



Effects of Herbal Vitamin C (Herbo C) on Growth Performance and Anti-Stress Potential in Commercial Broilers

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Abstract

Stress has a significant impact on the performance of the commercial broiler birds. Vitamin C is an essential micronutrient and a stress reliever. Although, birds can synthesize vitamin C, they require vitamin supplement in their ration. Vinayak Ingredients (India) Pvt. Ltd has formulated an herbal vitamin C formulation, Herbo C, an alternative of synthetic vitamin C. In the present trial, day-old Cobb 430 Y broiler chicks (n=500) were procured and randomized into 5 groups (100 birds/group). The treatment group 1 was allotted for the control birds obtaining basal diet only. The birds of remaining groups received Herbo C at a concentration of 100, 200 g/ton, a competitor product of 200 g/ton and one synthetic vitamin C at 100 g/ton along with basal diet. The study duration was 42 days. The growth performance, hematological, biochemical and immunological parameters were evaluated. Our trial results showed that Herbo C supplementation in broiler ration improved weight gain and FCR value of the treated birds. It exerted low HL ratio among the birds as compared to control and other treatment groups. Herbo C supplementation showed low cortisol level among the birds. All immune parameters like lymphoid organ weights, antibody titres of IBD and ND were observed higher in group treated with Herbo C as compared to other groups. Herbo C at a concentration of 200 g/tonne showed superior results among all treatment groups.

Keywords: Stress; Herbal Vitamin C; Herbo C; Commercial Broiler; Growth Performance; HL Ratio; Cortisol Level; Immunology

Introduction

Stress is delineated as “nonspecific response of the body to any demand” [23]. Stress is one of the crucial factors that exerts marked influence on the broiler production. The stressors may be of varied types like extreme climatic conditions, improper handling, transportation, deprived nutrition, sub-standard housing, overcrowding, poor health condition, immunization etc. [2]. Stress generates reactive oxygen species (ROS) in tissues causing cellular damage and disrupting the body's homeostasis. It disturbs the functional integrity of the hypothalamic-pituitary-adrenal (HPA) axis, a key component of the stress regulatory system [24]. Hence, the serum corticosterone level is raised leading to the impairment of the thermoregulatory mechanism [22]. Stress causes metabolic

distress, low immune response and fatality among the poultry birds [11]. It develops significant impact on the growth and efficiency of the birds.

Vitamin C supplement in diet exerts beneficial effect in relieving stress among the broiler birds. It is an essential micronutrient required for the growth and performance of the birds. Vitamin C helps to maintain the serum cortisone level and regulate HPA axis [15]. Thus it retains body's homeostasis. It works as anti-oxidant and boost immune response among the birds. It helps to lessen the release of stress hormone, cortisol from the adrenal gland [13]. Therefore, the metabolic function of the birds is restored.

Our study is focussed on to evaluate the herbal vitamin C supplement (Herbo C) in broiler ration to improve the health and productivity of the birds. Herbo C is an excellent product, developed by Vinayak Ingredients Pvt. Ltd and showed superb efficacy when added in broiler feed. The objectives of the present trial are to assess the growth performance, immune status and stress management quality of Herbo C in commercial broilers.

Methods

Birds

Day-old Cobb 430 Y broiler chicks (n=500) were procured from a reputed hatchery registered with the Committee for the Control and Supervision of Experiments on Animals (CCSEA). The birds were housed in the housing facility of Omega Laboratories, Satara,

India for further experiments. The experimental protocols were reviewed and approved by the experts of the Institutional Animal Ethics Committee (IAEC). The standard brooding, feeding and watering were performed among the birds in the entire trial. The birds were vaccinated with the Lasota and IBD vaccines as per the schedule mentioned in table 1.

Vaccine	Time
Lasota, 1 drop by intraocular route	day 7, 21
IBD vaccine, by intraocular route	day 14

Table 1: Vaccination schedule.

Diet

The birds were fed according to the standard feed formulation described in table 2.

RM	Ingredient	STARTER	FINISHER
	MAIZE (9% MOISTURE)	551.9	621.9
	HIPRO SOYA 49%	365	300
	SOYA/ RB OIL (FFA<5, PV<4)	35	40
	LSP	10	8
	DCP	16	10.7
	SODA	2.5	2.8
	Salt	2.8	2.2
ADDITIVES	VITAMIN PREMIX (VITAMIX)	0.55	0.5
	PREMIX - WITHOUT ZINC	0.5	0.5
	ANTI OXIDANT (ENDOX DRY)	0.15	0.15
	CHOLINE CHLORIDE 60%	1.5	1.4
	TOXIN BINDER (AVATOX)	1	1
	EMULSIFIER (LIPTIVO XT)	0.5	0.45
	ANTICOCCIDIALS- MADURA+ NICARB (GLAVITRO)	0.6	0.6
	LYSINE SULPHATE	3	2
	D L METHIONINE	3.2	2.5
	L THREONINE	1.5	1
	BMD 10%	0.5	0.5
	PHYTASE 5000 FTU (QUANTUM BLUE)	0.15	0.15
	PROTEASE WITH NSPase (GLAVENZA DUO)	0.35	0.35
	MOULD INHIBITOR (MOLDZAP)	0.6	0.6
	LIVER TONIC (LIV52)	0.5	0.5
	BETAINE HCL	0.7	0.7
	ACIDIFIER	1	1
	ANTIDIARRHOEAL/ GUT MODULATOR	0.5	0.5
	VITAMIN C / (HERBAL C)*	00	00
	Zinc	00	00
	TOTAL	1000	1000

Table 2: Feed formulation - Basal diet formula.

*Required quantity was added in the respective groups as per the trial protocol given by the Vinayak Ingredients.

Study design

The present study was conducted at Omega Labs farm at Lonand, District Satara, Maharashtra, INDIA located at 18°02'33.3"N and 74°10'52.6"E in the Western region of Maharashtra, about 518 meters above the mean sea level (MSL). A total number of 500 Cobb 430 Y broiler birds were used in the trial. The appropriate measures of quarantine and acclimatization were implemented before onset of the study. All birds received standard brooding, feeding and watering procedures from day old till end of the trial. The experimental birds were randomized into 5 groups (100

birds/group). The treatment group 1 was allotted for the control birds obtaining basal diet only. The birds of remaining four groups received herbal/synthetic vitamin C (vit. C) along with basal diet (table 3). The study duration was 42 days.

The study was carried out during the natural heat season and the environmental temperature was between 38 to 41°C (<https://www.accuweather.com/en/in/lonand/204580/april-weather/204580?year=2024>)

Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
	Basal diet	Herbo C	Herbo C	Competitor 1	Synthetic Vit. C
	No Vit. C	100 g/tonne	200 g/tonne	200 g/tonne	100 g/tonne
No of birds/replicate	20	20	20	20	20
No of replicates	5	5	5	5	5
Total no of birds	100	100	100	100	100
Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
	Basal diet	Herbo C	Herbo C	Competitor 1	Synthetic Vit. C
	No Vit. C	100 g/tonne	200 g/tonne	200 g/tonne	100 g/tonne
No of birds/replicate	20	20	20	20	20
No of replicates	5	5	5	5	5
Total no of birds	100	100	100	100	100

Table 3: Experimental design.

Assessment parameters

The experimental birds were routinely examined for body weight, feed consumption, feed conversion ratio (FCR), clinical signs, morbidity and mortality and all observations were recorded in trial register. Feed consumption of the birds was estimated by deducting the value of feed residue (remaining at the end of week) from the feed offered to the birds. The corrected FCR was calculated as

$$FCR = \frac{\text{Total feed consumed}}{\text{Final weight - initial weight}}$$

The birds died during and at the end of the trial were undergone detail postmortem examination and the findings were recorded. At the end of the trial, 10 birds were selected randomly from each

experimental group and sacrificed for further assessment procedures. The aseptic clot activator tubes were used to collect blood from the birds and the serum was separated for the biochemical evaluation. The parameters like cortisol, cholesterol, total proteins, albumin, globulins, AST, ALT and titers of New castle disease (ND) and Infectious bursal disease (IBD) antibody were assessed. The hematological parameters viz. HB, PCV, heterophils, lymphocytes, monocytes and eosinophils were measured. The organs like spleen, thymus and bursa were collected and weighed.

Statistical analysis

The raw study data were compiled and expressed as Mean. The standard error of mean (SEM) was calculated. Dunnett's test was performed for comparing the treatment groups. *p* <0.05 was accepted as statistically significant value.

	Parameters	Method used
1	Serum ALT IU/L	Merill Biochemistry analyser
2	Serum AST IU/L	
3	Serum Cholesterol mg/dl	
4	Total serum Protein g/dl	
5	Serum Albumin g/dl	
6	Serum globulin g/dl	
7	HA and HI – ND and IBD Titers	Haemagglutination and Haemagglutination inhibition test
8	Serum cortisol ng/ml	ELISA kit
9	Organ weights- (Thymus, spleen, bursa)	Digital weighing balance
10	Statistical analysis	Dunnett's test for comparing several treatments

Table 4: Methods used to assess various parameters.

Results

In the present trial, the experimental birds were assigned for 5 treatment groups. The treatment group 1 received basal diet only. However, the birds of treatment group 2 to 5 obtained various concentration of herbal or synthetic vitamin C admixed with basal diet.

Growth parameters

The growth performance of the birds under trial was assessed using the tools like weekly body weight, feed consumption and FCR value. Results showed that the overall growth performance

of group T3 was excellent and significantly comparable with other treatment groups. At 6th week of age, the body weights of the birds were significantly higher in T3 and lower in T1 as compared to other groups (Table 5, Figure 1A). FCR value was significantly higher in T1 as compared to other groups. Although, group T3 showed significantly lower FCR value as compared to other treatment groups (Table 6, Figure 1B). The all week average body weight of the birds was highest in T3 group and lowest in T1 (Figure 2A). However, the average FCR value of T2 and T3 was significantly higher ($p < 0.01$) than other treatment groups (Figure 2B). No mortality was observed among the birds of the treated groups.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Week 6 body weights	2544 ^e	2768.2 ^b	2831.267 ^a	2649.867 ^d	2728.1 ^c
SEM	24.24022	25.19851	26.272098	26.255099	23.88655

Table 5: Body weights (6th week).

*Body weights were significantly higher in Treatment 3 as compared to other groups, Treatment 1 showed significantly lower body weights as compared to other treatment groups.

	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Week 6 FCR value	1.768082 ^a	1.657033 ^d	1.627893 ^e	1.726879 ^b	1.685422 ^c
SEM	0.002832637	0.002784251	0.002056902	0.00226407	0.001684112

Table 6: FCR (6th week).

*FCR values were significantly higher in Treatment 1 as compared to other groups, Treatment 3 showed significantly lower FCR as compared to other treatment groups.

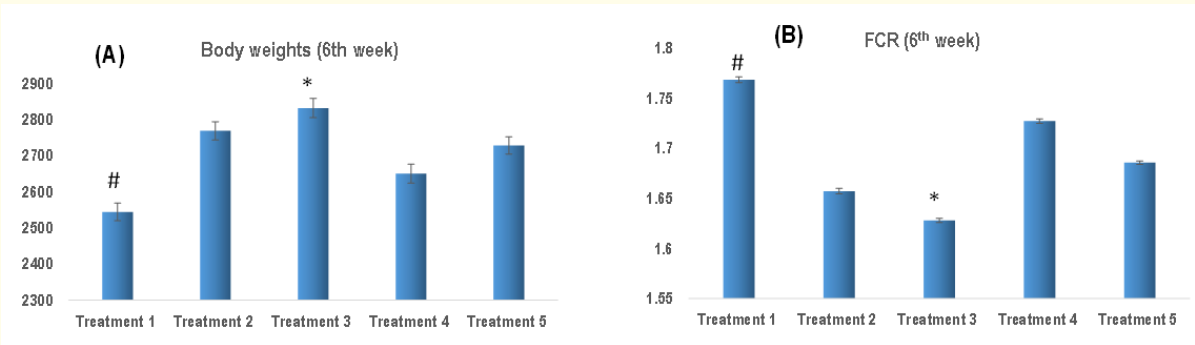


Figure 1: Histograms showing Body weights (A) and FCR (B) of the broilers (6th week) at various treatment groups. Data represent mean \pm SEM. Asterisk indicates significant difference in Body weight and FCR in treatment groups as compared to other groups. Number sign indicates significant change in Body weight and FCR in treatment 1 (control) in comparison of other groups ($p < 0.05$).

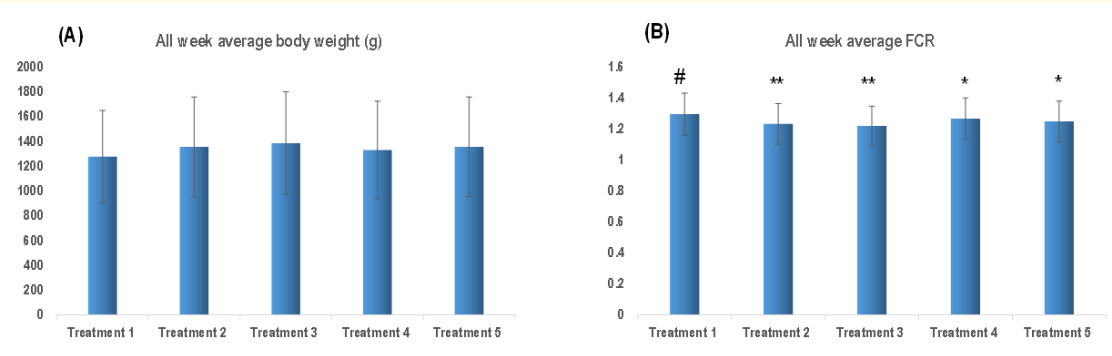


Figure 2: Histograms showing Body weights (A) and FCR (B) of the broilers (all week average) at various treatment groups. Data represent mean \pm SEM. Asterisk indicates significant difference in Body weight and FCR in treatment groups as compared to other groups. Number sign indicates significant change in Body weight and FCR in treatment 1 (control) in comparison of other groups (* indicates $p < 0.05$; ** indicates $p < 0.01$).

Haematological parameters

The haematological parameters like Haemoglobin (g%), Packed cell volume, Heterophils %, Lymphocyte %, Monocyte %, Eosinophil % and HL ratio were estimated. All parameters were statistically significant and comparable in group T3 as compared to other treatment groups. The treatment group 1 showed significant difference in comparison of other groups (Table 7). However all the values in all the groups were within the normal physiological range.

The HL ratio was significantly higher in T1 group as compared to other treatment groups, whereas T3 showed lowest HL ratio than the other groups (Table 8, Figure 3).

Serum biochemistry

The serum biochemical parameters like Total proteins (g/dl), Albumin (g/dl), Globulin (g/dl), Plasma cholesterol (mg/dl), ALT (IU/L) and AST (IU/L) were estimated. In all evaluations the birds of T3 group showed significant differences in biochemical param-

Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Haemoglobin (g%)	7.79 ^d ± 0.0567	8.43 ^b ± 0.0396	8.85 ^a ± 0.0703	8.1 ^c ± 0.0596	8.13 ^c ± 0.0448
Packed cell volume	25.1 ^d ± 0.2333	27.2 ^b ± 0.1333	28.9 ^a ± 0.3786	26 ^c ± 0.3333	26.8 ^c ± 0.3266
Heterophils %	54.6 ^a ± 0.4761	36.9 ^c ± 0.9	33 ^d ± 0.5774	42.7 ^b ± 0.4230	42.4 ^b ± 0.3055
Lymphocyte%	35.4 ^d ± 0.4522	53.2 ^b ± 0.3887	54.8 ^a ± 0.3266	47.3 ^c ± 0.9434	47.2 ^c ± 0.8
Monocyte %	4 ^c ± 0.2582	4.9 ^b ± 0.2769	5.4 ^a ± 0.2211	4.2 ^c ± 0.2	4 ^c ± 0.2981
Eosinophil %	6 ^a ± 0.4944	5 ^b ± 0.8819	6.8 ^a ± 0.6960	5.8 ^a ± 1.1813	6.4 ^a ± 0.7483

Table 7: Haematological parameters.

Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
H/L ratio	1.542373 ^a	0.693609 ^d	0.60219 ^d	0.902748 ^b	0.898305 ^c
SEM	0.0308	0.0200	0.0111	0.0181	0.0200

Table 8: H/L ratio.

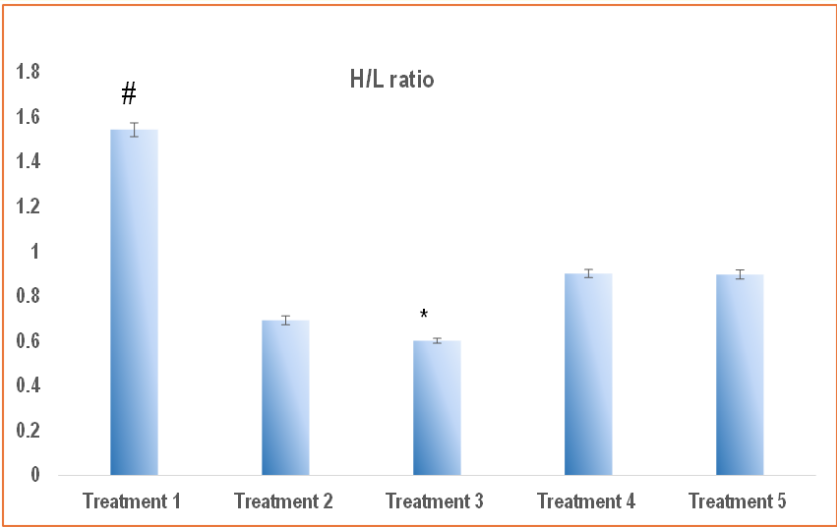


Figure 3: Histograms showing H/L ratio of the broilers at different treatment levels. Data represent mean ± SEM. Asterisk indicates significant difference in treatment groups as compared to other groups. Number sign indicates significant change in treatment 1 (control) in comparison of other groups (p < 0.05).

eters as compared to the control (T1) and other treatment groups (Table 9).

Serum cortisol values were significantly higher in treatment group 1 (control) as compared to other groups. T3 group showed significantly lower serum cortisol values (Table 10, Figure 4).

Immunological parameters

All immune parameters like lymphoid organ weights, antibody titres of IBD and ND were significantly higher in treatment group 3 as compared to other groups.

Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Total proteins g/dl	4.502 ± 0.0571	5.606 ± 0.0409	5.922 ± 0.0579	5.541 ± 0.0335	5.548 ± 0.0348
Albumin g/dl	2.411 ± 0.0615	3.088 ± 0.0280	3.363 ± 0.0728	3.134 ± 0.0485	3.13 ± 0.0454
Globulin g/dl	2.091 ± 0.0149	2.518 ± 0.0326	2.559 ± 0.0396	2.407 ± 0.0331	2.411 ± 0.0176
Plasma cholesterol mg/dl	201.9 ± 2.0894	190 ± 1.4142	186.3 ± 1.3503	192.5 ± 1.4472	191.5 ± 1.2931
ALT- IU/L	7.329 ± 0.1517	6.413 ± 0.0585	6.377 ± 0.0404	6.437 ± 0.0359	6.458 ± 0.0430
AST - IU/L	231.6 ± 1.8631	219.1 ± 1.6224	216.9 ± 1.6697	220.7 ± 1.1160	220.6 ± 1.5290

Table 9: Biochemical parameters.

Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Serum cortisol ng/ml	2.171 ^a	1.653 ^d	1.634 ^e	1.668 ^c	1.681 ^b
SEM	0.0156	0.0215	0.0120	0.0099	0.0106

Table 10: Serum cortisol level (ng/ml).

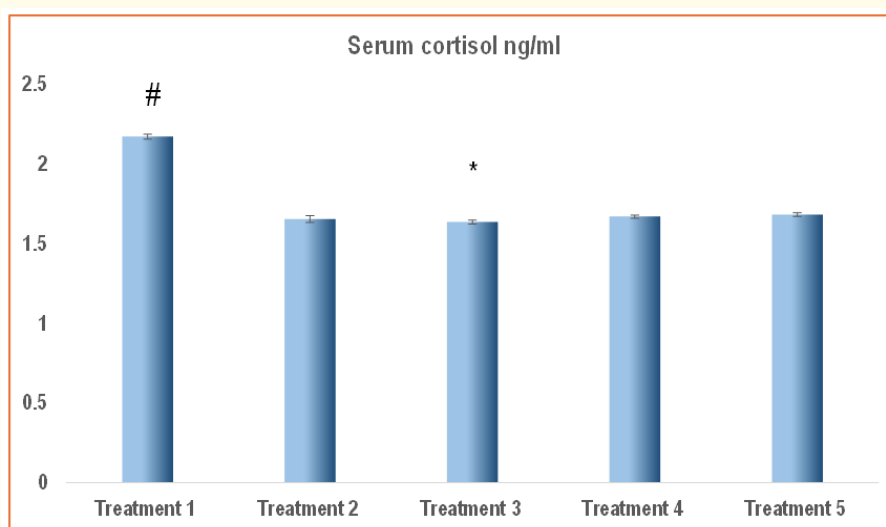


Figure 4: Histograms showing Serum cortisol level of the broilers at different treatment levels. Data represent mean ± SEM. Asterisk indicates significant difference in treatment groups as compared to other groups. Number sign indicates significant change in treatment 1 (control) in comparison of other groups ($p < 0.05$).

Spleen and thymus weights were significantly higher in groups T2 and T3 as compared to other treatment groups. Whereas, the bursal weight was significantly higher in group T3 as compared to other treatment groups. T1 group showed significantly lower values of lymphoid organ weights (Table 11, Figure 5).

The antibody titre of ND was significantly higher in group T3 as compared to other treatment groups. IBD antibody titre was significantly higher in group T2 and T3 as compared to other treatment groups. T1 group showed significantly lower ND and IBD titre values (Table 12, Figure 6).

Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Weight of spleen	2.266 ^e ± 0.0593	2.982 ^a ± 0.0727	2.986 ^a ± 0.0784	2.427 ^c ± 0.0323	2.414 ^d ± 0.0240
Weight of bursa	3.784 ^e ± 0.0450	4.748 ^b ± 0.0490	5.022 ^a ± 0.0322	4.517 ^c ± 0.476	4.509 ^d ± 0.0385
Weight of thymus	6.443 ^e ± 0.0745	8.637 ^b ± 0.1065	8.861 ^a ± 0.0720	8.569 ^d ± 0.0574	8.585 ^c ± 0.0547

Table 11: Immune organ weight (g).

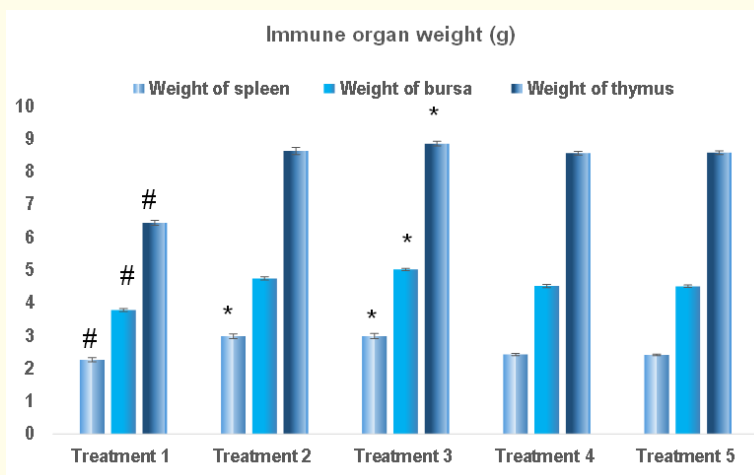


Figure 5: Histograms showing Immune organ weight of the broilers at different treatment levels. Data represent mean ± SEM. Asterisk indicates significant difference in treatment groups as compared to other groups. Number sign indicates significant change in treatment 1 (control) in comparison of other groups ($p < 0.05$).

Groups	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
ND titres	89.6 ^d ± 10.45116	334 ^b ± 39.71566	386 ^a ± 43.33333	295.2 ^b ± 38.9041	282.4 ^c ± 42.29505
IBD titres	76.8 ^e ± 8.533333	282 ^a ± 26	282 ^a ± 26	243.6 ^c ± 35.77932	217.6 ^d ± 19.55232

Table 12: Antibody titres.

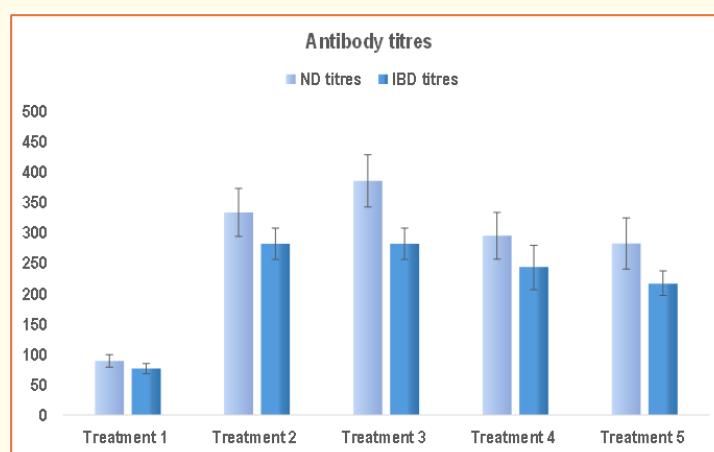


Figure 6: Histograms showing Antibody titres of the broilers at different treatment levels. Data represent mean ± SEM. Asterisk indicates significant difference in treatment groups as compared to other groups. Number sign indicates significant change in treatment 1 (control) in comparison of other groups ($p < 0.05$).

Discussion

Vitamin C or ascorbic acid, a water-soluble vitamin, serves as a potential anti-oxidant in the biological system. The enzyme, L-gulonolactone oxidase present in living beings excluding primates and guinea pigs, bio-catalyses the synthesis of vitamin C from glucose [7]. In stressful condition, vitamin C is rapidly depleted in body leading to its deficiency in the circulation [10]. Though, poultry birds can synthesize vitamin C, they need the vitamin supplementation in their ration routinely. Vitamin C has a vital role in boosting up the immune response of the birds. It mitigates the oxidative damage caused by stress and helps to improve performance

among the broilers. Commercial broiler production is affected by varied type of stress impacting on the birds' health and metabolism. Stress causes diminished activity of the anti-oxidant enzymes viz. heme oxygenase-1 (HO-1), glutathione sulfotransferase (GST) and superoxide dismutase 2 (SOD2) leading to oxidative stress [14]. The anti-oxidant system has a leading role to resist the cellular oxidative damage due to the harmful free radicals generated in the stressful environment. Stress also plays a crucial role in impairing the defense mechanism of the [8]. The immunocompromised status of the birds triggers susceptibility to many diseases resulting in poor health condition.

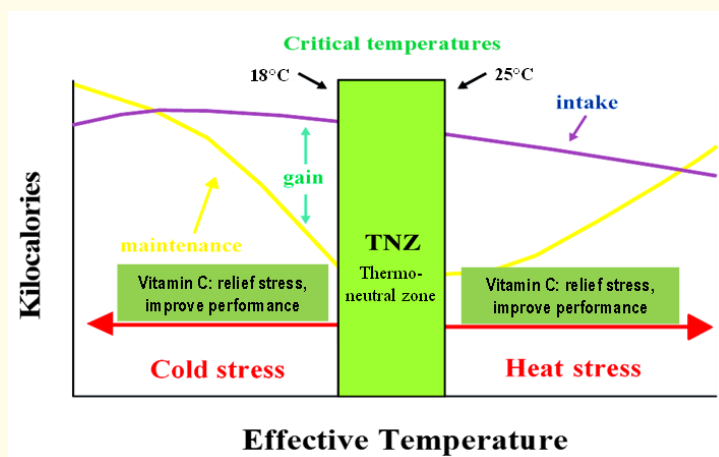


Figure 7: Role of Vitamin C in broiler ration to combat heat stress.

Vinayak Ingredients (India) Pvt. Ltd. has prepared an herbal Vitamin C formulation, Herbo C as a natural stress reliever for the commercial broilers to promote growth and performance. Vitamin C is easily degraded due to the moisture, heat, pelleting temperature and pressure generated in the feed processing technique. Herbo C is a stable, safe and non-toxic formulation that improves weight gain, FCR, immune response and stress parameters of the birds. In the present study, 500 commercial broiler birds (Cobb 430 Y) were randomized and allotted into 5 groups viz. control, Herbo C (100 & 200 g/tonne), competitor or marketed reference product (200 g/tonne) and synthetic vitamin C (100 g/tonne). The trial data revealed that the treatment group Herbo C 200 g/tonne of feed was superior in growth performance parameters of the birds. At 6th week of age the birds fed ration containing Herbo C 200 g/tonne showed significantly better body weight and FCR

values as compared to other treatment groups. Earlier, researchers reported that polyherbal formulation improved performance of the poultry birds [22]. Our findings were in agreement with the previous results.

HL ratio or heterophils and lymphocytes ratio is a reliable tool to evaluate the immune status of the birds. Scientists reported that stress is responsible for deprived immune function in poultry birds. Heterophils are the granulocytes responsible for the innate immunity. Whereas, lymphocytes are known to develop adaptive immunity among the birds [18]. In response to the infectious or non-infectious inflammation, heterophils are rapidly accumulated in the injured tissue to initiate phagocytosis. Thus, HL ratio has an inverse relationship with stress and a useful biomarker [12]. In the present trial, HL ratio was observed significantly lower in birds

fed ration containing Herbo C 200 g/tonne, as compared to other treatment groups. Other hematological and biochemical parameters were also found superior among birds of Herbo C 200 g/tonne treatment group. Earlier research also revealed that plant may replace synthetic vitamin C supplement by modulating immune response, improving HL ratio and phagocytic activity [1].

Stress is responsible for stimulation of sympathetic adrenomedullary (SAM) pathway leading to the secretion of catecholamines, like epinephrine and norepinephrine [6]. Eventually, the hypothalamic-pituitary-adrenal (HPA) axis is activated causing the release of glucocorticoids (GC) such as cortisol and corticosterone [4], [18]. Hence, serum cortisol level is identified as a valuable marker of stress status of the broiler birds. In our study, the serum cortisol level was significantly reduced in broilers supplemented with 200 g/tonne Herbo C. This is in concord with the previous research where plant extract exhibited marked response in regulating HPA axis and serum corticosterone level [17].

Enhanced cortisol level in chicken is responsible for reduced muscular mass and increased visceral weight [21]. It also develops anxious behaviour among the birds. High rise of serum cortisol reportedly causes low immune response [9], [25]. Body's immune response against pathogen is comprised of innate and adaptive immune system [5]. Innate immunity is the primary defence mechanism in response to any infection. Adaptive immunity is again of two types viz. cell mediated (through T lymphocyte) and humoral (through B lymphocyte) immunity. Lymphocytes are produced and matured in the immune organs like bursa of Fabricius and the thymus. The secondary lymphoid organ, spleen is the house of many immune cells and plays an important role in regards of innate and adaptive immune response [3]. Stress has a significant impact on the immune organs leading to their reduced weight and diminished activity. Moreover, antibody titre is the assessment of antibody level in blood against any specific pathogen. Stress is responsible for the lowered immune response leading to the development of a negative impact on the antibody production [19]. Furthermore, stress often develops aggressive response among the poultry birds leading to altered feeding pattern, poor performance and reduced productivity [20]. Stress disturbs the lipid metabolism and muscular growth of the birds and affects the nutritional quality of the broiler meat [16]. The birds show a state of immune-suppression, susceptible to various pathogens, even mortality. This gives rise to

huge economic loss of the poultry farmers. In the recent experiment, supplementation of vitamin C showed improved immunity among the commercial broilers. It showed noticeable increase in the immune organ weights and antibody titre against IBD and ND infections among the birds. The birds receiving Herbo C 200 g/tonne of feed exerted significant rise of immune status as compared to other treatment groups.

These results are in accordance with the earlier experimental reports. Scientists suggested that plants have a significant impact on broiler's growth and FCR value through mitigating stress and promoting anti-oxidant property. Another study showed that plant extract has immunomodulating property when added in birds' ration [1].

Conclusion

In conclusion, Herbo C is an excellent alternative of synthetic vitamin C supplement in commercial broiler ration. Herbo C exerted anti-stress potential by optimising serum cortisone level in birds. It enhanced immune response of the broilers by improving HL ratio, lymphoid organ weights and antibody titre level. Herbo C improved growth and performance of the commercial broilers noticeably. Our results showed that Herbo C at a concentration of 200 g/tonne of feed exhibited the superior performance.

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