immune boosters in starter or finisher phase simultaneously improved production indices and economic performance of broiler chickens.

Key words: growth performance, immune modulators, nucleotides, vitamin C, vitamin E

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INTRODUCTION

Commercial broiler farming is influenced by various environmental, biological and nutritional stressors causing deregulations of physiological/homeostasis processes (Corsello *et al*. 2018; Santoro, 2018) results in compromised physiological and immune status and declined productive performance of broiler chickens (Surai *et al*., 2019). Excessive production of free radicals adversely influences antioxidant defense system, resulting in oxidative stress (Corsello *et al*., 2018; Santoro, 2018). Various feed additives can be supplemented as nutritional elements for enhancing protective competency of birds with beneficial immunomodulatory and stress releasing properties (Kuldeep *et al*., 2014). Generally, farmers and feed manufacturers use antibiotics as growth promoting agents to get better feed efficiency and to minimize the mortality. These practices may result in antibiotic resistant microorganisms' species due to which certain restrictions has been imposed on poultry industry for the judicious use of antibiotics as growth promoters (Rafeeq *et al*., 2017). Strict regulations and increasing consumer concerns regarding the use of antibiotics as growth promoters in the poultry production, have thrown the industry under mounting pressure to limit its use in broiler chicken production before marketing. The denial to the application of antibiotics as growth promoter is leading to higher disease out breaks and declined production performance in the developing countries where still open shed conventional farming system is the common way of broiler production (Huff *et al*., 2013a).

Supplementation with phytochemicals (Valenzuela-grijalva *et al*., 2017), dietary symbiotics (Chen *et al*., 2018), vitamin and minerals (Watson *et al*., 2016) and antioxidants, such as vitamin E, vitamin C (Sheikh *et al*., 2020) have become an essential component of the poultry diets to cope the physiological requirements of stressed birds, that may also work as growth promoting agents, an alternative to antibiotics. Hence, it is a thought-provoking job to develop a method of judicious antioxidant supplementation to support growth in broiler chickens and to sustain effective antioxidant defense and redox balance in the body.

The main source of vitamin E is double lipid cell membrane that is closely resembling to oxidase enzymes,

responsible to produce free radicals (Sahin and Kucuk, 2001; Sahin *et al*., 2003). Main chain-breaking antioxidants particularly polyunsaturated fatty acids protect the cell membranes and tissues from the damage of free radicals (Vlojc *et al*., 2011). Similarly, vitamin C is also found to be useful in inhibiting copper-induced oxidative harm in poultry birds (Ajuwon and Idowu, 2010). Supplementation of such modulators in diets may enhance health conditions and growth performance, enabling economic benefits.

The scenario creates a dire need for finding alternative ways to control the diseases with least dependency on the use of antibiotics. A number of prospective immune modulators are of worth use as substitute to antibiotics that are not only giving growth boost but also defending the birds against the diseases (D'Costa *et al*., 2011). In this regard immunomodulators having antioxidant properties might be a good option that will enhance immune functions (Sheikh *et al*., 2020), improves growth performance and meet quality (Chen *et al*.. 2018). The current study was conceded to compare the role of different immune modulators on growth improvement and their economic impact in broiler chickens.

MATERIALS AND METHODS

Three experiments were conducted simultaneously with day old Hubbard broiler chickens (n=240/experiment) divided into five groups of 48 chicks. Each treatment group was further replicated 4 times (12 chicks/replicate). The group one was kept as control and offered basal diet and plain water (no treatment in drinking water, whereas treatment groups two, three, four and five viz vitamin C (500mg/L), vitamin E (200mg/L), *Saccharomyces cerevisiae* nucleotides (100mg/L) and DNA, extracted from chicken liver (100mg/L) respectively were offered basal diet and immunomodulators in drinking water. The birds were kept under standard management conditions and were offered basal diet i.e. Pre-starter feed during first 12 days, continued with starter feed from day 13th to 25th and thereafter till the end of trial with finisher feed unanimously to all treatment groups. The drinking water was provided *ad-libitum* and the experimental shed temperature was maintained initially at about 95ºF during first week then gradually decreased 5°F/week till 70-75 ºF at 5th and 6th weeks of age. All the experimental birds were vaccinated against Newcastle disease virus (NDV) at 7th and 21st days of age and for infectious bursal disease virus (IBDV) at day 14th and 28th.

In the experiment I, immunomodulators treated water was offered from day 1 to 21 (starter phase). In experiment II immune modulators were offered throughout 1-42 days of rearing period and in experiment III immune modulators treated drinking water was commenced from day 22nd and continued till day 42 (finisher phase). At the end of experiments (day 42nd) 12 birds from each group (3 birds/replicate) were humanely slaughtered. Growth performance parameters; average weight gain (AWG), feed conversion ratio (FCR), feed intake (FI) was evaluated on the weekly basis whereas, dressing percentage (DP) and relative organ weight (ROW) were recorded on day 42 (Owosibo *et al.,* 2013).

Economic efficiency of these immune modulators was evaluated using European Production Efficiency Factor (EPEF) following the methodology reported previously (Huff *et al*., 2013b).

$$EPEF = \frac{\text{Mean Live Body Weight (kg)} \times \text{Livability (\%)} \times 100}{\text{Age} \times \text{FCR}}$$

Statistical analysis: Collected data were analyzed using one-way analysis of variance (ANOVA) using statistical analysis software SPSS 20 for Windows, and to compare means of the treatments by applying Duncan's Multiple Range test. The results were presented as mean ±SE.

RESULTS

Experiment I: The results of experiment 1 are given in Table 1. The weight gain during starter phase of experiment I showed a significant difference ($P<0.05$) among the treatment groups. Highest weight gain (723.92±4.46gm), ADG (34.47±0.21gm) and improved FCR (1.18±0.01) were noted in the group supplemented with nucleotides. In this phase, weight gain of other groups including vitamin C, vitamin E, DNA and control were noted 702.14±3.01, 708.55±7.29, 696.41±11.07 and 681.09±6.15 respectively. During the finisher phase of experiment, I, non-significant effects were observed regarding AWG, ADG and FCR on growth parameters. In experiment I, significant effect on overall growth performance of nucleotide, vitamin C, vitamin E and DNA supplemented groups was observed as compared to control. The effect of immune modulator on relative organ weight (ROW) of liver, gizzard, proventiculous, intestinal length and carcass percentage at the end of experiment was noted non-significant ($P>0.05$) among treatments (Table 4).

Table 1. Body weight gain (gm), feed intake (gm), feed conversion ratio (FCR) and average daily weight gain (ADG) during different phases of Experiment 1.

	Treatments	Weight gain (gm)	Feed intake (gm)	FCR	ADG (gm)
Starter Phase (1-21 days)	Control	681.09±6.15^{c}	866.25±5.39	1.27±0.01^{a}	32.43±0.29^{c}
	Vitamin C	702.14±3.01^{ab}	864.75±7.39	1.23±0.01^{ab}	33.44±0.14^{ab}
	Vitamin E	708.55±7.29^{ab}	861.50±8.11	1.22±0.02^{bc}	33.74±0.35^{ab}
	Nucleotides	723.92±4.46^{a}	855.25±4.52	1.18±0.01^{c}	34.47±0.21^{a}
	DNA	696.41±11.07^{bc}	847.25±8.96	1.22±0.01^{bc}	33.16±0.5^{bc}
Finisher Phase (22-42 days)	Control	1774.03±25.84	2939.75±6.57	1.66±0.02	84.48±1.23
	Vitamin C	1821.33±14.32	2925.75±9.45	1.61±0.01	86.73±0.68
	Vitamin E	1807.19±13.80	2934.75±8.27	1.62±0.00	86.06±0.66
	Nucleotides	1850.53±20.92	2928.75±6.34	1.58±0.02	88.12±0.99
	DNA	1833.58±18.17	2924.50±15.88	1.59±0.02	87.31±0.87
Overall (1-42 days)	Control	2455.12±20.44^{c}	3806.00±9.34	1.55±0.02^{a}	58.46±0.49^{c}
	Vitamin C	2523.48±12.78^{ab}	3790.50±5.87	1.50±0.01^{bc}	60.08±0.33^{ab}
	Vitamin E	2515.74±15.91^{b}	3796.25±12.67	1.51±0.01^{bc}	59.90±0.38^{b}
	Nucleotides	2574.45±17.71^{a}	3784.00±3.08	1.47±0.01^{c}	61.30±0.29^{a}
	DNA	2529.98±12.53^{ab}	3771.75±24.71	1.49±0.01^{bc}	60.23±0.20^{ab}

^{abc} Shows in the same row with different superscripts are significantly different (P < 0.05).

Experiment II: The results of broiler chicken performance of experiment 2 are presented in Table 2. In 2nd experiment, immune modulators were supplemented throughout the rearing period (day 1 to 42). The average weight gain during the starter phase showed a significant difference (P<0.05) among the treatment groups. In starter phase, highest weight gain (724.90±6.84gm), ADG (34.52±0.33gm) and improved FCR (1.19±0.01) were gained in group supplemented with nucleotides while non-significant difference was observed within vitamin C, vitamin E and DNA supplemented groups. During finisher phase, supplementation of vitamin C, vitamin E, nucleotides and DNA showed significant difference (P<0.05) on growth parameters in comparison to control while non-significant difference within all immune modulator supplemented groups. The results of growth indices at the end of the experiment (day 42) showed significant difference (P<0.05) among various treatments. Highest average weight gain (2526.56±15.76gm), ADG (60.16±0.38gm) and lowest FCR (1.50±0.01) was noted in group supplemented with nucleotides while non-significant difference in comparison to other immune modulators used in the experiment. The impact of immune modulator on ROW (Table 4) during the experiment was also non-significant (P>0.05).

Experiment III: In the 3rd experiment, immune modulators were supplemented in drinking water only during finisher phase i.e. from day 22nd to 42nd. The results of growth parameters are presented in Table 3. Initially, no significant differences in WG, FI, FCR and ADG were noted during starter phase. While at the end of the finisher phase, treatment groups supplemented with immunomodulators showed significantly (P<0.05) higher WG, ADG and improved FCR compared to control group. However, immunomodulators supplemented groups revealed a non-significant (P>0.05) difference with each other. Higher WG (1767.87±24.14), ADG (84.18±1.15) and FCR (1.66±0.03) were observed in vitamin E supplemented group. The results regarding the effect of immune modulators on intestinal length, carcass percentage and ROW of liver, gizzard, and proventiculous, revealed a non-significant (P>0.05) difference among groups (Table 4).

Economic performance: Economic efficiency of these immune modulators was evaluated in terms of European Production Efficiency Factor is given in Table 5. In all three experiments highest EPEF values 384, 384 and 357 were observed in experiment I, II and III respectively in nucleotide supplemented groups whereas lowest EPEF values (341, 337 and 300) were observed in control groups.

Table 2. Body weight gain (gm), feed intake (gm), feed conversion ratio (FCR) and average daily weight gain (ADG) during different phases of Experiment II.

	Treatments	Weight gain (gm)	Feed intake (gm)	FCR	ADG (gm)
Starter Phase (1-21 days)	Control	685.38±5.82^{c}	868.75±3.04	1.27±0.01^{a}	32.64±0.28^{c}
	Vitamin C	701.64±4.80^{ab}	870.25±7.25	1.24±0.01^{ab}	33.41±0.23^{bc}
	Vitamin E	716.92±5.54^{ab}	869.00±5.08	1.21±0.02^{bc}	34.14±0.26^{ab}
	Nucleotides	724.90±6.84^{a}	860.00±3.08	1.19±0.01^{c}	34.52±0.33^{a}
	DNA	713.03±4.37^{ab}	863.00±10.59	1.21±0.02^{bc}	33.96±0.21^{ab}
Finisher Phase (22-42 days)	Control	1736.55±10.58^{b}	2938.25±10.87	1.69±0.01^{a}	82.69±0.50^{b}
	Vitamin C	1801.38±22.35^{a}	2941.25±5.66	1.63±0.02^{b}	85.78±1.06^{a}
	Vitamin E	1801.20±16.53^{a}	2938.75±5.27	1.63±0.01^{b}	85.77±0.79^{a}
	Nucleotides	1804.66±13.62^{a}	2948.25±9.42	1.63±0.02^{b}	85.79±0.65^{a}
	DNA	1802.65±15.93^{a}	2950.00±10.21	1.63±0.01^{b}	85.84±0.76^{a}
Total (1-42 days)	Control	2421.93±6.40^{b}	3807.00±8.80	1.57±0.00^{a}	57.67±0.15^{b}
	Vitamin C	2503.02±18.29^{a}	3811.50±9.97	1.52±0.01^{b}	59.60±0.44^{a}
	Vitamin E	2518.12±21.0^{a}	3807.75±2.10	1.51±0.01^{b}	59.95±0.50^{a}
	Nucleotides	2526.56±15.76^{a}	3808.25±3808	1.50±0.01^{b}	60.16±0.38^{a}
	DNA	2515.81±15.97^{a}	3813.00±17.76	1.51±0.01^{b}	59.90±0.38^{a}

^{abc} Shows in the same row with different superscripts are significantly different ($P < 0.05$).

Table 3. Body weight gain (gm), feed intake (gm), feed conversion ratio (FCR) and average daily weight gain (ADG) during different phases of experiment III.

	Treatments	Weight gain (gm)	Feed intake (gm)	FCR	ADG (gm)
Starter Phase (1-21 days)	Control	667.83±6.35	867.25±4.94	1.29±0.02	31.80±0.30
	Vitamin C	680.31±6.68	868.25±13.31	1.28±0.03	32.40±0.32
	Vitamin E	692.68±6.18	865.00±6.79	1.25±0.02	32.98±0.29
	Nucleotides	686.63±14.22	852.00±5.45	1.24±0.02	32.70±0.68
	DNA	675.33±13.13	843.00±8.88	1.25±0.03	32.16±0.63
Finisher Phase (22-42 days)	Control	1677.38±6.50^{b}	2953.00±4.26	1.77±0.00^{a}	79.87±0.31^{b}
	Vitamin C	1754.45±7.82^{a}	2942.75±14.63	1.68±0.01^{b}	83.55±0.37^{a}
	Vitamin E	1767.87±24.14^{a}	2931.25±8.87	1.66±0.03^{b}	84.18±1.15^{a}
	Nucleotides	1755.71±14.26^{a}	2963.50±1.94	1.69±0.01^{b}	83.61±0.68^{a}
	DNA	1756.37±13.54^{a}	2949.00±6.52	1.68±0.01^{b}	83.64±0.64^{a}
Total (1-42 days)	Control	2345.21±11.92^{b}	3820.25±6.57	1.63±0.01^{a}	55.84±0.28^{b}
	Vitamin C	2434.75±10.27^{a}	3811.00±14.46	1.57±0.01^{b}	57.97±0.24^{a}
	Vitamin E	2460.55±20.70^{a}	3796.25±12.70	1.54±0.02^{b}	58.58±0.49^{a}
	Nucleotides	2442.34±12.38^{a}	3815.50±4.25	1.56±0.01^{b}	58.15±0.29a^{a}
	DNA	2431.70±3.62^{a}	3792.00±10.23	1.56±0.01^{b}	57.90±0.09^{a}

^{ab} Shows in the same row with different superscripts are significantly different ($P < 0.05$).

Table 4. Relative weights (%) of body organs of Experiment I, Experiment II and Experiment III under the effects of different treatments.

	Treatments	Liver	Heart	Gizzard	Proventiculous	Intestine	Carcass Percentage
I	Control	0.0233±0.0008	0.005±0.0005	0.025±0.001	0.003±0.0002	6.719±0.29	62.20±0.509
	Vitamin C	0.0225±0.0009	0.0048±0.003	0.025±0.002	0.0048±0.0009	7.689±0.32	61.14±0.638
	Vitamin E	0.0218±0.0007	0.005±0.0004	0.022±0.002	0.003±0.0003	7.56±0.61	62.56±0.194
	Nucleotides	0.0228±0.0016	0.0048±0.0003	0.0266±0.002	0.0039±0.0003	7.72±0.27	62.69±3.279
	DNA	0.023±0.0009	0.005±0.0001	0.026±0.002	0.0040±0.0002	7.032±0.20	61.28±0.896
II	Control	0.022±0.001	0.0043±0.0002	0.0282±0.001	0.005±0.0002	7.29±0.36	60.11±0.540

	Vitamin C	0.023±0.001	0.0042±0.0003	0.027±0.001	0.005±0.0006	8.17±0.44	61.76±0.556
	Vitamin E	0.023±0.001	0.0043±0.0005	0.026±0.002	0.004±0.0005	8.51±0.97	61.04±1.177
	Nucleotides	0.020±0.002	0.0048±0.0007	0.0238±0.001	0.0038±0.0004	7.18±0.39	60.67±1.003
	DNA	0.025±0.002	0.0046±0.00	0.0267±0.0019	0.0043±0.0005	8.15±0.38	61.29±1.992
	Control	0.020±0.001	0.0044±0.0005	0.023±0.002	0.004±0.0002	7.08±.0045	61.20±0.630
	Vitamin C	0.020±0.0004	0.0046±0.0003	0.022±0.001	0.004±0.004	7.60±0.50	62.49±0.850
III	Vitamin E	0.0230±0.003	0.004±0.0002	0.027±0.002	0.004±0.0002	9.38±1.66	62.17±0.929
	Nucleotides	0.025±0.002	0.0046±0.0003	0.022±0.001	0.004±0.0003	8.06±0.62	61.67±2.420
	DNA	0.023±0.0009	0.004±0.0003	0.023±0.002	0.004±0.0003	7.84±0.19	63.94±0.314

Table 5. Evaluation of Economic effects of immune modulators using European Production Efficiency Factor (EPEF).

Treatments	EFEP Experiment 1	EFEP Experiment 2	EFEP Experiment 3
Control	341	337	300
Vitamin C	364	359	346
Vitamin E	381	381	357
Nucleotides	384	384	357
DNA	377	372	348

EPEF=Mean Live Body Weight (kg) ×Livability (%)/Age*×FCR×100
*42 days in these experiments

DISCUSSION

In experiment I, II and III, significant effect ($P<0.05$) of immune modulators on growth performance (AWG, ADG and FCR) was noted as compare to control. Amongst supplements (nucleotide, vitamin C, vitamin E and DNA), best growth performance was observed in nucleotides groups simultaneously in all three experiments. The results of the present experiments are in accordance with the findings of many researchers (Kejie *et al*., 2014; Suganya *et al*., 2015; Cheng *et al*., 2017; Hossain *et al*., 2018), reporting that the dietary supplementation of vitamin E showed positive impact on productive performance, weight gain and FCR of broiler chickens. Vitamin E supplemented broiler chicken diets may decrease stress by reducing the catabolic reaction of the body and, in that way, spare nutrients otherwise utilized for combating the stress and enhanced growth and increased body weight (Bhatti *et al*. 2016;).

The findings are also in agreement with the observations of Ismail *et al*. (2014); Bhatti *et al*. (2016); and Selvam *et al*. (2017) whom supplemented vitamin E in the diet of broiler chickens and noted improvement in growth, compared to the birds not offered vitamin E, whereas, vitamin E supplementation showed non-significant effect on feed intake (Ismail *et al*., 2014). In the same context, higher daily gain, superior body weight, better FCR were observed in broiler chicken supplemented vitamin C diet compared to control group (Kutlu and Forbes 1993[1]; Kutlu and Forbes 1993[2]; Sahin *et al*., 2003; Imik *et al*., 2012; Motasem, 2012; Zeferino *et al*., 2015). Similarly, the addition of vitamin C and vitamin E in diet showed enhanced growth performance in broilers. Sheikh *et al*., (2020); Khattak *et al*., (2012) and Lohakare *et al*. (2005) reported that feed supplemented with ascorbic acid have strong economic impact as well as improvement in growth performance of broilers.

Contrary to our results, addition of dietary ascorbic acid in combination with vitamin E as feed supplement did not improved body weight gain and FCR (Ali *et al*., 2010; Jang, 2014; Ali, 2012). Surprisingly, significant reduction in feed intake and improved body weight and feed conversion ratio was observed with vitamin E in diet (Habibian *et al*., 2016; Habibian *et al*., 2014; Leskovec *et al*., 2018). These differences in results might be due to variations in the experimental duration, management practices adopted during rearing of birds, source of vitamins and nucleotides, strain of broiler breed and also the environmental conditions that were maintained during the experiments (Baracho *et al*., 2018). Endogenous production of nucleotides might become inadequate because a considerable energy is essentially required for de novo production of nucleotides (Hess *et al*., 2012; Wu *et al*., 2018). In the present study, supplementation of nucleotides significantly improved the growth performance indices of birds and showed higher WG (4.62% and 4.14%) experimental groups I, II and III respectively than the control. Similar to these findings, previously the supplemented yeast nucleotide improved live weight gain and FCR during early growth phase (1 to 10) compared to that of control birds (Grimble *et al*., 2001; Morales *et al*., 2010; Yalçin *et al*., 2013;Huff *et al*., 2013b). Similar results have been reported by several other researchers (Esteve *et al*., 2007; Chiofalo *et al*., 2011). However, observations of Alizadeh *et al*., (2016) are contrary to our findings who reported non-significant effect of nucleotide

supplementation on broiler chicken growth performance. The variations might be due to the source of nucleotides used in the experiment (Esteve *et al*., 2007). Addition of immune modulators had non-significant effect on relative organ weight of liver, gizzard, proventiculous, and intestinal length and carcass percentages in all three experiments. Findings are in hand with other scientists (Hernandez *et al*., 2004). Islam [1] *et al*., 2004 used probiotics and plants extract in their studies and observed no significant effect on visceral organs. Furthermore, improvement in growth performance of broiler chicken directly or indirectly with the supplementation of immune modulators might be related to improved immune status of the birds, reduced nutrient lose that might have been used to maintain certain immune status (Sarangarajan *et al*., 2017; Niki, 2015; Islam [2] *et al*).

Conclusions: Based on results, it can be concluded that the supplementation of immune modulators either in the starter or finisher phase had positive impact on growth parameters which ultimately results in better profit margins.

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