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Research Article

Effects of Feeding Anti-stressor and Immunizer to Broiler Chickens on Growth Performance, Meat Yield and Immunity

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ABSTRACT

To examine the influence of anti-stressor and immunizer on the growth of broiler chicken, their meat yield features, and immune response, a total of 360 Cobb 500 day-old commercial broiler chicks were used in a 35-day feeding trial. Experimental birds were randomly assigned to three dietary treatments having six replications each of 20 birds. During the starter and grower periods, corn-soya-based plant protein mash diets were provided. Starter and grower diets were provided in two phases: day-old to 21 days and 22-35 days, respectively. The first group of chicks was treated as control, the second group was offered a control diet supplemented with an anti-stressor and the third group received a control diet to which an immunizer was incorporated. Growth performance data were recorded weekly. Meat yield traits were determined and blood samples were collected on termination of the experiment to determine antibody titer. Data were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD). Results showed that significantly higher body weight and lowest FCR values were found in the anti-stressor incorporated group ($P<0.05$). Giblet content was significantly ($P<0.01$) increased due to addition of anti-stressor and immunizer in the diet. The immunizer supplemented group significantly improved antibody titer against Infectious Bursal Disease ($P<0.05$). It was therefore concluded that both anti-stressor and immunizer as used in this study could be considered to improve growth performance, giblet weight and immunological response.

Introduction

Animal proteins are superior and come principally from eggs, meat, milk, and fish. It is assumed that the demand for poultry meat will be doubled in the world by 2050 (Herrero et al., 2015). The poultry nutritionists put endeavors to fulfill this demand by supplying safe meat and eggs originated from poultry. But environmental stress resulting from climate change, particularly the heat stress hinders poultry food production during summer. When tropical and subtropical areas are taken into consideration, heat stress is the major cause of lower productivity of broilers (Moustafa et al., 2021). The high environmental temperature along with high humidity reduces the birds' growth rate, and meat quality while affecting intestinal histology, antioxidant status, and immunity (Lara and Rostagno, 2013; Xie et al., 2014). Recently, the environmental impact on poultry production has received much attention from poultry researchers. Generally, heat-tolerated breed, environment-controlled shed, and nutritional manipulation are effective tools to combat heat stress. But constructing an automated poultry shed is very costly and the development of a heat tolerant breed is time-consuming. Conversely, nutritional strategy is being more viable during heat stress to ensure productivity (Vandana et al., 2021). These strategies are helpful in order to balance the needs of stressed birds for amino acids, energy, and electrolytes. Several feed additives are being considered for inclusion in poultry diets with the objective of preventing animals from different kinds of environmental stresses as well as for enhancing feed quality, growth performance, palatability, and productivity (McDonald et al., 2010). Presently, several

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biotechnological tools have been developed and are being applied to ameliorate heat stress. Of them, several kinds of anti-stressors, immunizers, probiotics, prebiotics, acidifiers, phytobiotics, herbs and, enzymes are widely used in poultry feeding. Some of these products have shown promising effects on production by combating heat stress (McDonald et al., 2010). Among these, anti-stressor and immunizers as feed additives, have a great impact on improving broiler growth performance, meat yield, and boosting immunity (Bortoluzzi et al., 2021; He et al., 2021). Different kinds of anti-stressors have beneficial effects on broiler growth, productivity, and immune response. They are treated to be effective in reducing the harmful impact of heat stress (Hosseini et al., 2012). This anti-stressor alleviates the production of corticosterone and malondialdehyde during heat stress and neutralizes free radicals that are being produced in the cell wall of heat-stressed birds (Ajakaiye et al., 2010; Musa-Azara et al., 2012). Immunizers are the biological or synthetic substances that can stimulate/suppress either innate, adaptive, or both arms of the immune system. Immunizers have antimicrobial properties with no direct effect on microbes but vacate the problem of the rapid incarnation of resistance and thus invigorate the immune system (McDonald et al., 2010). Vitamin A, D, E & B complex, zinc, chromium, betaine, propils and several other bioactive components, either singly or in a combination of two or more are being normally incorporated in commercially available immunizers and anti-stressors. Application of immunizers in feed or its water treatment reduces heat stress, because they stimulate immune cells and enhance their phagocytic activity against various microorganisms like bacteria, viruses, and other foreign particles (Niu et al., 2009; Bahrami et al., 2012). Heat stress hampers the immunity of broiler by reducing the capability of antibody production against specific diseases and changes heterophil/lymphocyte (H/L) ratio (Prieto and Campo, 2010; Soleimani et al., 2011), resulting in high mortality and lower production performance of broiler. To authors' knowledge, anti-stressors and immunizers considered for inclusion in broiler diets in Bangladesh are not normally examined experimentally for their efficacy, hence trial results are scanty. Furthermore, because of variation in composition, it is quite unlikely that results will be similar for all available materials irrespective of their source. Therefore, the objective of this study was to investigate whether feeding a commercially available anti-stressor and an immunizer to broilers affect their growth performance, meat yield traits, and the status of antibody titer against Infectious Bursal Disease (IBD) during the summer when the possibility of heat stress naturally existed.

Materials and Methods

Animal Ethics

The birds were handled in accordance with the guidelines of Animal Welfare and Experimentation Ethics Committee at Bangladesh Agricultural University, Mymensingh, Bangladesh.

Experimental design and diets of birds

Three hundred sixty, one-day-old Cobb 500 straight-run broiler chicks were procured from a commercial hatchery. Three different diets were considered for comparison. Each group contained a total of 120 birds and was further divided into six sub-groups. Corn-soybean meal-based mash diets were formulated satisfying the requirements of nutrients (metabolizable energy, crude protein, calcium, available phosphorus) close to recommendation for Cobb 500 (Vantress, 2018). Birds of different treatment groups received control (Basal diet), Basal diet supplemented with an anti-stressor, 100g/100kg (composition: analogin, Vitamin C, Nacl, KCl, sodium salicylate, calcium gluconate, zinc oxide, betain, aspirin as stated by the manufacturer) and Basal diet to which added an immunizer, 100g/100kg (composition: *Bacillus* sp., *Lactobacillus* spp., *Saccharomyces cerevisiae*, amylase, lipase, protease, glucanase, phytase, yeast, glucans, glycine, lysine, methionine vita A, E & C, antioxidants as stated by the manufacturer). Table 1 shows the ingredient and nutrient composition of the basal diet.

Table 1. Ingredient and nutrient contents of control diet (kg/100kg)

Ingredients (%)	Feeding Period (0-35 days)	
	Starter diet (0 to 21 days)	Grower diet (22 to 35 days)
Yellow corn	58.17	62.20
Soybean meal (44%)	28.00	25.75
Protein concentrate (62%)	8.00	5.75
Soybean oil	3.00	3.60
Limestone	1.20	1.00
Di-calcium phosphate	0.40	0.50
Salt	0.25	0.30
Sodium-bi-carbonate	0.10	0.08
Broiler premix[1]	0.15	0.15
DL-Methionine	0.28	0.22
Blend acidifier	0.15	0.15
L-lysine	0.10	0.10
Shark liver oil	0.10	0.10
Choline chloride-60	0.10	0.10
Nutrient composition (%)		
ME, kcal/kg	3000	3100
Crude protein	22.0	20.0
Lysine	1.36	1.07
Cystine	0.40	0.31
Methionine	0.50	0.45
Methionine+cystine	0.90	0.76
Calcium	0.90	0.90
Available phosphorus	0.45	0.45

ME=Metabolizable energy, kcal=kilocalorie, kg=kilogram,

[1]Each 2.5kg broiler premix contained: Vitamin A 12.50 MIU, Vitamin D 2.50 MIU, Vitamin E 25g, Vitamin K 4g, Iron 24g, Zinc 40g, Manganese 48g, Selenium 0.12 g and Cobalt 0.30g. Blend acidifier contained formic acid 65 %, sodium 32% and silica 3 %.

Housing and management

The feeding trial was conducted in a housing environment where the ambient temperature ranged from 28-34°C naturally during summer. The peak temperature persisted for five consecutive days although the fluctuation in temperature at the other time of rearing was also observed. Before the arrival of the chicks, the house was properly cleaned, washed, disinfected, and dried. The floor was washed with bleaching powder. All feeders, waterers, plastic buckets, and other necessary equipment were properly cleaned, washed, and disinfected thoroughly with a disinfectant (TH4+ solution, manufactured by-Sogeval, France). Fresh and dry rice husks were used as litter materials at a depth of about 3cm. The birds were exposed to 23 hours of light and 1 hour of darkness each day. For the first 14 days, each pen was provided with a round feeder and a small drinker. Small drinkers were replaced by large round drinkers on day 15. While feed was offered to chicks more frequently (six times a day) at the early stage of growth (seven days), serving was reduced to three times in such a way that birds had always free access to feed except at the time of high temperature (32^0C or more). During hot weather, feeding was practiced during the cooler part of the day, mostly in the early morning and at late night). Birds of all groups received similar management. Strict biosecurity and stipulated vaccination program against the common viral diseases of broiler were followed. Birds were vaccinated against New Castle Disease (ND) on day 5. Vaccination for IBD was performed on day 10 and a booster dose was applied on day 17. Vaccination with a booster dose of ND was done on day 21. An automatic thermo-hygrometer was hung at chick level to record the room temperature and relative humidity (RH) four times a day (7:00 AM, 1:00 PM, 7.00 PM, and 1.00 AM).

Dressing parameters

For the determination of dressing parameters, six birds from each treatment (one bird per replication), close to average weight, were selected at the end of the trial (35 days). Following sacrifice, each bird was allowed to bleed for five minutes and then kept immersed in hot water (51-55˚C) for 120 seconds in order to lose the feathers. After de-feathering, the breast, thigh (meat+bone), drumstick, wing, heart, liver, gizzard and giblet of dressed broilers were weighed and documented.

Blood sample collection and antibody titer determination

Blood sample was taken from the jugular vein at the time of animal sacrifice for meat yield traits. Eighteen samples comprising six from each treatment were taken in test tubes and properly marked in each case. The samples were then centrifuged for five minutes at 3000 rpm to obtain more clear serum from blood. The serum was transferred to a screw-capped serum vial and stored at -20°C. The antibody titer was determined using the Enzyme-Linked Immune Sorbent Assay (ELISA). The method reported by De Wit et al. (2001) was followed.

Statistical analysis

Data collected from different treatment groups were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) employing a statistical computer package program (SAS, 2009). Duncan's Multiple Range Test (DMRT) was performed to compare mean values where ANOVA showed significant difference (P<0.05 or better).

Results

Growth performance

Figure 1, 2 and 3 depict the effects of anti-stressor and immunizer on growth performance. The anti-stressor-treated birds had the highest body weight and body weight gain. When compared to the immunizer and control groups, final body weight and body weight gain were significantly improved (P<0.05) in the anti-stressor treatment (T_2) group. The immunizer group did not show any significant difference in weight gain as compared to the control group (Figure 1). A numerically higher value of weight gain was observed in the anti-stressor fed group as compared to immunizer receiving birds. There was no significant difference in feed consumption, although a higher trend was observed in the anti-stressor supplemented group (Figure 2). The FCR in the anti-stressor fed group outperformed the control group significantly (Figure 3, P<0.05). The anti-stressor fed group had no mortality, while the other two groups had a survival rate of 99%, which was treated to be highly satisfactory.

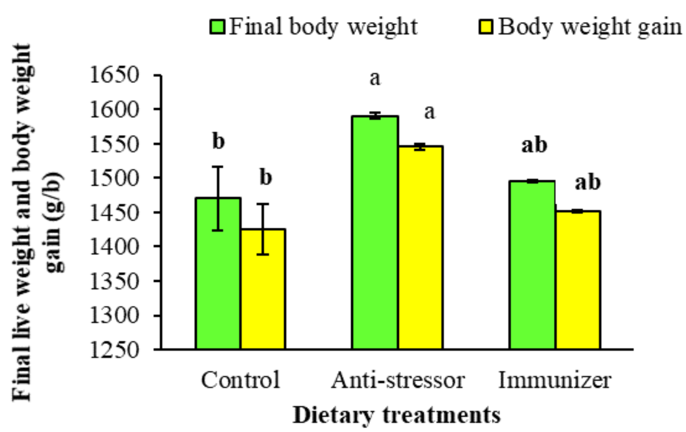

Figure 1. Final body weight and body weight gain of birds fed anti-stressor and immunizer at 35 days: g/b= gram per bird, a, b= superscript, mean values of dietary treatments bearing not a common alphabet as superscript differ significantly (p<0.05). Bar indicates standard error of mean (SEM).

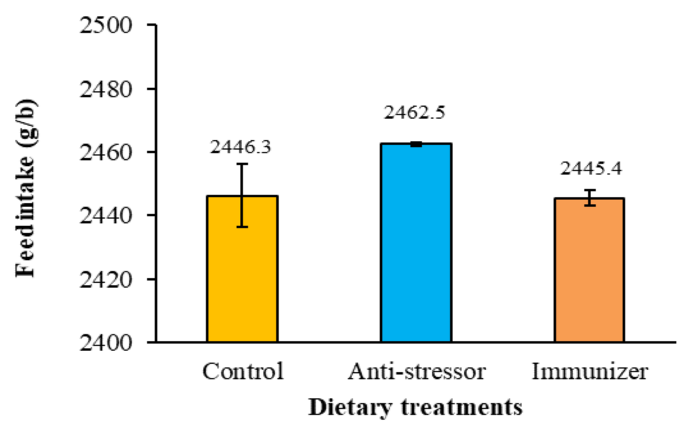

Figure 2. Feed intake of birds fed anti-stressor and immunizer (0-35 days): g/b=gram per bird, Bar indicates standard error of mean (SEM). Differences in mean values among dietary treatments were not significant (P>0.05).

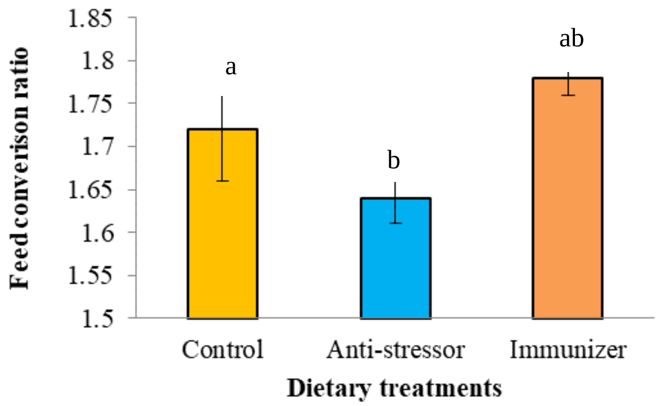

Figure 3. Feed conversion ratio of birds fed anti-stressor and immunizer: a, b= superscript, means bearing superscript not a common alphabet among dietary treatments differ significantly (p<0.05). Bar indicates standard error of mean (SEM).

Dressing parameters

The effects of feeding anti-stressor and immunizer on dressing parameters are shown in Table 2. Anti-stressor and immunizer-treated birds had the highest giblet value, with a significant difference from the control group (P<0.01). However, no significant differences in other dressing parameters (dressing yield, thigh (meat+bone), drumstick, breast, wing, liver, and gizzard) were observed among the treatment groups.

Antibody titer level

Antibody titer levels were found to differ significantly (P<0.01) among the dietary groups (Figure 4). In both the immunizer and anti-stressor treatment groups, birds had the higher antibody titer against IBD, as would be expected. The control group had the lowest antibody titer value.

Table 2. Effect of feeding anti-stressor and immunizer on dressing parameters of broiler at 35 days

Variables (% of body weight)	Dietary treatments			P-value
	Control	Anti-stressor	Immunizer	
DP	74.3±0.14	77.9± 1.82	76.9± 1.06	0.17
Thigh(meat+bone) weight	14.8±0.49	15.4± 0.25	15.3± 0.26	0.44
Drumstick weight	11.9±0.33	12.4± 0.41	12.2± 0.13	0.57
Breast weight	29.1±0.24	30.6± 0.99	30.2± 0.89	0.40
Wing weight	10.3±0.06	10.7± 0.45	10.5± 0.19	0.64
Heart weight	0.6±0.03	0.6± 0.02	0.6± 0.01	0.68
Liver weight	3.3±0.06	3.4± 0.21	3.5± 0.06	0.65
Gizzard weight	2.6±0.02	2.8± 0.20	2.7± 0.04	0.68
Giblet weight	6.4^{b} ±0.03	6.7^{a} ± 0.03	6.7^{a} ± 0.03	0.01

DP=Dressing percentage, a, b: superscript, means bearing dissimilar superscript in a row differ significantly at the stated level of probability, (±) indicates standard error of mean (SEM)

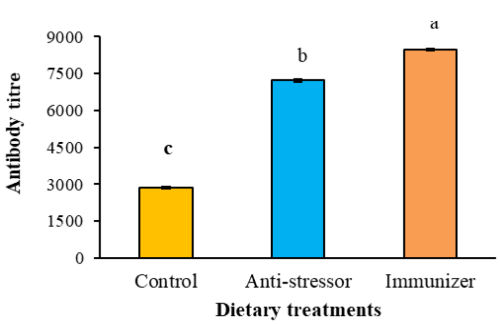

Figure 4. Antibody titre (AT) against IBD as obtained by ELISA test at 35 days of age: a,b= superscript, mean values among dietary treatments bearing dissimilar superscript differ significantly (p<0.05). Bar indicates standard error of mean (SEM).

Discussion

Birds receiving diets supplemented with anti-stressor showed positive results on body weight and body weight gain, feed intake, and FCR compared to the control diet. The anti-stressor had antioxidant, osmoregulatory functions, and digestion-enhancing properties that regulated the homeostasis of birds even in stress conditions and thus improved the growth performance of broilers. This was in agreement with the report of Nadeem et al. (2014), who stated that anti-stressor containing chia seed (Tiny black *seeds* of the *chia* plant; *Salvia hispanica*) have anti-oxidant properties which inhibited the production of free radical during heat stress and enhanced feed intake. Several other researchers (Kettunen et al., 2002; Khattak et al., 2012 and Yago et al., 2013) also reported similar results indicating that anti-stressors such as betaine have osmoregulatory properties that lower the dehydrated condition of birds and increase the concentration of hydrochloric acid in the stomach. This has resulted in better digestibility of birds by enhancing utilization of nutrients, which aided in increasing body weight and better FCR of broiler. The anti-stressor used in this study also contained betaine. Attia et al. (2014) found that vitamin C (ascorbic acid) containing anti-stressor in broiler diet at a level of 250 mg/kg, reduced the effects of heat stress on birds. It also improved feed intake, and reduced FCR. The finding of Borges et al. (2004) was that feed supplemented with different types of anti-stressor like ascorbic acid, vitamin E, and potassium chloride enhanced body weight gain of birds which agreed with our result. The tested anti-stressor also contained ascorbic acid and potassium chloride.

Both anti-stressor and immunizer showed positive effects on giblet yield of broiler chickens. Diets containing increasing doses of betaine improved giblet yield in this study which closely matched the findings of Attia et al. (2005). Ascorbic acid in immunizer also showed a numerical improvement of breast meat yield (Lohakare et al*.,* 2005) similar to our results. Research results of Behboodi et al. *(*2021) showed a significant increase in thigh meat and numerically greater breast meat yield when a mixture of betaine, vitamin C, St John's wort (an effective dietary supplement for treating various nervous system related disorders), lavender, and *Melissa officinalis* extracts were fed to commercial broilers. Both the anti-stressor and the immunizer used in the current study contained betaine and vitamin C.

Antibody titers were higher in the immunizer-treated group compared to the anti-stressor and control groups, as would be expected. Immunizer and anti-stressor were such feed additives that have immune-modulatory, antioxidative, antibacterial, antiviral and antifungal properties. Immunizer improved antibody titre of birds against IBD, similar to the findings of Newairy et al. (2009) and Orsatti et al. (2010). Immunizer enhances the phagocytic activity of birds that boost immunity (Cetin et al., 2010; Shreif and El-Saadny, 2016) which was in agreement with our results. Anti-stressor that contains substances like Zn and vitamin C (ascorbic acid) when supplemented in the diet probably enhanced immune cells activity thus improved ELISA titers against IBD of broilers in hot weather condition (32-34^{0}C). This result coincided with the results of several authors (Amakye -Anim et al., 2000; Bartlett and Smith, 2003; Lohakare et al., 2005).

The result of this study clearly indicated that anti-stressor and immunizer had performance-enhancing effects. Both the tested ingredients were composed of several well recognized anti-stressor elements, as already mentioned, that might have improved performance and immune response against IBD. In the current study, no artificial heat stress was induced for the experimental birds. Rather, they were reared in such a housing environment that hot weather naturally exposed them to high internal house temperature (34˚C maximum) and high humidity (75% RH maximum) intermittently causing a stressful condition during the course of the experimentation. It appears that the fluctuating temperature (28 to 34°C) and humidity (55% to75% RH) caused discomfort affecting the performance more or less for all birds but the treated groups could overcome this situation to an extent that allowed the birds to perform better. Thus, the tested ingredients could be considered for feeding broiler chicks during natural stressful conditions resulting from the high ambient temperature and high humidity and more importantly at the time of fluctuating temperature in summer.

Conclusion

Anti-stressors in broiler diets were found to have promising effects on growth and feed conversion. Immunizers may also be used to boost immunity. Anti-stressor and immunizer had positive effects on giblet yield. It was therefore concluded that both anti-stressor and immunizer as used in this study, could be considered to improve growth performance, giblet weight and immunological response. Enhanced performance in terms of weight gain, FCR and satisfactory survival rate are the advantages derived from this experiment. Whether or not these benefits outweigh the increased production costs associated with the inclusion of an anti-stressor and an immunizer will require further research involving a comparatively larger flock of broiler chickens.

Authors contribution

SDC, AK, BCR[1], BCR[2] and SMSG were involved in the conception and design of the experiment. AK, BCR[1] and BCR[2] collected data and were involved in laboratory tests. AK analysed data while SDC, AK, BCR[1] and BCR[2] interpreted the results. SDC and AK contributed to the initial drafting and revision of the article while all authors have gone through it and approved the final version for publication.

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Conflict of interest

None to declare.

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