

# Comparative effect of African locust bean, baobab and *Prosopis africana* pulp meal supplementation as natural ascorbic on blood constituent of broiler chickens raised under high temperature

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**ABSTRACT:** The experiment was conducted to evaluate the effect of African locust beans, baobab and *Prosopis africana* pulp meal supplementation as natural ascorbic acid on blood constituent of broiler chickens raised during the hottest months April-May with an average temperature of about 40°C. One hundred and eight (108) broiler chickens (marshall) were allotted to four dietary treatments replicated three times with (9) birds per replicate in a completely randomized design (CRD). The inclusion levels of the Synthetic Ascorbic Acid (SAA) and Natural Ascorbic Acid (NAA) in the water were 0 g/l, 40 g/l, 40 g/l and 40 g/l designated as treatments 1 (control), 2 (African locust beans), 3 (baobab), and 4 (*Prosopis africana* pulp) respectively. The experiment lasted for eight (8) weeks. The haematological and biochemical indices of the birds were determined. The result revealed significant ( $p>0.05$ ) difference in the haematological indices. The serum biochemistry indicated significantly ( $p<0.05$ ) higher: glucose (163.34 g/dl); cholesterol (183.26 g/dl and urea (4.33 mg/dl) in all the treatments. There were no significant ( $p>0.05$ ) difference among the treatment for total protein, albumin and globulin. This study revealed that SAA and NAA could be fed to broiler chickens up to 40 g/l without detrimental effect and boost the defensive mechanism of the bird raised in hot climatic environment.

**Keywords:** Anti-oxidants, blood parameters, broiler Chickens, heat stress.

## INTRODUCTION

Heat stress is of great concern in the poultry industry since it can cause major economic losses (Panaite *et al.*, 2018). Their thermo neutral zone for maximize growth is between 18–21°C (Naga Raja Kumari and Narendra Nath, 2018). Any environmental temperature higher than 25°C elicits heat stress in poultry (Wasti *et al.*, 2020). Rafiee *et al.* (2016) showed that heat stress results to reduced feed intake, body weight gain, carcass yield, feed conversion ratio of broilers and increased mortality. Heat stress resulted in a marked change of bacterial composition in chicken intestine, which was subsequently associated with depression of body-weight gain. Cumulative feed intake and weight gain were depressed in old broiler chickens by about 16 - 53 gm per bird per every 1°C rise in temperature, respectively (Sabah Elkheir *et al.*, 2008).

Generally, in hot environment, emphasis should be placed on diets to increase intake or to alter levels of vitamins, protein, amino acids or other nutrients to improve the conversion of feed units into production units (Sabah Elkheir *et al.*, 2008). Since poultry farmers cannot afford expensive artificially temperature-controlled sheds, heat stress effects on broiler production and physiology are negative (Khan *et al.*, 2011). Although poultry can synthesize vitamin C, the amount is limited during heat stress conditions (Khan *et al.*, 2012a). Thus, dietary supplementation of vitamin C is an effective strategy to reduce the harmful effects of heat stress in poultry. Supplementation of vitamin C (250 mg/kg of feed) improved growth rate, nutrient utilization, egg production, and quality, immune response, and antioxidant status in

heat-stressed birds (Wasti *et al.*, 2020). Studies have indicated that the tamarind fruit contains elevated titratable acidity, is rich in pectin, B-complex, vitamins and minerals, and contains carotenoids and vitamin C. Thus, it can be considered as important food in traditional diets (Hamacek *et al.*, 2012). Hence the work was carried out to investigate the effect of tamarind pulp meal as a source of vitamin C in reducing the effect of heat stress on biochemical and haematological parameter in broiler chickens. Therefore, the objective of the study is to determine the effectiveness of tamarind pulp in ameliorating heat stress in broiler chickens.

## MATERIALS AND METHODS

The study was carried out at the poultry unit of Federal University Gashua Teaching and Research Farm, Yobe State. Located between latitude 12.8765°N and longitude 11.0316°E. The area falls within the Sahelian region of West Africa which is noted for its great climatic and seasonal variations. Annual rainfall ranges from 500-1000 mm. While the average ambient temperature varies range between 23-28°C in December -January and reaches up to 38-45°C in March -April. The mean relative humidity ranges from 30-35% with minimum in February-April when it drops to as low as 10% and maximum of about 70% in August (Google map, 2023).

### Preparation of the test solution

African locust beans, baobab and *Prosopis africana* pulp meal were collected from Federal University Gashua, sun dried for 72 hours and the pulp was separated from the seed. The pulp was grinded and sieved to make it into powder and store in polythene bags before used.

### Experimental bird's management

One hundred and eight (108) broiler chickens were allotted to four treatments replicated three times with (9) birds per replicate in a completely randomized design (CRD). The experiment lasted for eight (8) weeks (four weeks each for the starter and finisher). Feed and clean water were supplied *ad libitum*. The birds were reared intensively on deep litter (dried wood shavings) housing system. Normal vaccination program and medication schedule were strictly adhered to.

### Experimental diets

The inclusion levels of the Natural Ascorbic Acid (NAA) were 0 g/L, 40 g/l, 40 g/l and 40 g/l for treatment 1 (control), 2 (African locust beans), 3 (baobab), and 4 (*Prosopis africana* pulp) respectively.

## Data collection and laboratory analysis

Blood sample were collected through the wing vein using sterile needle (2 guage syringe from birds fasted overnight. Sample collected with Ethyl diquine tetra acetic acid (EDTA) for hematological analysis while bottle without EDTA were used to collect sample for serum biochemistry. Haematological procedure were carried out according to Jain (1986) in determining the total red blood cell (RBC), white blood cell (WBC) and packed cell volume (PCV). From the data generated on the Hb, RBC and PVC, the mean corpuscular heamoglobin, MCH [(PCV-RBC) $\times 10^3$ ], mean corpuscular volume MCV [(PCV-RBC)  $\times 10^3$ ] and mean corpuscular heamoglobin concentration, MCHC [(Hb-PCV)  $\times 100$ ] were calculated (Jain 1986). Serum biochemical analysis; blood sample collected without EDTA were used to determining the biochemical component such as total protein, albumin, globulin, glucose, cholesterol and urea using the method described by Uko *et al.* (2000).

### Data analysis

All data collected were subjected to analysis of variance using general linear model SAS (2007). Comparative analysis of variance for the responses of birds to the control, ascorbic acid and tamarind fruit pulp were performed. Where significance difference observed, means were separated using Duncan Multiple range Test (Steel and Torrie, 1980).

## RESULT AND DISCUSSION

### Hematological indices of broiler chicken fed ascorbic acid and tamarind pulp meal

The values recorded for PCV (36.00 – 39.99%), Hb (13.56 – 14.40 g/dl) and RBC (4.10 – 4.84  $\times 10^6$ /ml) were presented in Table 1. The values recorded fall within the normal range of PCV (35.9-41.0%), Hb (11.60-13.68 g/dl), RBC (4.21-4.84  $\times 10^6$ /ml, WBC (4.07-4.32  $\times 10^3$ /ml), MCV (81.60-89.10 Fl), MCH (27.20-28.90 pg) and MCHC (32.41-33.37%) in broiler chickens (Campbell, 2013). Significantly ( $p < 0.05$ ) lower values of PCV (%) and Hb (g/dl) were recorded in birds fed test diet compared with control. The finding is in agreement with the work of Odo *et al.* (2019) who found significance ( $p < 0.05$ ) increase in PCV and Hb in the heat stress broiler chickens when compared with the control. They postulated that it could be probably due to decrease in oxygen uptake as a result of high ambient temperature or by increased haemo-concentration following possible dehydration occasioned by the heat stress. The significantly ( $p < 0.05$ ) higher PCV and Hb values recorded in birds fed control diet compared with those fed test diets in the present experiment could be an adaptive mechanism to consumed more water that was necessary for evaporative cooling process, which

**Table 1.** Effect of feeding ascorbic acid and tamarind pulp meal on hematological indices of broiler chicken in hot-dry season.

Parameters	T1 (0g)	T2 (40g)	T3 (40g)	T4 (40g)	SEM
PCV (%)	39.99 <sup>a</sup>	36.33 <sup>b</sup>	36.00 <sup>b</sup>	37.66 <sup>b</sup>	2.35
Hb (g/dl)	14.40 <sup>a</sup>	12.93 <sup>b</sup>	13.90 <sup>b</sup>	13.56 <sup>b</sup>	2.37
RBC (10 <sup>6</sup> /ml <sup>3</sup> )	4.00 <sup>b</sup>	4.17 <sup>a</sup>	4.25 <sup>a</sup>	4.10 <sup>a</sup>	0.65
MCV (fl)	89.67 <sup>b</sup>	85.33 <sup>a</sup>	84.13 <sup>a</sup>	85.67 <sup>a</sup>	4.57
MCH (pg)	27.30 <sup>b</sup>	28.13 <sup>a</sup>	28.00 <sup>a</sup>	28.29 <sup>a</sup>	0.64
MCHC (%)	33.36 <sup>b</sup>	32.50 <sup>a</sup>	32.13 <sup>a</sup>	32.95 <sup>a</sup>	1.87
WBC (10 <sup>3</sup> /ml <sup>3</sup> )	4.30	4.15	4.14	4.04	0.59

PCV=Pack cell volume, Hb=heamoglobin, MCV=mean corpuscular volume. MCH=mean corpuscular heamoglobin, MCHC= mean corpuscular heamoglobin, WBC=white blood cell, RBC=red blood cell.; Heamoglobin (Hb).

agreed with the work of Jaiswal *et al.* (2017) who reported significant ( $p < 0.05$ ) higher PCV and Hb in birds exposed to heat stress than unexposed birds but contrary to the work of Altan, *et al.* (2000) who reported that broilers subjected to heat stress had reduced PCV and Hb.

Significantly ( $p < 0.05$ ) higher values of RBC were recorded in birds fed test diets when compared with control. This may be attributed to the ability of the ascorbic acid in maintaining the integrity of erythrocyte (RBC) membrane and also been able to scavenge for enormous amount of free radicals which is more in the test ingredient. This finding agreed with the work of Al-Haidary (2004) who reported that RBC was higher in broiler that received vitamin C than the control and also with the report of Dinu *et al.* (2007) who stated that exposure to thermal stress significantly ( $p < 0.05$ ) reduced the level of RBC. The size of RBC will alter the viscosity of blood fluids, which might interfere with the activity and smooth blood circulation, and frequently influenced by ambient temperature (Biswas *et al.*, 2012).

The MCV, MCH and MCHC values ranged from 84.33 – 89.07 fl, 27.30 – 28.00 pg and 32.13 – 33.36% respectively as shown in Table 1. All the parameters fall within the normal range reported by Campbell (2013) who found MCV (81.60-89.10 Fl), MCH (27.20-28.90 pg) and MCHC (32.41- 33.37%) in broiler chickens. The results indicate significantly ( $p < 0.05$ ) that higher values were recorded in birds fed test diets when compared with the control. This finding agreed with the work of Jaiswal *et al.* (2017) who reported that broiler chickens fed vitamin C recorded significantly ( $p < 0.05$ ) higher values in terms of MCV, MCH and MCHC when compared with the control treatment in hot temperature. MCV can rise as a results of heat stress (Mohammed *et al.*, 2012). MCH value was used to determine RBC and Hb content, whereas the MCHC values was used to determine the RBC and Hb concentration (Pandian *et al.*, 2012).

The WBC recorded in this study (Table 1) range between 4.04 – 4.30 (10<sup>3</sup>/ml<sup>3</sup>) which fall within the normal range of 4.07-4.32 X 10<sup>3</sup> /ml (Campbell, 2013) and found to be similar ( $p > 0.05$ ) across the treatments groups but numerically decreased in birds fed control diet. The

decreased in WBC in heat stress treatment probably was as the result of immuno-supressive effect of corticosteroids which are usually secreted in large amount during stress including heat stress (Mitral *et al.*, 1972 cited by Odo *et al.*, 2019). The finding is in agreement with the work of Jaiswal *et al.* (2017) who reported significantly ( $p < 0.05$ ) higher values in birds fed vitamin C when compared with the control. The amount of WBC is heavily influenced by a variety of factors including heat stress, the WBC is an active component of the body defense mechanism.

### Serum biochemistry indices of broiler chicken in hot-dry season

The values of total protein, albumin and globulin recorded in this study ranges between 4.00 – 4.72 g/dl, 1.98 --- 2.20 g/dl and 2.02 – 2.53 g/dl respectively (Table 2). Although numerically higher values were recorded in birds fed test diets compare to control, however, their levels did not differ significantly ( $p > 0.05$ ) among the treatment group and fall within the normal range of 4.63 – 4.81 g/dl (Wikivet, 2013). This indicated that experimental diet had positive effect on serum chemistry of broiler chicken raise under heat stress. This agreed with the work of Nayila (2020) who reported increase in serum chemistry when broiler chickens were fed vitamin C supplement. Similar report was observed by Gharieb and Moursi (2013) who found the increase in values of total protein, albumin and globulin from 4.19 to 4.33 g/dl, 2.42 to 2.45 g/dl and 1.77 to 1.88 g/dl respectively when vitamin C was administered to broiler chicken under heat stress compared to control. The result also agreed with the report of Biradar *et al.* (2017) who found non-significant ( $p > 0.05$ ) different when layers fed diet containing tamarind pulp meal. This study disagreed with the work of Evelyn (2014) who recorded decreased in total protein, albumin and globulin level with increasing tamarind levels in the diet.

The values recorded for glucose range between 146.76 – 163.34 g/dl (Table 2). Significantly ( $p < 0.05$ ) lower values were recorded in bird fed test ingredient compared to

**Table 2.** Effect of feeding Natural ascorbic acid on serum biochemistry indices of broiler chicken in hot-dry season.

Parameters	T1 (0g)	T2 (40g)	T3(40g)	T4 (40g)	SEM
TP (g/dl)	4.64	4.66	4.73	4.78	2.80
Alb (g/dl)	1.98	2.19	2.10	2.20	0.98
Gluc. (g/dl)	163.34 <sup>a</sup>	152.43 <sup>b</sup>	150.90 <sup>b</sup>	149.76 <sup>b</sup>	6.91
Glob. (g/dl)	2.46	2.47	2.63	2.53	1.04
Chol. (g/dl)	183.26 <sup>a</sup>	181.76 <sup>b</sup>	174.03 <sup>b</sup>	170.13 <sup>b</sup>	10.9
Urea(mg/dl)	4.33 <sup>a</sup>	2.70 <sup>b</sup>	1.76 <sup>b</sup>	1.70 <sup>b</sup>	0.95

TP= Total protein ALB= Albumin, Gluc = Glucose, Glob = Globulin, Chol = Cholesterol.

control and fall within the normal ranges of 152 to 182 g/dl (Mitruka and Rawnsley, 1977). This study agreed with the work of Gharieb and Moursi (2013) who reported significant ( $p < 0.05$ ) increase in glucose levels in birds raise under heat stress (180 g/dl) compare with birds fed vitamin C supplement (160 g/dl) and also Borges (2007) who found significant ( $p < 0.05$ ) increased in glucose levels of birds raise under heat stress and explained that high glucose levels is an indication of direct response of birds to greater adrenalin, noradrenalin and glucocorticoid secretion in stressful condition which was needed to prepare birds for a fight and flight response (Gharieb and Moursi, 2013).

The values for globulin recorded in this experiment ranges from 2.46 – 2.63 (g/dl) and found to be insignificant ( $p > 0.05$ ) across the treatments group though numerically high values were found in birds fed test ingredients.

The values for cholesterol ranges from 170.13 to 183.26 (Table 2). Significantly ( $p < 0.05$ ) lower values were recorded in birds fed test diets which indicates efficient protein metabolism, this might be due to the function of vitamin C in transforming cholesterol to bile acids by controlling the microsomal  $7\alpha$ -hydroxylation as the reaction is the rate limiting step of cholesterol catabolism (Opoola *et al.*, 2018). The decreased in cholesterol concentration observed as dietary levels of ascorbic acids increased in this study was in agreement with the work of Sahin *et al.* (2003) while significantly ( $p < 0.05$ ) higher value recorded in the control diet might be as a results of ascorbic acid deficiency that induces a marked slowing down of this reaction leading to cholesterol accumulation in the lever and blood (Naidu, 2003). It also indicates that test ingredient possesses hypocholesterolaemic properties, which lower serum cholesterol level (Evelyn, 2014). Similar results was reported by Evelyn (2014) who observed significant ( $p < 0.05$ ) different in cholesterol when *Tamarindus indicus* fruit were fed to layers but contrarily to the work of Shinde *et al.* (2015) who found a significantly ( $p < 0.05$ ) reduction in total cholesterol levels after supplementation of tamarind pulp extract at different levels in broilers.

The blood urea recorded in this study ranged from 1.70 to 4.33 mg/dl (Table 2). Significantly ( $p < 0.05$ ) higher value were recorded in birds fed control diet but found to be similar ( $p > 0.05$ ) across the treatment groups fed test diets.

Significantly ( $p < 0.05$ ) lowered levels of urea was due to roles of vitamin C present in test materials in reducing the negative impact of heat stress, and fall within the normal range of 1.9 – 12.5 given by Clinical Diagnostic Division (1990) for uric acid. A low urea indicated more efficient metabolism, proper renal and hepatic function (Adeyemo and Sani, 2013). Xue, *et al.* (2017) found an increased in urea production while broiler chickens underwent heat stress. This study agreed with the work of Olugbemi *et al.* (2019) who reported significantly ( $p < 0.05$ ) lowered values in birds fed Boabab (*Adonsonia digitata*) pulp meal.

## Conclusion

From the results obtained in this study, it can be concluded that African locust beans, baobab and *Prosopis africana* pulp in drinking water of broiler chickens can beneficially influenced heamatology and blood chemistry of broiler chickens raised under adversely heat stress which mostly experience in the semi-arid zones that limited broiler chicken production.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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