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Comparative Efficacy of Supplementation of Natural (*Citrous limon* Juice), Herbal and Synthetic Vitamin C on the Immune Response of Broiler Chicken During Summer Stress

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Abstract: The hematological and immunological study was undertaken on 488 Ven- cobb straight run commercial broiler chicks for period of one week divided into four treatments viz., T_0, T_1, T_2 and T_3 on the onset of fifth week of age. The treatment T_0 was the control without supplementation of vitamin C. The birds in treatment T_1, T_2 and T_3 were supplemented with 5 mL *Citrus limon* juice, 2 mL herbal vitamin C and 1 g synthetic vitamin C per 100 birds, respectively through drinking water. The results of the study indicated that Heterophill percentage was significantly ($p \leq 0.05$) decreased in treatment T_1, T_2 and T_3 as compared to control T_0. Similarly, the Lymphocyte percentage was significantly ($p \leq 0.05$) increased in treatment T_1, T_2 and T_3 than the control T_0. The Heterophil: Lymphocyte ratio was significantly ($p \leq 0.05$) lower in treatment T_1 followed by T_3, T_2 and T_0. The weight of bursa of fabricious and spleen was significantly ($p \leq 0.05$) higher in T_1 followed by T_3, T_2 and T_0. The rectal temperature of birds was decreased significantly ($p \leq 0.05$) T_1, T_2 and T_3 as compared to control group. Carcass traits did not differ significantly among the different groups except the percent giblet weight was significantly higher in T_1. The overall study conclude that, the *Citrus limon* juice, herbal vitamin C and synthetic vitamin C has beneficial significant effect in alleviating the Summer stress in broiler chicken. However, the better results were found with *Citrus limon* juice as compared to herbal and synthetic vitamin C.

Key words: Heat stress, vitamin C, *Citrous limon juice,* immune response

INTRODUCTION

Heat stress is one of the major concerns in the poultry industry, since it causes high mortality and/or low productivity, especially during the Summer season in India. As compared to other domestic animals, broiler chickens are more susceptible to changing environmental conditions (Nolan *et al*., 1999) in particular, high ambient temperature (Ayo *et al*., 1996). Maximum performance was demonstrated in chicks at temperatures ranging from 18 to 24°C while low performance observed in broiler chickens raised under heat stress (32°C) from 21 to 42 days of age (Alleman and Leclerq, 1997).

Various studies reveled that broiler exposed to acute heat stress had more effects on immune response, lymphoid organs (bursa, thymus and spleen) and pathophysiology of white blood cells, increased percentage of monocytes and increased percentage of heterophil and Heterophil/Lymphocyte ratio (H/L ratio) (Mogenet and Simo, 1998;

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Borges *et al*., 1999; Altan *et al*., 2000; Naseem *et al*., 2005; Chamdi, 2008). It is generally agreed that heat stress reduces immune response (Savic *et al*., 1993). The heat stress led to significant decrease in plasma ascorbic acid, antibodies (IgG and IgM) levels. Heat stress affects the humoral and cellular immune response. Suppression of immune response may be due to temporary or permanent damage to primary lymphoid organs (Muneer *et al*., 1988).

Under climatic stress, ascorbic acid demands become greater than that provided by tissue synthesis and therefore dietary supplementation of vitamin C is be beneficial (Khan and Sardar, 2005). The chicken may not have ability during stress to produce enough ascorbic acid to meet physiological demand (Cheng *et al*.,1990). Several studies have been conducted to determine whether supplemented ascorbic acid can improve performance of broiler chicks exposed to heat or management stressors. The ascorbic acid as reported earlier was found to maintain the normal leucocyte count, lower H/L ratio and increase immune response in heat stressed broilers (Karthiyaini and Philomina, 2009; Lohakare *et al*., 2005). A possible approach to counteracting the negative effects of heat stress among chicken could be the supplementation of birds with vitamin C.

In the market number of synthetic vitamin C (ascorbic acid) and herbal vitamin C preparations are available. The lemon fruit is one of rich source of vitamin C (53 mg/100 g of lemon juice). The aim of the present study was to investigate the comparative efficacy of natural (*Citrous limon* juice), herbal and synthetic vitamin C on the immune responses (H%, L% and H/L ratio, lymphoid organs) of broiler under Summer stress.

MATERIALS AND METHODS

Experimental Design

The 488 day-old straight-run Ven-cobb broiler chicks were reared commercially up to a period of 4 weeks under deep litter housing system. The experiment was conducted at Department of Poultry Science, K.N.P. College of Veterinary Science, Shirval, Maharashtra State, India in the month of February-March 2009. The feed consumption and body weight, gain in weight and feed efficiency data was maintained on weekly basis. At the end of 4th week the broiler birds were randomly assigned to 4 treatment groups viz., T_0, T_1, T_2 and T_3 containing 122 birds in each treatment. The commercial broiler prestarter was fed up to 10 days and broiler starter was fed for next 10 days and then switched over to broiler finisher diet up to the end of 5th week. The different sources of vitamin C were provided to the birds for only last week (5th week) of age. The treatment T_0 was the control, T_1 provided with the Natural Vitamin C (NVC) i.e., fresh lemon juice at 5 mL/100 birds through drinking water, T_2 was provided with Herbal-C an Herbal Vitamin C (HVC) at 2 mL/100 birds through drinking water and T_3 was provide with Microcee an Synthetic Vitamin C (SVC) at1 g/100 birds through drinking water. The vitamin C added drinking water given to the birds in such a way that, they can drink the water within half an hour. Afterwards the fresh water was provided *ad libitum*. The data on the body weight, feed consumption, weight gain and feed efficiency was maintained treatment wise for 5th week of age.

Meteriological Parameters

The minimum and maximum temperature during forenoon and afternoon was recorded during the experimental period. Similarly, the dry bulb and weight bulb temperature was recorded to arrive percent relative humidity.

Differential Leucocytic Count and H/L Ratio

At the end of 5th week the blood smears were prepared form randomly selected 5 birds from each treatment for differential leukocytes count recording H%, L% and H/L ratio (Gross and Siegel, 1983) to study the immune status of the bird.

Carcass Study

At the end of 5th week five birds from each treatment were slaughtered for the evaluation of carcass study like eviscerated yield and giblet yield.

Immune Organ Study

The weight of immune organs; bursa of fabricious, thymus and spleen was recorded from 5 birds from each treatment group at the time of slaughter.

Statistical Analysis

Data collected on various parameters were statistically analyzed as per method described by Snedecor and Cochran (1994).

RESULTS

The Differential Leucocytic Count (DLC) was estimated and Heterophil: Lymphocyte ratio (H:L ratio) was calculated at the end of 5th week of age which is presented in Table 1. The statistical analysis of the data indicated that the H% was decreased significantly ($p \leq 0.05$) in birds supplemented with NVC (T_1), HVC (T_2) and SVC (T_3) than the control T_0. The H% was decreased more significantly ($p \leq 0.05$) in birds supplemented with NVC at 5 mL/100 birds as compared to HVC and SVC group. The lymphocyte count was increased significantly ($p \leq 0.05$) in T_1, T_2 and T_3 than the control T_0. However, the lymphocyte count was more in the birds supplemented with NVC than HVC and SVC group. The values for H:L ratio were significantly ($p \leq 0.05$) lower in T_1, T_2 and T_3 than the control T_0. Among the vitamin C treated group, H:L ratio was decreased more predominantly in NVC group than that of HVC and SVC group.

The statistical analysis of the data on spleen, bursa of fabricious and thymus weight (Table 2) indicated that, the weight of spleen and bursa of fibricious was increased

Table 1: Effect of vitamin C on blood immune parameters of broilers (Mean±SE)

	Treatments			
Parameters (%)	T_0	T_1	T_2	T_3
Heterophil	36.60±0.24^{c}	26.80±0.37^{a}	31.80±0.31^{b}	31.80±0.66^{b}
Lymphocyte	47.00±0.78^{a}	57.40±0.87^{b}	54.20±0.91^{b}	54.20±0.96^{b}
Eosinophil	10.00±0.22^{ab}	10.60±0.67^{b}	9.20±0.86^{b}	7.40±0.77^{a}
Basophil	3.00±0.44^{ab}	3.40±0.50^{ab}	2.60±0.24^{a}	4.20±0.48^{b}
Monocyte	2.20±0.37	1.80±0.20	2.20±0.37	2.40±o.24
H:L ratio	0.73±0.06^{c}	0.46±0.06^{a}	0.58±0.03^{b}	0.58±0.01^{b}

The means with different superscripts in a row differ significantly ($p \leq 0.05$)

Table 2: Effect of vitamin C on immune organs of broilers (Mean±SE)

	Treatments			
Parameters (g)	T_0	T_1	T_2	T_3
Spleen	2.29±0.14^{a}	3.70±0.35^{b}	2.39±0.12^{a}	2.49±0.14^{a}
Bursa of fabricious	3.34±0.29^{a}	5.26±0.25^{b}	3.94±0.49^{ab}	4.27±0.63^{ab}
Thymus	5.10±0.36^{a}	6.34±0.35^{b}	4.76±0.25^{a}	6.00±0.14^{b}

The means with different superscripts in a row differ significantly ($p \leq 0.05$)

Table 3: Effect of vitamin C on the carcass traits (Mean±SE)

	Treatments			
Parameters	T_0	T_1	T_2	T_3
Slaugher weight (g)	1862.00±38.79	1811.20±25.62	1851.00±52.88	1761.20±23.56
Giblet weight (%)	4.08±0.87[a]	4.67±0.14[b]	4.24±0.15[a]	4.18±0.15[a]
Eviscerated weight (%)	63.83±0.39	63.69±0.41	64.20±0.76	62.96±0.76
Total loss (%)	32.08±0.43	31.63±0.41	31.55±0.73	32.85±0.85

The means with different superscripts in a row differ significantly ($p \leq 0.05$)

significantly ($p \leq 0.05$) in T_1 as compared to control T_0, T_2 and T_3. However, there was numerical increase in the weight of spleen and bursa of fabricious in T_2 and T_3 as compared to control T_0. Weight of thymus in T_1 and T_3 showed significant ($p \leq 0.05$) increment as compared to T_0 and T_2.

The slaughter weight, percent eviscerated weight; percent total loss (Table 3) did not differ significantly among the different groups. However, the giblet weight was significantly higher in T_1.

DISCUSSION

The data on the temperature indicated that the experimental broiler birds were under the Summer stress. Decrease in performance by broiler kept at 32°C was reported by Alleman and Leclerq (1997). However, maximum performance in chicks was reported at temperatures ranging from 18 to 24°C (Charles and Spencer, 1976).

Birds in control group were under the stress which was indicated by increased percentage of hetrophils, decreased percentage of lymphocytes and increased H:L ratio in the control T_0 as reported by Puvadolpirod and Thaxton (2000a-c). The results of present study was in agreement with Karthiyaini and Philomina (2009) reported that supplementation of vitamin C at 0.03% in the diet significantly lowered the H/L ratio in broiler chicken under the stress and concluded that the dietary supplementation of vitamin C reduces stress. Gross and Siegel (1983) and Zulkifi *et al.* (2003) reported that H:L ratio is reliable indicator of avian stress. A low H:L ratio indicates low levels of stress. The results of the present study for heterophil and lymphocyte percentage are in agreement with Borges *et al.* (2003, 2004), while for H/L ratio with McFarlane and Curtis (1989). Supplementations of natural, herbal and synthetic sources of vitamin C through drinking water alleviate the heat stress and thereby improve immune response in broiler chicken. Gross (1992) and Naseem *et al.* (2005) reported that ascorbic acid improved immune response in birds under stress and disease condition. Moreover, it was observed that the supplementation of natural source of vitamin C supplementation through drinking water helps to reduce stress more effectively as compared to other sources of vitamin C used in this experiment.

Gross *et al.* (1980) reported that exposure of birds to high environmental temperature causes an increase in the plasma corticosterone resulting in to depressed activity of the lymphoid organs which was found in the present study. Increase in weight of spleen, bursa of fabricious and thymus in vitamin C supplemented groups attributed to improved activity of lymphoid organs. The better results were shown by the bird in T_1 (NVC) followed by SVC (T_3) and HVC (T_2) group.

The percent giblet weight was significantly ($p \leq 0.05$) higher in NVC supplemented group (T_1) than T_0, T_2 and T_3. Sahin *et al.* (2003) reported increased giblet weight with supplementation of vitamin C. The slaughter weight and carcass weight was not affected in the present study. Similar result was reported by Mehmet *et al.* (2005) in quails. However, Kutlu (2001), Sahin *et al.* (2003) and Lohakare *et al.* (2004) reported 250 ppm vitamin C supplementation in broiler ratio, increased the carcass weight.

On the basis of overall study it was concluded that the natural, herbal and synthetic vitamin C supplementation through drinking water helps to reduce the Summer stress and thereby improve the immune status of broiler chicken. Moreover, supplementation of fresh lemon juice which is natural source of vitamin C used in this study had additional beneficial effect in allivating stress and improving immune response in broiler birds during Summer stress. The effect of natural vitamin C is due to the antioxidant property of fresh lemon juice. Thus, the supplementation of fresh lemon juice at 5 mL/100 birds is useful emergency tool in combating the Summer stress.

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