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Effect of Heat Stress and Ascorbic Acid on Gut Morphology of Broiler Chicken

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Abstract: The experiment was conduct d to evaluate the effect of heat stress and ascorbic acid (AA) on the gut morphology of broiler chickens. In this regard, macro and micromorphomatric study of the three segment of intestine such as duodenum, jejunum, ilium were performed along with the nutrients retention in the intestine. For this purpose 180 birds were purchased and divided into four equal groups i.e. normal temperature with and without AA, high temperature with and without AA supplementation. Our finding showed that birds reared under heat stress free environment and provided with AA showed significantly higher weight gain and size of intestinal parts along with higher nutrient retention than all other groups ($P<0.05$). Moreover, weight and length of Duodenum, jejunum and ileum was observed significantly ($P<0.05$) lower in heat stress control group than the AA supplemented group. Thus it was concluded that AA supplementation improves the villi size and nutrients digestibility when supplemented in normal temperature, whereas significantly ($P<0.05$) improves the weight and length of intestine when supplemented under higher temperature.

Keywords: Broilers, Heat Stress, Ascorbic acid, Gut Morphology

1. INTRODUCTION

Heat stress is an environmental condition which is adversely affecting poultry industry worldwide. A broiler chicken is sensitive to heat stress due to large size and absence of sweat glands along with rapid metabolism, and high body temperature. Researchers are investigating the effect and solution of high environmental temperature on the performance of the meat type of. Pakistan's most of the farming is located at tropical zone and thus our poultry industry suffers from the ambient temperature of May to September. Heat stress occurs when high temperature of the environment accompanies high humidity during these months and which adversely effects the productivity of broiler chicken and make broiler rearing conditions risky and not profitable (Fouad, *et al.* 2016).

Broilers have versatile heat regulatory system when they are exposed to high ambient temperature, their body temperature increases more than the normal body temperature and the corticosterone stored in adrenal cortex are released into the blood circulation to help broilers increase metabolism (Ready, 2000). This hormone might cause cell mediated and humoral immunity failure because changes in the plasma concentrations of corticosteroids and ACTH affected the lymphoid tissues; for example, a diminution in the mass of the spleen, thymus and bursa of fabricius might be observed. Moreover, glucocorticoids cause dissolution of lymphocytes which may lead to lymphopenia and more heterophils are released in the blood circulation but heir phagocytic and bactericidal activities may decreases. Thus, there is a need to improve the thermo tolerance of the broiler chickens in the hot climatic conditions without affecting the production of meat purpose broiler chickens. Nutritional strategy in the heat stress conditions plays crucial part in reducing the negative impact of heat stress. The offered diet should fulfill the requirements of birds for proteins, electrolytes and energy (Balnave 2004) . Ascorbic acid is being used largely in the poultry industry during heat stress due to its anti-oxidant effect, which neutralizes the free radicals which are produced during the environmental stress (Ramnath *et al.,* .2008). Broilers themselves can synthesize their own ascorbic acid but its quantity is insufficient to reduce the free radicals (Maurice *et al.,* 2002). Review of literature shows not enough work has been carried on previously on the relationship among environmental stressors (such as heat stress etc) on the gut microbial environment in chicken. A broiler subjected to heat stress has been reported to altered morphology and altered microbial community in the intestinal tract. Under heat stress conditions, increased concentrations of reactive oxygen species (ROS) occur which lead to increased intestinal permeability, which in turn facilitates the translocation of bacteria from the intestinal tract (Lara and Rostagno 2013).

Keeping in views the importance of commercially used ascorbic acid, the research trials were conducted to

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observe the effects of ascorbic acid on gut morphology (Intestinal villus size, intestine weight, length and nutrients retention) of broiler chickens supplemented with ascorbic acid in heat stress and heat free condition.

For this purpose 180 birds were purchased and divided into four equal group's i.e. normal temperature with and without AA, high temperature with and without AA supplementation. The experiment was performed in the month of June-July for higher temperatures and in the moderate month of August-September, thus two research trials were conducted under this research work to know the effects of ascorbic acid (Vitamin-C) supplementation. Birds were reared on the litter housing system and both A , and B groups were reared without heat and the later was provided with vitamin ascorbic acid150mg/kg supplemented in the feed. The group C and D were subjected to hot environmental to produce heat stress but the later one was provided with AA. All the groups consisted of three replicates. Feed and water were provided *ad libitum.* Feeding program was consisted over starter diet (1-21days) and a finisher diet (22-42days).

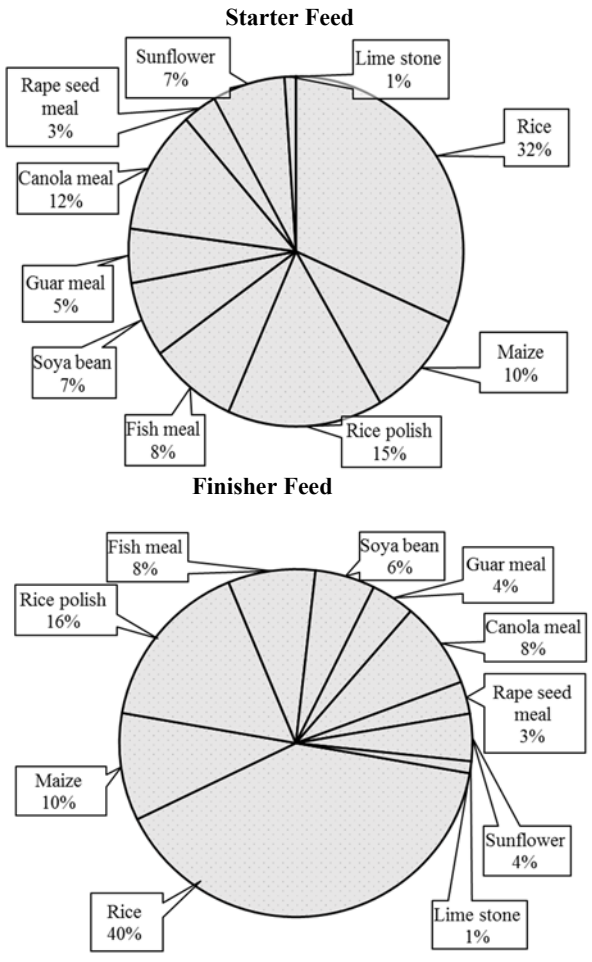


Fig.1: Ingredients and feed formulation.

The house was entirely cleaned, washed and sanitized then painted with lime stone and dried for 24 hours. One squire feet per bird floor space provided. The artificial brooding preparation practiced 24 hours before the arrival of chicks. Six thermometers were placed in every replicate to monitor the temperature in the heat stress charged groups (Control and Supplementary). Exhaust fans were also installed for maintaining optimum oxygen level and proper ventilation.

Intestinal Histomorphology

A transverse section of 1cm tissue was removed from different segments of gut and then fixed in the preservative solution and further processed till tissue embedding in wax. Embedded tissues were sectioned into 5µm pieces and then mounted on glass slides. After gradual processing of slides in ethanol and xylene, hematoxylin and eosin were used for staining. Stained slides were observed under microscope for morphometry.

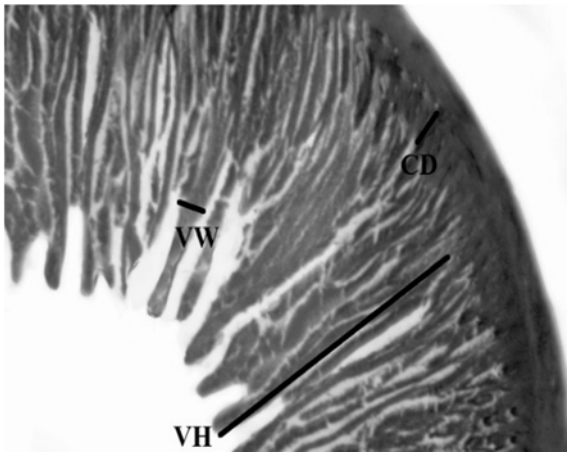


Fig.-2: Measurement of intestinal

Nutrients Digestibility (CP, ME, EE, DM, Ash)

At the end of 36 d of age, one bird per replicate will be transferred to a separate pen and after three days of adaptation period; feces were collected at morning, noon and evening for three consecutive days (39 to 41 d for finisher period). Samples were pooled and stored at −20℃ and further analysis performed by the method of AOAC (2000).

Intestine weight and length

Three birds per every replicates of the four groups were slaughtered and the whole intestinal tract was removed safely, afterward the parts of small intestine (Duodenum, jejunum, ilium) and large intestine. All parts were cut with the help of scissor and faeces were washed with the help of normal saline. After all this steps weight and length of (Duodenum, jejunum, ilium) were recorded.

Data Analysis

The raw data was tabulated in Microsoft excel then analyzed in Two-way analysis of variance (ANOVA) with (JMP, software 7.0 Version) and significant differences were compared through Tukey comparison test.

3. RESULTS AND DISCUSSION

Intestinal Histomorphology

The result regarding effects of heat stress and ascorbic acid supplementation on length, width of villi of duodenum, jejunum and ilium of broiler chickens is presented in **(Table-01).** The average villi size, height, width and crypt to depth of intestine (Duodenum, jejunum, ilium) was observed significantly (P<0.05) higher in heat stress free AA supplementary group.

Table-1. Effects of heat stress and ascorbic acid supplementation on length, width and crypt to depth size of villus of duodenum, jejunum and ileum of broiler chickens.

Items (µm)	Normal Temperature		High Temperature	
	Cont.	AA	Cont.	AA
Duodenum Height	1430±26[b]	1573±25[a]	1383±76[b]	1416±40[b]
Duodenum Width (µm)	157±2[b]	173±5[a]	150±5[b]	151±7[b]
Duodenum Crypt to Depth(µm)	6±0.5[b]	8±0.5[a]	5±1[b]	5±0.57[b]
Jejunum Height (µm)	1266±51[b]	1366±28[a]	1233±20[b]	1250±50[b]
Jejunum Width	153±2.88[b]	166.66±7.63[a]	149±4.50[b]	150.66±4.04[b]
Jejunum Crypt to Depth	5.73±0.64[b]	7.33±0.57[a]	5±1[b]	5.33±0.57[b]
Ilium Height	1163±54.83[b]	1300±50[a]	1100±50[b]	1150±50[b]
Ilium Width	136±5.29[b]	160±5[a]	126±7.63[b]	131±9.64[b]
Ilium Crypt to Depth	5.500±0.5[b]	7±1[a]	4.8±0.35[b]	5±1[b]

[a-b] Means within a column not sharing a common superscript differ at P < 0.05.

Ascorbic acid supplementation significantly increased jejunum muscle layer thickness of birds before heat stress (Hajati *et al.* 2014). There was no difference among jejunum muscle layer thickness, villi width, crypt depth and villi height: crypt depth ratio of broilers after heat stress, However, broilers fed with grape seed extract had higher jejunum villi heights compared to the control group after heat stress (Hajati *et al.* 2014). On the other hand, previous studies on pigs indicated that blood flow to the gastrointestinal tract was reduced during heat stress condition (Hajati *et al.* 2014). This can lead to tissue hypoxia which depletes ATP stores, intra-cellular acidosis, changes ion pump activity and increased permeability of tight junctions of the intestinal epithelium, which can cause the translocation of bacterial and endotoxin across the membrane and into the circulation.

Nutrients Retention

Effects of heat stress and ascorbic acid supplementation on nutrients retention of broiler chickens is presented in (Table-02). Nutrients retention (crude protein, ether extract, crude fiber and metabolized energy) observed significantly (P<0.05) higher in heat stress free AA supplementary group.

Table-2. Effects of heat stress and ascorbic acid supplementation on nutrients retention of broiler chickens.

Nutrients (%)	Normal Temperature		High Temperature	
	Cont.	AA	Cont.	AA
CP	77±1[b]	79±1[a]	73±1.25[c]	75±0.85[b]
EE	69±1[b]	75±1[a]	70±1[b]	73±0.36[a]
CF	33±1[a]	33.30±1[a]	29±0.52[b]	31±0.70[a]
ME	78±1[a]	80±1[a]	70±1[b]	79±1[a]

[a-b] Means within a column not sharing a common superscript differ at P < 0.05.

The researchers stated that lengthening of the villi and a short crypt can lead to better nutrient absorption, decreased secretion in the gastrointestinal tract, increased disease resistance and greater overall performance (Viveros *et al* 2011).Utilization of dry matter, crude protein, and ether extract decrease in laying hens when kept under low ambient temperature (6.2ºC) and ascorbic acid supplementation alleviated negative values to a higher extent than control group and digestibility of nutrients increased with dietary ascorbic acid supplementation in Japanese quail under stress conditions (Sahin, and Kücük 2001) .

Intestine weight and length

Effects of heat stress and ascorbic acid supplementation on weight, length of intestine (Duodenum, jejunum and ilium) of broiler chickens is presented in **(Table-03).** Weight and length of intestine (Duodenum, jejunum, ilium) observed significantly (P<0.05) lower in heat stress control group.

Table-3. Effects of heat stress and ascorbic acid supplementation on weight (g), length (cm) of duodenum, jejunum and ilium of broiler chickens.

Items	Normal Temperature		High Temperature	
	Control	AA	Control	AA
Large Intestine Weight	4±0.1[a]	4.26±0.25[a]	3.30±0.26[b]	3.76±0.5[a]
Large Intestine Length	12.10±0.26[a]	14.20±0.2[a]	11.23±0.25[b]	12.70±0.26[a]
Duodenum Weight	15±1[a]	16.66±1.52[a]	13±1[b]	14±1[a]
Duodenum Length	30±1[a]	31.66±1.52[a]	27±1[b]	28±1[a]
Jejunum Weight	20.66±1.52[a]	22±2[a]	18.66±0.57[b]	19.66±0.57[a]
Jejunum Length	81.66±1.52[a]	85.33±1.52[a]	78.33±1.52[b]	80±1[a]
Ilium Weight	18.33±1.52[a]	19.33±0.57[a]	16±1[b]	18±1[a]
Ilium Length	66.33±1.52[a]	68.33±2.08[a]	65±1[b]	67±1[a]

[a-b] Means within a column not sharing a common superscript differ at $P < 0.05$.

Thermal stress results adverse effects on the absorptive epithelium of the gut, causing decreased villi height and crypt depth (Yamauchi *et al*.2006). (Burkholder et al. 2008) reported decrease in crypt depth when chickens were raised at 30°C for 24 h in comparison to the chickens raised at 23°C and villi height decreased to 18.8% after thermal stress in chicken. Moreover, reports have also indicated that mucosal thickness; instestinal brush border and villi height is also affected by the feed constituents. In addition to this literature on feed additives derived from plant source is not clear on their alleriovating effect on the gut health. Thus it can be inferred that feeding meat type of chickens with feed constituents taken from plant source can result in either increase ,decrease or no change in villi length and crypt depth in intestine. It has been suggested that some components of feeds can affect the mucosa thickness and villi height and intestinal brush border (Jamroz, *et al*.2006). Concerning phytogenic feed additives literature does not draw a consistent picture. Based on literature, feeding broiler chickens and pigs with phytogenic products could cause to increased, unchanged as well as reduced villus length and crypt depth in gut.

It was concluded that 150mg/kg of ascorbic acid supplementation significantly improved the villus size and nutrients digestibility when supplemented in normal temperature, whereas non-significantly improved the weight and length of intestine when supplemented under higher temperature.

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