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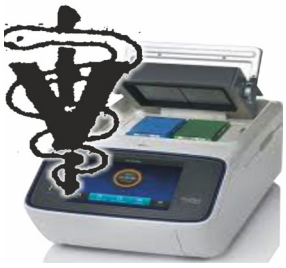

Effects of Crude hesperidin and Ascorbic acid on Carcass characteristics and Haematological parameters of Heat stressed Broiler finisher Chickens

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Abstract

Two hundred and forty-day old un-sexed Arbor acre broilers' chicks were randomly divided into four treatment groups (T1–T4) for the purpose of investigating the effect of crude hesperidin and ascorbic acid supplementation either singly or in combination on carcass characteristics and haematological parameters of broiler finisher chickens. The study was caried out in between the month May and June during hot dry season. Crude hesperidin was extracted from dried orange using the protocol developed by [1] while ascorbic acid was obtained commercially. The test materials were administered through out experimental period at the rate of 20 mg/liter and 200 mg/ liter of water. There was improvement (P<0.05) in carcass characteristics and primal cut of the supplemented groups with highest value obtained in T3 respectively. Pack cell volume, haemoglobin concentration and red blood cell count were significantly (P<0.05) difference relative to the control. The result shows that, crude hesperidin and ascorbic acid either singly or in combination affect carcass characteristic and haematological indices of heat-stressed broiler chickens.

Keywords: Crude hesperidin, Ascorbic acid, Heat-stressed, Finisher chickens

INTRODUCTION

One of the major challenges of poultry production, particularly in the tropical environment, is the problem of heat stress. The broiler chicken industry faces the challenge of heat stress, which induces high metabolic rate, increase production cost and severely reduce meat quality [2] Metabolic changes occur in broiler chickens reared in a heat stress-induced environment, leading to considerable decrease in breast muscle size and protein content [2]. Both acute and chronic heat stresses could cause a sharp decline in the metabolism of birds, resulting in impaired growth performance and carcass quality such as a change in colour, decreases in muscle pH, water-holding capacity (WHC) and juiciness [3]. High ambient temperature causes reactive oxygen species (ROS), which is responsible for lipid peroxidation and severe negative effects on skeletal muscle development [4]. There is, therefore, the need to mitigate the effects of heat stress on broiler production and the quality of the carcass. To achieve this will require oral supplementation with high antioxidant compounds.

Hesperidin, a phytochemical and polyphenolic bioflavonoid flavanone glycoside is a natural compound found abundantly in citrus fruits such as oranges, lemons, tangerines and limes, and it is known to possess significant benefits, such as anti-inflammatory, anti-stress, antioxidant, growth promoting, anticancer and immunological properties [5]. It is chemically referred to as hesperitin-7-O-rutinoside or hesperitin-7-O-rhamnosyl (1,6) glucoside. Oral administration of hesperidin is found to be efficient against inflammation and immunosuppression in poultry [6]. Inspite of the numerous attributes and benefits of broiler chicken production, the performance of the bird is limited by heat stress during the critical dry season, necessitating search for effective antioxidant to ameliorate this problem and its consequences on the productive performance, meat quality and body physiology of the broiler chickens.

Blood is an important index of physiological and nutritional status of animals. A number of studies have been carried out on hematological indices in birds [7]. Reference blood profiles of birds in natural condition at different ages of husbandry period are essential for interpretation of hematological tests [8]. Avian blood differs in cell characteristics from its mammalian counterpart [9]. In chicken, blood constitutes about 12% of the weight of newly hatched chicks and about 6-8% in mature chickens [10]. The platelets (thrombocytes), red blood cells (erythrocytes), white blood cells (leukocytes), non-granular leukocytes and the granular leukocytes are suspended in plasma [11]. Also, many substances are dissolved in the plasma including electrolytes, proteins, lipids, carbohydrates (particularly glucose), amino acids, vitamins, hormones, nitrogenous breakdown products of metabolism and gaseous oxygen, carbon dioxide and nitrogen [11]. Blood serves as vehicle in the body, as it carries oxygen and nutrients to tissues and waste products of metabolism,

which include carbon dioxide and urea to the lungs and kidneys where they are removed [12]. Blood also carries hormones from the endocrine glands to the target organs and its circulation helps to distribute heat around the body from metabolically active and warmer organs to peripheral organs, thereby helping to maintain an even body temperature [12]. Buffers in the blood like haemoglobin, plasma proteins and bicarbonate help to keep the hydrogen ion concentration of the extra cellular fluid at a constant pH. Blood plays a vital and protective function against infection by virtue of its leukocytes and antibodies (immunoglobulins) in the plasma [11]. The homeostatic balance of blood constituents may be upset by impaired function in a multiple of disorders, particularly those involving the lungs, the cardiovascular system, kidney, liver and endocrine organs [13]. Haematological changes are usually used to determine nutritional and pathological status of the body due to impact of environmental stressors [14].

The blood profile of healthy bird is affected by several factors which include induced molting in laying hens [15], acute and extremely high environmental temperature [16], diet contents [17], water and feed restriction [18], fasting [19], age [20], drugs [21], anti-aflatoxin premixes [22] and vitamin supplements [23].

MATERIALS AND METHOD

Determination of Meteorological Conditions of the Experimental Pen

The ambient temperature and relative humidity of the experimental site were measured daily using a hygrometer and mercury thermometer. Measurements were taken five times a day at four-hour intervals (08:00, 12:00, 16:00, 20:00, and 24:00 hours) for seven consecutive days prior to the start of the experiment. Also the same procedure measurement was subsequently repeated throughout the study period. THI was used to measure the thermal comfort of the broiler chickens. It was determined using the equation reported by [24].

$$THI = Tdb - \{(0.31 - 0.31\ RH)\ (Tdb - 14.4)\}$$

Where: THI = temperature humidity index, Tdb = dry bulb temperature (°C), RH = relative humidity (%)/100.

Interpretations:- < 26 = comfort limit, 26-29 = heat stress, > 29 = severe heat stress

Management of experimental birds and treatment

Two hundred and forty un-sexed day old broiler chicks (Arbo acre) were obtained from a commercial hatchery and transported to a poultry farm in Chikuku, Gwagwalada Local Government Area in Abuja. On arrival, the chicks were administered anti-stress (Vitalyte®) and glucose in drinking water to reduce the effect of stress due to transportation. The chicks were weighed individually at the beginning of the experiment using electronic scale. They were then divided into 4

treatment groups (T1-T4) and subsequently assigned to treatments in a completely randomised design. There were 60 chicks per treatment with 3 replicates comprising 20 chicks per replicate. The birds were randomly transferred into a brooder house based on the treatments and were brooded for two weeks in a deep liter. Thus, there were total of 4 brooders (equal to treatment groups) made of plywood board, with. Each brooder partitioned into three replicates to align with the treatments design. Broiler starter diet was fed to the chicks from day 0 to 56 day. Birds were served the experimental diets twice a day, in the morning and in the evening, *ad libitum*. The quantity of feed consumed by the birds were calculated on daily basis by subtracting the leftover of the feed from the quantity initially given. The quantity consumed weekly was calculated by addition of daily intake for seven days' period. The test materials, hesperidin and ascorbic acid, were administered in water to the chicks according to experimental design. Daily dose of crude hesperidin and ascorbic acid were based on recommendations of [25] and [26] at the rate of 20mg and 200mg/kg respectively. Treatment 1 was the control group (T1), with no test ingredient administered. Treatment 2 was crude hesperidin only (T2) administered at 20mg/litre of water. Treatment 3 was crude hesperidin + ascorbic acid (T3) at the dosage rate of 20mg and 200mg/litre respectively. Treatment 4 was ascorbic acid only at the dosage rate of 200mg/litre. To ensure rapid consumption of the test material, water was withdrawn from the birds every evening of the experiment. Electric bulb of 100-Watt capacity was used as a source of lighting for the first 2 weeks of brooding. The lightening duration was 22 hours for the first week of brooding and was stepped down with advance in the age of the chicks. The temperature and relative humidity of the brooder were taken with mercury thermometer and hygrometer throughout brooding period. At the expiration of brooding, chicks were transferred to battery cage following distribution pattern of the brooding stage. Birds were kept under similar conditions of management throughout the experimental period. Strict biosecurity measures were followed throughout the experimental periods. Water troughs and other equipment used were cleaned daily. Birds were kept under close observation throughout the experimental period for abnormal signs. Sick birds were promptly isolated to a sick bay separated from main pen. Dead birds were disposed in an incinerator built at a distance from the poultry house.

Experimental diets

Birds were fed a basal diet formulated according to [27]. Starter diet (0-28 days) containing a crude protein of 22.12 % and metabolisable energy (ME) of 30254 kcal/kg and finisher diet (29 – 56 days) containing 20.46 % crude protein and metabolisable energy of 31279 kcal/kg were given. Table 1 shows the chemical composition of the experimental basal diets.

Table 1: Chemical composition of experimental diets (% DM)

Parameters	Starter mash	Finisher mash
Crude protein (%)	22.12	20.46
Crude fibre (%)	5.04	5.27
Ether extract (%)	4.09	3.12
Ash (%)	5.42	5.87
Nitrogen free extract (%)	52.08	57.11
Calcium (%)	1.42	1.33
Phosphorus (%)	0.75	0.82
Metabolizable energy (Kcal/kg)	30254	31279

Data collection

The following data were collected and determined over a period of four weeks:

Feed intake: Daily feed intake was calculated by subtracting the leftover feed from the quantity initially given.

Body weight gain: Body weight gain was calculated by subtracting the initial body weight from the final body weight.

Feed conversion ratio: Feed conversion ratio was calculated using the formula: FCR = Feed consumed (g) / Body weight gain (g)

Mortality: Mortality was recorded as encountered throughout the experimental period.

Nutrient digestibility: The nutrient digestibility test was carried out according to the method described by [28].

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test post hoc test using the General Linear Model (GLM) procedure of SAS (2003). Mean differences were considered significant at $p<0.05$.

RESULTS

Thermal environmental observation of experimental house

The ambient temperature and relative humidity of the experimental house is as shown in Table 2. Results for Dry bulb temperature (DBT)/ambient temperature, relative humidity (RH) and temperature humidity index (THI) shows fluctuations in obtained values corresponding to different times of the day. However, the observed differences in numerical values of thermal environmental indices of the experimental house were not significantly ($p>0.05$) influenced by the period of collection. The Weekly results followed a similar pattern for DBT, RH and

THI which shows insignificant (P>0.05) differences in the listed variables across the groups for the set period recorded.

Effects of Crude Hesperidin, Ascorbic acid and the Combination of both on Carcass Characteristics and Primal Cut of Heat-stressed Broiler Chickens

Live weight was affected in the order: T3 > T2 > T4 > T1 (P<0.05). Slaughter weight dressed weight, dressing percentage drumstick and thigh were higher in T3 (P<0.05), similar in T2 and T4 but lowest in T1. Breast muscle, wing and back were similar (P>0.05) among the supplemented group but higher when compared with control (P<0.05). Head, neck and shank values were not affected by the treatment (P>0.05) (Table 6).

Table 2: Thermal environmental observation of experimental house

	8:00 hour			12:00 hour			16:00 hour			20:00 hour			24:00 hour		
WK	DBT (°C)	RH (%)	THI	DBT (°C)	RH (%)	THI	DBT (°C)	RH (%)	THI	DBT (°C)	RH (%)	THI	DBT (°C)	RH (%)	THI
1	27.43	57.53	25.62	39.14	62.27	36.24	39.42	62.23	36.49	31.29	62.20	29.21	27.00	63.47	25.57
2	27.08	57.08	25.39	35.28	61.96	32.82	39.00	61.39	36.06	31.42	62.73	29.45	28.02	61.62	29.45
3	26.33	56.33	24.71	37.86	63.96	35.24	39.11	63.01	36.28	30.85	61.01	28.86	24.85	63.08	23.65
4	25.50	59.01	24.09	35.33	60.73	32.78	40.03	65.22	37.27	27.83	64.14	26.34	22.66	64.37	21.75
5	25.91	55.97	24.33	35.02	62.09	32.60	39.72	61.73	36.77	28.01	62.40	26.42	23.07	62.17	22.05
6	24.17	59.45	22.94	38.28	62.11	35.47	40.12	60.97	37.01	29.15	63.05	27.46	24.30	63.92	23.19
7	27.14	60.03	25.56	36.04	63.04	33.55	40.73	63.28	37.73	25.16	60.94	23.86	23.28	60.84	22.20
8	26.02	56.75	24.46	38.11	62.75	35.37	38.41	62.45	35.61	27.13	62.18	25.63	24.62	62.17	23.42
x	26.19	57.77	24.64	36.88	62.36	34.26	39.57	63.54	36.65	28.86	62.33	27.15	24.73	62.71	23.91
±	0.38	0.54	0.32	0.58	0.33	0.52	0.26	0.43	0.24	0.79	0.37	0.69	0.67	0.43	0.90

DBT (ºC) = Dry bulb temperature; RH (%) = Relative humidity; THI = Temperature humidity index; x=Mean; ± = standard error of the mean.

Table 3 : Crude hesperidin, ascorbic acid and the combination on carcass characteristics and primal cut of heat-stressed broiler chickens

Parameter	Treatment				SEM
	T1	T2	T3	T4	
Live weight (g)	2115.06[a]	3410.82[b]	3847.25[a]	3197.09[c]	368.25
Slaughter weight (g)	1997.72[c]	3280.75[b]	3718.49[a]	3072.21[b]	365.56
Dressed weight (g)	1549.28[c]	2764.86[b]	3262.14[a]	2521.84[b]	359.74
Dressing percentage (%)	77.55[c]	84.28[b]	87.72[a]	82.08[b]	2.13
Drumstick (%)	14.47[c]	16.0[b]	19.64[a]	17.93[b]	1.12
Breast muscle (%)	17.57[b]	23.15[a]	27.85[a]	22.64[a]	2.10
Thigh (%)	12.22[c]	15.12[b]	18.79[a]	14.51[b]	1.36
Wing (%)	5.20[b]	8.63[a]	9.14[a]	9.61[a]	1.00
Head (%)	2.24	2.52	2.27	2.48	0.07
Neck (%)	3.81	4.05	3.62	4.13	0.11
Back (%)	14.52[b]	18.04[a]	19.79[a]	17.58[a]	1.10
Shank (%)	3.07	3.85	3.96	3.19	0.23

abc superscripts on the same row = significantly differences at $P<0.05$.

Haematological parameters of heat-stressed broiler chickens administered crude hesperidin, ascorbic acid and the combination

Haematological parameters of heat-stressed broiler chickens administered crude hesperidin, ascorbic acid and the combination is displayed in Table 4. Packed cell volume, haemoglobin concentration and red blood cell values were similar among supplemented groups (P>0.05), The obtained values were significantly (P<0.05) different relative to the control. White blood cell, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were not affected (P>0.05) by the treatments. The outcome of the test ingredients on leucocyte profile of heat-stressed broiler chickens is displayed in Table 5. Monocyte, eosinophil, basophil and lymphocytes values were not significantly ($P<0.05$) affected by supplementation across treatment groups. Heterophil was similar in T2, T3 and T4 while T1 had the highest value. Heterophil/lymphocyte ratio was similar in T2, T3 and T4, however the values were significantly ($P<0.05$) lower in T3.

Table 4: Haematological parameters of broiler chickens administered crude hesperidin, ascorbic acid and the combination

Parameter	Treatment				SEM
	T1	T2	T3	T4	
Pack cell volume (%)	25.91[b]	32.94[a]	35.41[a]	30.18[a]	2.04
Haemoglobin (g/dL)	11.57[b]	15.24[a]	16.71[a]	14.18[a]	1.11
Red blood cell (10^6/µL)	2.69[b]	3.35[a]	3.60[a]	3.20[a]	0.19
White blood cell (10^4/µL)	2.44	2.32	2.65	2.55	0.07
Mean corpuscular volume (fl)	104.57	111.17	108.26	109.01	1.37
Mean corpuscular haemoglobin (pg)	44.97	47.36	49.81	47.75	0.99
Mean corpuscular haemoglobin concentration (%)	42.97	44.19	43.81	41.35	0.63

Means with abc superscripts on same row are significantly (P<0.05) different.

Table 5: Effects of crude hesperidin, ascorbic acid and the combination on leukocyte profile of heat-stressed broiler chickens

Parameter	Treatment				SEM
	T1	T2	T3	T4	
Lymphocyte (%)	48.84	59.14	57.20	55.33	2.24
Monocyte (%)	2.13	3.09	3.50	3.52	0.33
Heterophil (%)	28.16[a]	19.87[b]	18.17[b]	19.63[b]	2.16
Eosinophil (%)	4.38	3.06	3.18	4.29	0.35
Basophil (%)	5.19	4.71	3.96	4.09	0.45
Heterophil/lymphocyte	0.58[a]	0.34[b]	0.32[b]	0.35[b]	0.06

abc superscripts on same row = significant differences at P<0.05; SEM = standard error of the mean

DISCUSSION

The higher intake of feed and nutrients (crude protein, ether extract, crude fibre, calcium and phosphorus) of the treatment groups (hesperidin, ascorbic acid and hesperidin+ascorbic acid) compared to the control group indicates that supplementation of broiler chickens diet with antioxidants at the Finisher phase reduced metabolic rate and heat stress in the birds, and thus enhanced feed intake. Generally, birds eat to meet up with their energy requirements but reduce their feed intake during hot dry season in order to minimize heat increment and metabolic rate, which tend to increase heat loss with consequent enhancement in feed intake. It is documented that heat stressed chickens would usually lower their body temperature by reducing feed intake [29], indicating that control birds must have experienced more heat

stress than the treated birds. Therefore, control birds without antioxidant supplementation to ameliorate heat stress probably reduced their feed intake in order to suppress metabolic rate, excessive heat production and body temperature.

The antioxidants supplementation in this study either singly or in combination had profound, positive effects on slaughter weight, dressed weight, dressing percentage and primal cuts such as drumstick, breast muscle, thigh, wing and back relative to the control carcass. Dressing percentage is generally influenced by several factors particularly live weight and dressed weight. The higher dressing percentage of the antioxidant-supplemented groups could, therefore, be attributed to their higher slaughter and dressed weights, which are the two major determinants of dressing percentage. The result obtained is similar to the work of [30], who found significant increase in dressing yield, breast meat, total meat and wing meat of heat-stress broiler chickens that received vitamin C in drinking water. It also supports the works of [31], who recorded higher carcass yield and performance in birds supplemented with ascorbic acid (250 mg/kg) under high ambient temperature. The similar result obtained in crude hesperidin and ascorbic acid indicated that neither of the test material (hesperidin nor ascorbic acid) is superior to each other in affecting carcass characteristics. This implies that crude hesperidin could serve as alternative antioxidant to ascorbic acid, without negatively affecting carcass characteristics of broiler chickens. There was a positive synergy between both antioxidants making the combination, more potent than either of the separate antioxidants compounds. This synergy corraborates the findings of [32] who documented an increase in carcass weight of heat stress broiler chickens administered a mixture of genistein and hesperidin. However, there is a contradiction with the result of [33], who find no significant effect in the carcass characteristics of broilers chicken given quercetin and rutin mixture. The non-significantly affected head, neck and the shank across the treated groups was in consonance with the work of [33], who find no difference in the primal cut of broiler chickens administered mixtures quercetin and rutin supplementation.

The use of blood analysis may be the readily and faster means of assessing clinical and health status of animals. This is because ingestion of dietary components has a profound effect on the blood chemistry [34]. The use of natural substances on haematological status has been shown to have significant effect on blood profile [35].

The higher PCV, Hb and RBC in the antioxidant supplemented birds relative to control may be as a result of protective effects of the antioxidant on the stem cell against heat stress, which in turn influences haematopoiesis. These values were comparable to earlier results [36], which indicated that the inclusion of oregano essential oil at 500 ppm significantly increased RBC, Hb and PCV. A

similar result was also reported [37], and it was found that broiler chicks fed thyme oil (100–200 ppm) had significantly ($P<0.05$) higher RBC, PCV and Hb counts compared to the control. The values of RBC, PCV and Hb concentrations in the treatment groups were within the normal physiological range for broiler chickens [38]. Previous studies remarked that normal RBC, Hb, PCV and platelets values are indications of the absence of haemolytic anaemia or depression of erythropoiesis and this removed possible fears of imminent hazard that may be associated with the use of phytochemicals [39].

WBC, MCV, MCH and MCHC were not affected significantly ($P>0.05$) by the treatment across the groups but were within the normal physiological range for broiler chickens [38]. According to [38] abnormal counts of RBC and volumes of PCV, MCV, MCH and MCHC may indicate anaemia, liver damage and distortion of the immune status or low protein in feed. Therefore, the normal values may reveal adequate nutrient consumption and uncompromising immune status of the birds.

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