Comparative Clinical Pathology (2022) 31:427–438
https://doi.org/10.1007/s00580-022-03342-4

ORIGINAL ARTICLE

Haematological and biochemical parameters of broiler chickens subjected to feed restriction during the hot-dry season and administered L-serine

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Received: 21 December 2021 / Accepted: 22 March 2022 / Published online: 5 April 2022

Abstract

Effects of L-serine on haematological and biochemical parameters in 120 broiler chicks subjected to feed restriction were investigated during the hot-dry season. Thirty birds each were used: group I, feed restriction; group II, feed restriction + L-serine (200 mg/kg); group III, *ad libitum*; and group IV, *ad libitum* + L-serine (200 mg/kg). Oral administration of L-serine and 20% feed restriction were performed on days 1–14 and 7–14, respectively. On days 21, 28 and 35, blood samples were evaluated for erythrocyte and leucocyte counts, erythrocytic indices, protein concentrations and activities of alanine (ALT) and aspartate aminotransferase (AST). Temperature-humidity index (31.25 ± 1.43) shows that broiler chickens were exposed to heat stress. Packed cell volume, erythrocyte and haemoglobin values were higher ($P < 0.05$) in AL + L-serine and FR + L-serine than AL or FR broiler chickens. Heterophil/lymphocyte ratio was lower ($P < 0.05$) in FR + L-serine than FR or AL broiler chickens. Mean corpuscular volume and mean corpuscular haemoglobin were highest ($P < 0.05$) in AL broiler chickens than any other group. Total protein concentration increased ($P < 0.05$) in FR + L-serine and AL + L-serine, compared to FR and AL broiler chickens. Activities of AST and ALT were higher ($P < 0.05$) in FR and AL than FR + L-serine and AL + L-serine broiler chickens. It was concluded that L-serine increased packed cell volume, erythrocyte, haemoglobin and protein values, but decreased heterophil/lymphocyte ratio and ALT and AST activities in broiler chickens, exposed to feed restriction and heat stress.

Keywords Haematology · Biochemistry · Feed restriction · L-serine · Broiler chickens · Hot-dry season

Introduction

Environmental and nutritional factors may adversely affect broiler chicken production by lowering its output. Heat stress occurs when broiler chickens are reared in pens with ambient temperatures above the thermoneutral zone (Ding et al. 2020), resulting in significant economic losses (Ma et al. 2021). Broiler chickens fail to maintain their normal body temperature under unfavourable thermal environment conditions of high ambient temperature and high relative humidity, which reduce heat loss and induce oxidative stress (Zeitz et al. 2020). Oxidative stress occurs due to increased production of reactive oxygen species (ROS), culminating in lipid peroxidation and cell damage (Toyomizu et al. 2019). Heat stress increases the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatine phosphokinase in broiler chickens (Hosseini-Vashan et al. 2016). Increased plasma or serum enzyme activity has been associated with tissue damage and disease states (Ghorbani et al. 2017). Nutritional stress such as starvation increases corticosterone release, an indicator of stress in broiler chickens, which may overwhelm antioxidant capacity and impair health and productivity (Yan et al. 2021). Antioxidants, such as L-serine, may ameliorate the negative effects of heat and nutritional stresses. L-serine is a naturally occurring amino acid that exhibits antioxidant activities and lowers corticosterone level in stressed situations in mice (Zhou et al. 2017; Wu et al. 2019). Several amino acids including glycine and cysteine, which are limiting substrates for glutathione synthesis, are formed from L-serine (Zhou et al. 2018). Therefore, supplementing with L-serine may reduce oxidative

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stress by raising antioxidant levels in broiler chickens. L-serine is involved in cellular proliferation and metabolism by enhancing biosynthesis of purines and pyrimidines (Ma et al. 2017). Feed restriction, especially early in life, is a management technique for adjusting the feeding schedule to alleviate the detrimental effects of heat stress in broiler chickens. It lowers the metabolic activity and body temperature, but increases the survival rate of broiler chickens raised under heat-stressed conditions (Wasti et al. 2020). Haematological and biochemical responses are a reliable diagnostic tool for evaluating stress and health states in broiler chickens (Abudabos et al. 2018). Stress is determined by haematological parameters particularly the heterophil/lymphocyte ratio (H/L), which is a biomarker of stress in broiler chickens (Scanes 2016; Egbuniwe et al. 2018a). The hot-dry season in the Northern Guinea Savannah zone of Nigeria, where Zaria is located, has been described to be thermally stressful to broiler chickens. It extends from March to April (Dzenda et al. 2013; Egbuniwe et al. 2018b) and shown to be thermally stressful, adversely affecting the physiological parameters in broiler chickens raised in the zone (Aluwong et al. 2017; Sinkalu et al. 2020). The effects of L-serine on haematological and biochemical parameters in broiler chickens have not been investigated. The current study was carried out to assess the efficacy of L-serine in reducing stress-induced responses on the parameters in broiler chickens during the hot-dry season.

Materials and methods

Experimental site

The research was carried out in a poultry pen in March and April, 2021 during the hot-dry season (Dzenda et al. 2013) in the Department of Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria (11°10′N, 07° 38′E), in the Northern Guinea Savannah zone of Nigeria.

Experimental animals and management

A total of 120 day-old, apparently, healthy broiler chicks (Arbor Acres), which consisted of both sexes, served as experimental subjects. The broiler chickens were kept in the Faculty of Veterinary Medicine's poultry pen, which was divided into four and littered with sawdust. They were given time to acclimatise before being fed commercial poultry feed and water. The broiler chickens were given broiler starter (days 0–28) and finisher (days 29–35) (Table 1).

The experimental broiler chickens were divided into four groups of 30 birds, each using basic randomisation: group I: water *ad libitum* + 20% feed restriction (that is, 80% of the feed consumed *ad libitum*); group II: feed and water *ad libitum*; group III: water *ad libitum* + 20% feed restriction (that is, 80% of the feed consumed *ad libitum*) + L-serine (200 mg/kg, Sim et al. 2015); and group IV feed and water *ad libitum* + L-serine (200 mg/kg). Broiler chickens were subjected to feed restriction for 1 week (days 7–14 of rearing). From the age of one day, L-serine was given daily and orally for only 14 days. Each broiler chick was tagged on the leg with masking tape for easy identification and correct record-keeping. The experiment was conducted under strict and standard biosecurity measures.

Table 1 Composition and proximate analysis of broiler chicken diets

Feed Composition	Starter	Finisher
Ingredients (%)		
Crude protein	22.00	18.0
Crude fat	4.50	5.50
Crude fibre	5.00	5.00
Calcium	1.10	1.0
Phosphorus	0.50	0.43
Lysine	1.33	1.05
Metabolisable energy (kCal/kg)	3000.00	3200.00
Proximate analysis (%)		
Dry matter	93.60	94.36
Crude protein	24.00	20.15
Crude fibre	5.10	5.23
Oil	3.60	3.10
Ash	6.50	5.00
NFE	60.80	66.52
Calcium	0.09	0.23
Phosphorus	0.46	0.59

NFE nitrogen-free extract

Analysed in the Nutrition Laboratory, Ahmadu Bello University, Zaria, Nigeria

Thermal environmental conditions of the experimental site

The dry-bulb temperature (DBT) and wet-bulb temperature (WBT) (Brannan®, Cumbria, England) were recorded thrice each day, at 07:00 h, 13:00 h and 18:00 h, inside the poultry pen by a wet- and dry-bulb thermometer (Brannan®, Cumbria, UK), placed 1 m above the ground. The relative humidity (RH) was calculated using Osmon's hygrometric table (Narinda Scientific Industries®, Haryana, India). The temperature-humidity index (THI) was calculated according to the following formula (Tao and Xin 2003):

$$\mathrm{THI} = 0.85\ (\mathrm{Tdb}) + 0.15\mathrm{Twb}\ \ (\text{for broilers}).$$

where THI = temperature-humidity index for broiler chickens, Tdb = dry-bulb temperature and Twb = wet-bulb temperature. The parameters were recorded inside the pen on each experimental day.

Determination of haematological parameters

Blood sample collection

Exactly 4 mL of blood sample was obtained from the wing vein of 7 broiler chickens from each group at 21, 28 and 35 days of age. Out of this volume, 2 mL was dispensed into a heparinised tube, while the rest 2 mL was placed in a non-heparinised tube. The blood samples were transferred to Research Laboratories of the Departments of Veterinary Physiology, and Veterinary Pathology, ABU, Zaria, Nigeria. Blood samples without anticoagulant were allowed to clot before centrifugation at 3000 *g* for 10 min, after which the serum was harvested and stored at 4°C until analysis (Ramnath et al. 2008).

Evaluation of packed cell volume

The packed cell volume (PCV) was measured using the method outlined by Rehman et al. (2003). Heat was used to seal the end of an empty capillary tube after it had been filled to about 3/4 of its length. Thereafter, using a Saitexiangyi TG12MX® Micro-haematocrit centrifuge (Shanghai, China), blood was spun for 5 min at 4,383 *g* for 5 min. The proportion of cells in the blood in percentages was determined using the Micro-haematocrit Reader (Hawksley®, Sussex, UK).

Determination of erythrocyte and total leucocyte counts

The erythrocyte and total leucocyte counts were evaluated using haemocytometer as described by Campbell and Ellis (2007). The heparinised blood was briefly agitated before being pipetted to the 0.5 marker with an erythrocyte dilution pipette. The tip of the pipette was cleaned with tissue paper before pipetting the diluting solution (Natt-Herrick) to the 101 mark (1:200), without entirely immersing the tip of the pipette in the fluid. It was poured into a clean bottle after shaking the mixture for 1 min to ensure even dispersion. A tiny portion of the mixture was extracted using a capillary tube after slight agitation. The haemocytometer was filled up, on both sides and left for 5 min.

The leucocytes were viewed by light microscope (Olympus-XSZ-107BN, Zenithlab Co., Ltd., Changzhou, China) at low power magnification (×40) and counted using tally counter. For total leucocyte count, the larger squares of the haemocytometer were counted and calculation was carried out as follows:

$$N/20 = Leucocytes \times 10^9/L,$$

where N = number of leucocytes in four large squares (64 tiny squares)

For erythrocyte count, cells counted in four corners were those in central squares and mid-section of the haemocytometer. The erythrocyte count was obtained as follows:

$$N/100 = Erythrocyte \times 10^{12}/L,$$

where N = number of erythrocytes counted in five squares attached to mid-section of the haemocytometer equivalent to 160 squares. The haemocytometer charged slides were counted for the erythrocytes and total leucocytes, and mean values were obtained.

Determination of differential leucocyte count

The leucocyte count was calculated as described by Campbell and Ellis (2007). Briefly, a pair of smear was prepared from each blood sample. Exactly 2 µL of blood was used to prepare each blood smear. The smears were air-dried and labelled clearly. Thereafter, the fixation was carried out for 3 min and air-dried in a fixing jar, which contained methanol. The slides were stained with Wright-Giemsa stain for 3 min. An equal amount of Sorensen's buffer (pH 6.8) was added, and the mixture was carefully mixed by blowing and using a pipette until a green metallic sheen appeared on the surface. Smears were left for another 6 min before being rinsed with Sorensen's buffer. To remove stains, the back of each smear was wiped with tissue paper and allowed to air dry. Then, the slides were stored in a box until they were examined under a microscope.

The blood smears were examined by a light microscope (Olympus-XSZ-107BN, Zenithlab Co., Ltd., Changzhou, China) using high-power magnification with oil immersion (×1000). A total of 100 leucocytes was counted and categorised based on morphologic characteristics (Campbell and Ellis 2007). The leucocytes were counted using the Marble® Blood Cell Calculator (Chicago, USA). The differential leucocyte count was calculated as a percentage of distinct cell groupings. The absolute counts were computed using the percentage and a reference to the total leucocyte count as follows:

$$Percentage\ of\ leucocyte\ counted \times Total\ leucocyte/100 = Absolute\ number \times 10^9/L.$$

Determination of haemoglobin concentration

The concentration of haemoglobin (Hb) in each blood sample was determined colorimetrically as cyanomethaemoglobin, adopting the Drabkin technique by Kwiecien et al. (2015). A total of 5 mL of Drabkin solution, containing of

potassium cyanide and potassium ferricyanide, was placed in test tubes using a 5-mL syringe. A micropipette was used to measure exactly 20 µL of blood sample, which was then added to the Drabkin solution and gently shaken. To avoid the empty erythrocytes from interfering with the readings, the tube was centrifuged at 1,509 × *g* for 15 min. The mixture was then absorbed into the haemoglobin meter (XF-1C, Perlong Medical Equipment Co., Ltd., Nanjing, China) after the supernatant was transferred to a sample vial. The haemoglobin concentration (g/dL) was obtained when the wiggling pump was turned off.

Evaluation of erythrocytic indices

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were obtained as follows (Campbell and Ellis 2007):

$$MCV = (PCV \times 10)/Erythrocyte = MCV\ (fl).$$

$$MCH = (Hb \times 10)/Erythrocyte = MCH(pg).$$

$$MCHC = (Hb \times 100)/PCV = MCHC(g/L).$$

Serum biochemical parameters

Total protein (TP) and albumin concentrations and alanine and aspartate aminotransferase (ALT and AST, respectively) activities were measured in the serum using a spectrophotometer (Agappe Diagnostic Ltd., Ernakulam, Kerala, India). The difference between total protein and albumin concentrations was globulin concentration (Coles 1986).

Data analyses

The data obtained from the laboratory experiment were expressed as mean ± standard error of the mean (mean ± SEM). Values were subjected to two-way analysis of variance (ANOVA), followed by Bonferroni *post-hoc* test to compare means of the group differences. Data were analysed by GraphPad Prism 8.02 Software for windows (GraphPad Software, San Diego, CA, USA). Values of $P <$ 0.05 were considered significant.

Results

Thermal environmental parameters in the poultry pen

The THI obtained during the study period inside the pen was 31.25 ± 1.43, with a range of 22.65–35.90.

Effect of L-serine on erythrocytic parameters of broiler chickens subjected to feed restriction during the hot-dry season

The PCV on day 21 was significantly ($P < 0.05$) higher in AL (36.00 ± 1.15%) and AL + L-serine (34.67 ± 1.33%) groups, when compared with FR (31.67 ± 0.88%) and FR + L-serine (30.33 ± 2.03%) groups. The highest PCV value was recorded in AL + L-serine group, compared to any other group on days 28 (42.0 ± 2.65%) and 35 (39.00 ± 6.81%). The overall mean PCV was highest in AL + L-serine group (38.56 ± 2.13%), compared to any other group (Table 2).

The RBC count was also highest in AL + L-serine broiler chickens on days 21 (5.32 ± 0.89 × 10^6/µL) and particularly on day 35 (5.43 ± 0.77 × 10^6/µL). On day 21, the haemoglobin concentration was highest in AL + L-serine broiler chickens (12.23 ± 1.63 g/dL), compared to any other day of the recording and other groups. On day 35, haemoglobin concentrations decreased and did not differ in all the groups. The overall mean RBC count (4.64 ± 0.74 × 10^6/µL) and Hb concentration (10.56 ± 0.98 g/dL) were highest ($P < 0.05$) in AL + L-serine group, compared to any other group (Table 2).

The highest MCV was recorded in AL broiler chickens on days 21 (145.96 ± 18.16 fl) and 28 (221.57 ± 81.69 fl), but the values did not differ ($P < 0.05$) on day 35 in all the groups. On days 21 and 28, the MCH was least in FR + L-serine group (19.49 ± 6.74 ρg and 29.25 ± 8.30 ρg, respectively) compared to AL (46.76 ± 7.43 ρg) and FR (46.22 ± 11.46 ρg) groups. The highest MCH was recorded in AL broiler chickens, compared to any other group which did not differ among themselves. On day 35 MCH did not differ ($P > 0.05$) among the groups. The MCHC was significantly ($P < 0.05$) lower in AL + L-serine broiler chickens compared to AL and FR broiler chickens, but did not differ from FR + L-serine chickens. The overall MCV and MCH were significantly ($P < 0.05$) highest in AL broiler chickens (145.50 ± 20.03 fl and 40.20 ± 11.81 ρg, respectively), compared to all other groups that did not differ among themselves. The overall MCHC was not significantly ($P > 0.05$) different in all the groups (Table 3).

Table 2 Effect of L-serine on erythrocytic parameters of broiler chickens subjected to feed restriction during the hot-dry season

Parameters	Days	FR	FR + L-serine	AL	AL + L-serine
PCV (%)	21	31.67 ± 0.88	30.33 ± 2.03[a]	36.00 ± 1.15	34.67 ± 1.33[b]
	28	36.00 ± 4.16	35.00 ± 6.43	38.67 ± 3.28	42.00 ± 2.65
	35	31.33 ± 0.33	35.00 ± 3.21[a]	34.33 ± 3.28	39.00 ± 6.81[b]
Overall mean ± SEM		33.00 ± 1.50	33.44 ± 1.56	36.33 ± 1.26	38.56 ± 2.13
RBC ($\times 10^6$/μL)	21	2.16 ± 0.33	3.70 ± 0.28[a]	2.78 ± 0.27	5.32 ± 0.89[b]
	28	2.56 ± 1.03	3.14 ± 0.68	3.06 ± 0.19	3.16 ± 0.73
	35	4.44 ± 0.17	4.97 ± 0.14	5.07 ± 0.75	5.43 ± 0.77
Overall mean ± SEM		3.05 ± 0.70	3.94 ± 0.54	3.64 ± 0.72	4.64 ± 0.74[b]
Hb (g/dL)	21	9.20 ± 1.40	9.60 ± 0.09	9.56 ± 0.57	12.23 ± 1.63[b]
	28	8.35 ± 1.87	10.5 ± 0.68	10.23 ± 0.53	10.63 ± 0.24
	35	7.74 ± 0.58	8.21 ± 0.76	8.01 ± 1.24	8.82 ± 1.91
Overall mean ± SEM		8.43 ± 0.42	9.44 ± 0.66	9.27 ± 0.66	10.56 ± 0.98

[a] = $P < 0.05$ as compared to FR group

[b] = $P < 0.05$ as compared to FR group

PCV packed cell volume, *RBC* red blood cell, *Hb* haemoglobin concentration, *FR* feed restricted, *AL* fed *ad libitum*, *FR + L-serine* feed restricted + L-serine, *AL + L-serine* fed *ad libitum* + L-serine; $n = 7$

Effect of L-serine on leucocytic parameters in broiler chickens subjected to feed restriction during the hot-dry season

On day 21, the total leucocyte count was highest ($P < 0.05$) in L-serine treated groups, that is, FR + L-serine ($5.03 \pm 1.17 \times 10^3$/μL) and AL + L-serine ($5.32 \pm 0.89 \times 10^3$/μL) groups. While on day 28 the AL + L-serine broiler chickens ($6.85 \pm 0.48 \times 10^3$/μL) had the highest TLC compared to all other group, the values did not differ. The heterophil count on day 35 was significantly higher ($P < 0.05$) in AL broiler chickens ($0.90 \pm 0.06 \times 10^3$/μL), compared to AL + L-serine ($0.63 \pm 0.07 \times 10^3$/μL) and FR + L-serine ($0.58 \pm 0.01 \times 10^3$/μL) broiler chickens. In all other days, the heterophil count did not differ ($P > 0.05$) among the groups. On day 21, the lymphocyte count was highest in FR + L-serine broiler chickens ($4.72 \pm 1.03 \times 10^3$/μL), followed by AL + L-serine broiler chickens ($4.38 \pm 1.01 \times 10^3$/μL), with the values significantly ($P < 0.05$) higher than that of FR ($3.31 \pm 0.07 \times 10^3$/μL) or AL ($3.48 \pm 0.32 \times 10^3$/μL) broiler chickens. On day 35, FR + L-serine ($5.17 \pm 0.92 \times 10^3$/μL) and AL + L-serine ($5.09 \pm 0.83 \times 10^3$/μL) groups had higher ($P < 0.05$) values

Table 3 Effect of L-serine on erythrocytic indices of broiler chickens subjected to feed restriction during the hot-dry season

Parameters	Days	FR	FR + L-serine	AL	AL + L-serine
MCV (fl)	21	127.83 ± 16.61	71.58 ± 11.53[a]	145.96 ± 18.16	86.38 ± 5.71[b]
	28	131.65 ± 40.03	113.36 ± 10.10	221.57 ± 81.69	127.10 ± 7.48[b]
	35	70.80 ± 3.05	70.31 ± 5.17	69.05 ± 4.38	71.34 ± 3.58
Overall mean ± SEM		110.10 ± 19.68	85.08 ± 14.14	145.50 ± 20.03	96.94 ± 16.66[b]
MCH (ρg)	21	46.22 ± 11.46	19.49 ± 6.74[a]	46.76 ± 7.43	26.22 ± 2.85[b]
	28	36.46 ± 9.10	29.25 ± 8.30	56.57 ± 22.34	34.91 ± 1.47[b]
	35	17.40 ± 0.80	16.58 ± 1.72	17.27 ± 5.00	17.36 ± 5.54
Overall mean ± SEM		33.36 ± 8.47	21.77 ± 3.83	40.20 ± 11.81	26.16 ± 5.07[b]
MCHC (g/dL)	21	25.84 ± 4.76	35.20 ± 4.09	30.17 ± 1.37	31.90 ± 1.86
	28	27.55 ± 1.01	24.95 ± 5.29	28.95 ± 2.48	25.01 ± 0.70
	35	24.71 ± 1.91	24.17 ± 3.96	24.38 ± 5.69	24.17 ± 7.60
Overall mean ± SEM		26.03 ± 0.83	28.11 ± 3.56	27.83 ± 1.77	27.03 ± 2.45

[a] = $P < 0.05$ as compared to FR group

[b] = $P < 0.05$ as compared to FR group

MCV mean corpuscular volume, *MCH* mean corpuscular haemoglobin, *MCHC* mean corpuscular haemoglobin concentration, *FR* feed restricted, *AL* fed *ad libitum*, *FR + L-serine* feed restricted + L-serine, *AL + L-serine* fed *ad libitum* + L-serine; $n = 7$

Table 4 Effect of L-serine on leukogram of broiler chickens subjected to feed restriction during the hot-dry season

Parameters	Days	FR	FR + L-serine	AL	AL + L-serine
WBCs ($\times 10^3$/μL)	21	3.82 ± 0.39	5.03 ± 1.17[a]	3.70 ± 0.28	5.32 ± 0.89[b]
	28	4.88 ± 0.66	5.58 ± 0.64	5.08 ± 1.20	6.85 ± 0.48[b]
	35	5.07 ± 0.42	5.90 ± 1.00	4.50 ± 0.26	5.73 ± 1.10
Overall mean ± SEM		4.59 ± 0.39	5.51 ± 0.25[a]	4.43 ± 0.40	5.97 ± 0.46[b]
MON ($\times 10^3$/μL)	21	0.07 ± 0.02	0.01 ± 0.01	0.05 ± 0.04	0.03 ± 0.02
	28	0.03 ± 0.03	0.00 ± 0.00	0.10 ± 0.09	0.06 ± 0.06
	35	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Overall mean ± SEM		0.05 ± 0.02	0.01 ± 0.01	0.08 ± 0.02	0.05 ± 0.01
HET ($\times 10^3$/μL)	21	0.58 ± 0.14	0.33 ± 0.20	0.59 ± 0.23	0.45 ± 0.27
	28	1.49 ± 0.53	0.83 ± 0.31	1.52 ± 0.14	1.13 ± 0.22
	35	0.82 ± 0.21	0.58 ± 0.01[a]	0.90 ± 0.06	0.63 ± 0.07[b]
Overall mean ± SEM		0.96 ± 0.27	0.58 ± 0.14	1.00 ± 0.28	0.74 ± 0.20
LYM ($\times 10^3$/μL)	21	3.31 ± 0.07	4.72 ± 1.03[a]	3.48 ± 0.32	4.38 ± 1.01[b]
	28	3.99 ± 0.37	5.23 ± 0.57	3.95 ± 0.98	4.02 ± 1.22
	35	4.55 ± 0.33	5.17 ± 0.92	3.51 ± 0.31	5.09 ± 0.83[b]
Overall mean ± SEM		3.95 ± 0.36	5.04 ± 0.16[a]	3.65 ± 0.15	4.50 ± 0.31

[a] = $P < 0.05$ as compared to FR group

[b] = $P < 0.05$ as compared to FR group

TLC total leucocyte count, *MON* monocyte, *LYM* lymphocyte, *HET* heterophil, *H/L* heterophil/lymphocyte ratio, *FR* feed restricted, *AL* fed *ad libitum*, *FR + L-serine* feed restricted + L-serine, *AL + L-serine* fed *ad libitum* + L-serine; $n = 7$

than FR (4.55 ± 0.33 × 10^3/μL) or AL (3.51 ± 0.31 × 10^3/μL) group (Table 4).

The heterophil/lymphocyte ratio was highest on day 28 in FR (0.41 ± 0.19) and AL (0.42 ± 0.10) groups, when compared with those recorded in FR + L-serine (0.15 ± 0.04) and AL + L-serine (0.23 ± 0.03) groups. On day 35, the highest ratio was obtained in AL group (0.26 ± 0.02), followed by FR group (0.18 ± 0.03), while the counts in FR + L-serine (0.12 ± 0.02) and AL + L-serine (0.13 ± 0.03) groups did not differ significantly ($P > 0.05$) (Fig. 1). On days 21 and 28, the monocyte counts did not differ ($P > 0.05$) among the groups. On day 35, no monocyte was recorded (Table 4).

Similarly, the overall mean count of TLC was highest ($P < 0.05$) in AL + L-serine group (5.97 ± 0.46 × 10^3/

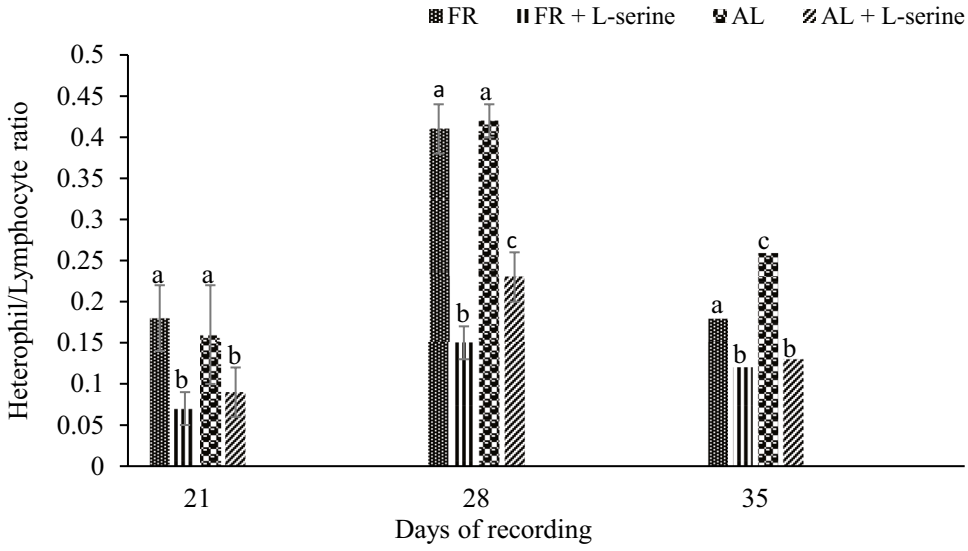

Fig. 1 Effect of L-serine on heterophil/lymphocyte ratio in broiler chickens subjected to feed restriction during the hot-dry season. Bars with different superscript letters (**a**, **b**, **c**) are significantly ($P < 0.05$) different; $n = 7$. FR, feed restricted; AL, fed *ad libitum*; FR + L-serine, feed restricted + L-serine; AL + L-serine, fed *ad libitum* + L-serine

µL), followed by FR + L-serine group ($5.51 \pm 0.25 \times 10^3$/µL). The highest overall lymphocyte count was obtained in FR + L-serine group ($5.04 \pm 0.16 \times 10^3$/µL) (Table 4). The overall lowest heterophil/lymphocyte ratio was recorded in FR + L-serine (0.11 ± 0.02) and AL + L-serine (0.15 ± 0.04) broiler chickens, and the ratio was significantly ($P < 0.05$) lower, when compared to that of FR (0.25 ± 0.08) or AL (0.28 ± 0.08) broiler chickens (Fig. 1).

Effect of L-serine on serum biochemical parameters in broiler chickens subjected to feed restriction during the hot-dry season

Protein concentrations

On days 21, 28 and 35, the total protein concentrations were significantly higher ($P < 0.05$) in FR + L-serine (4.03 ± 0.09 g/dL, 4.76 ± 0.62 g/dL and 6.29 ± 0.63 g/dL, respectively) and AL + L-serine (3.97 ± 0.12 g/dL, 4.67 ± 0.75 g/dL and 6.27 ± 0.64 g/dL, respectively) groups, compared to FR (3.27 ± 0.32 g/dL, 3.92 ± 0.38 g/dL and 4.32 ± 0.07 g/dL, respectively) or AL (3.30 ± 0.30 g/dL, 3.68 ± 0.63 g/dL and 4.48 ± 0.15 g/dL, respectively) group. In all the days of recordings, the difference in total protein concentrations between FR and AL broiler chickens was insignificant ($P > 0.05$). On days 21 and 28, the albumin concentrations were significantly ($P < 0.05$) higher in FR + L-serine (2.67 ± 0.20 g/dL and 2.54 ± 0.02 g/dL, respectively) than AL + L-serine (2.13 ± 0.13 g/dL and 2.82 ± 0.32 g/dL, respectively) groups. On day 35, the albumin concentration in AL + L-serine (3.09 ± 0.06 g/dL) group was the highest ($P < 0.05$) compared to other groups, whose values did not differ among themselves. On day 21, globulin concentration was highest ($P < 0.05$) in AL + L-serine group (1.83 ± 0.03 g/dL), followed by FR + L-serine group (1.36 ± 0.16 g/dL); while on days 28 and 35, globulin concentrations were highest in FR + L-serine group (2.23 ± 0.61 g/dL and 3.77 ± 0.66 g/dL, respectively), compared to any other group. Overall, FR + L-serine and AL + L-serine broiler chickens recorded significantly ($P < 0.05$) higher concentrations of total protein, albumin and globulin, when compared to FR or AL broiler chickens (Fig. 2).

Aspartate and alanine aminotransferase activities

On days 21, 28 and 35, AST and ALT activities decreased significantly ($P < 0.05$) in L-serine-administered groups than in any other group. The highest AST and ALT activities were recorded on day 35 in AL broiler chickens (54.03 ± 2.70 U/L and 49.95 ± 0.27 U/L, respectively), while the lowest occurred on day 21 in FR + L-serine broiler chickens (26.73 ± 0.43 U/L and 14.73 ± 1.39 U/L, respectively). Overall, the activities of AST and ALT were highest ($P < 0.05$) in AL broiler chickens compared to any other group, followed by those of FR broiler chickens, while the least activities were obtained in both FR + L-serine and AL + L-serine broiler chickens (Fig. 3).

Discussion

The THI recorded during the study period (31.25 ± 1.43) was higher than 20.8, fluctuating between 22.65 and 35.90. The value indicates that the hot-dry season was associated with environmental heat stress. The THI is an indicator of heat load. Values of 20.8 and above are associated with heat stress in broiler chickens (Tao and Xin 2003; Purswell et al. 2012).

The result of the erythrocyte parameters suggests that AL improved erythropoiesis more than FR as observed by increased PCV in AL group. The finding that L-serine administration increased the PCV in both AL and FR broiler chickens throughout the duration of the experiment demonstrated that L-serine may improve erythropoiesis in both *ad libitum* and feed-restricted broiler chickens. It shows that erythrocyte parameters may improve in broiler chickens subjected to 20% feed restriction and administered L-serine in order to compensate for the deficiency occurring due to inadequate supply of nutrients. The result agrees with the finding of Hassan and Asim (2020), who reported increased PCV in broiler chickens administered with antioxidant (acetylsalicylic acid) during heat stress. The PCV values obtained in the present study are toward the upper limit of the normal range of 20–40%, established for the avian species (Freeman 1971).

The RBC and Hb values were similarly enhanced in AL + L-serine broiler chickens, suggesting the beneficial effect exerted by L-serine on erythropoiesis. The increased RBC count obtained in the L-serine group reflected the increased PCV obtained in the L-serine administered groups. Improved RBC and Hb values in broiler chickens administered with antioxidants and reared under heat stress were demonstrated by Gouda et al. (2020) and Hassan and Asim (2020). The mechanism of action of L-serine in increasing the erythrocyte parameters was not investigated in the present study, but L-serine as a non-essential amino acid may be indirectly involved in the synthesis of Hb and nutrients required for the formation and maturation of the erythrocyte and its Hb component. This requires further investigations. The increase in the erythrocyte parameters during the hot-dry season may be an adaptive mechanism to mitigate the negative effects of both environmental and nutritional (feed restriction) stresses in broiler chickens (Olukomaiya et al. 2014; Egbuniwe et al. 2018a).

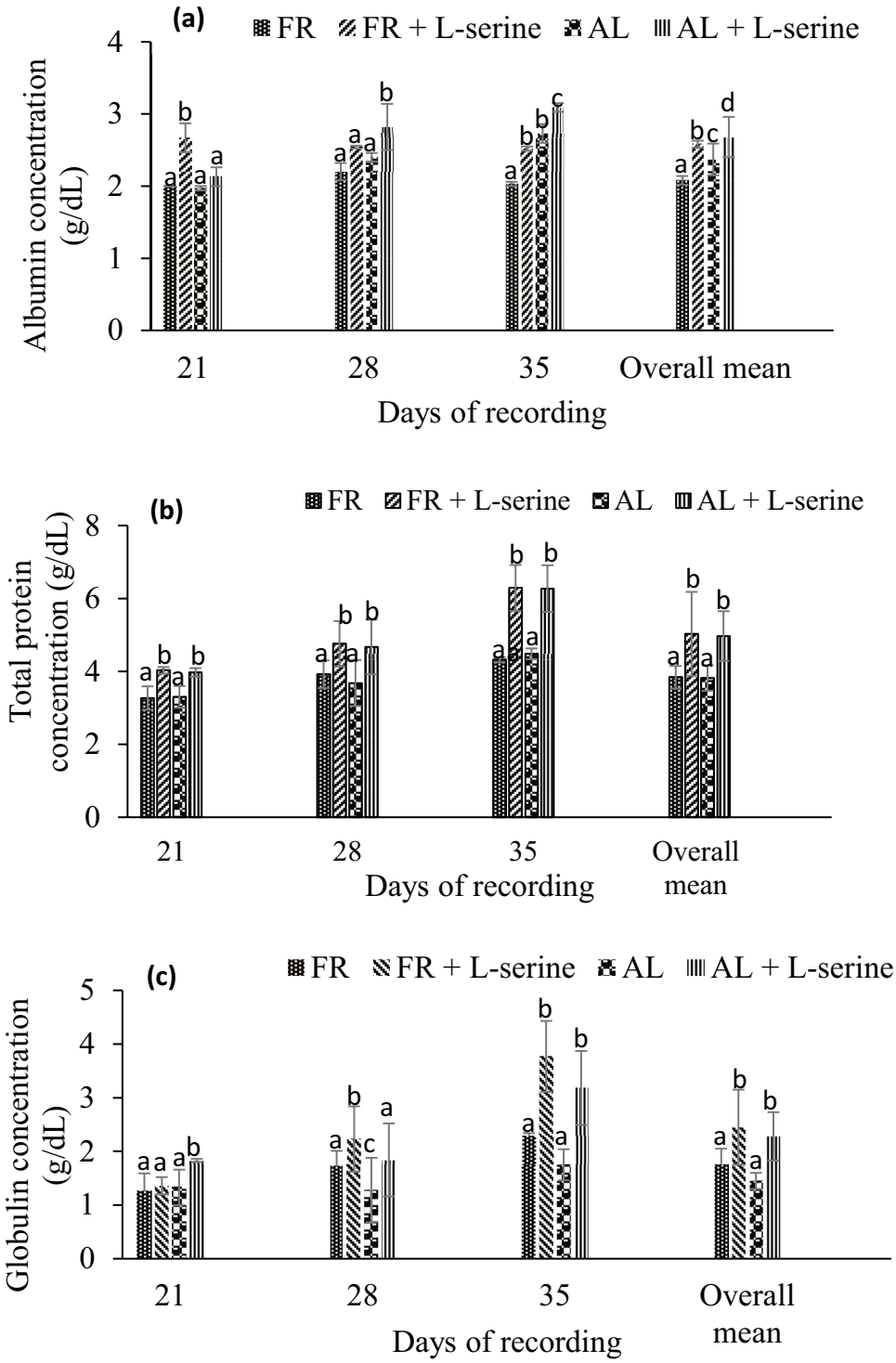

Fig. 2 **a-c** Effect of L-serine on serum protein concentrations in broiler chickens subjected to feed restriction during the hot-dry season. Bars with different superscript letters (**a**, **b**, **c**) are significantly ($P < 0.05$) different. FR, feed restriction; AL, fed *ad libitum*; FR + L-serine, feed restriction + L-serine; AL + L-serine, fed *ad libitum* + L-serine; $n = 7$

The result of the erythrocytic parameters shows increased MCV in FR and AL broiler chickens and reduced value in both AL + L-serine and FR + L-serine broiler chickens. Heat stress induces oxidative stress which increases ROS production leading to lipid peroxidation, damaging the RBC membrane and impairing its deformability. Decreased deformability contributes to the removal of RBCs from the circulation, thus reducing the number of RBCs (Altan et al. 2003; Mohanty et al. 2014). The reduction may trigger the movement of young RBCs, which are large in size (Chalfin 1956) from the reserved pool, into the circulation. This fact was, apparently, responsible for the increase in MCV recorded in broiler chickens that were not administered L-serine. There was no significant difference in

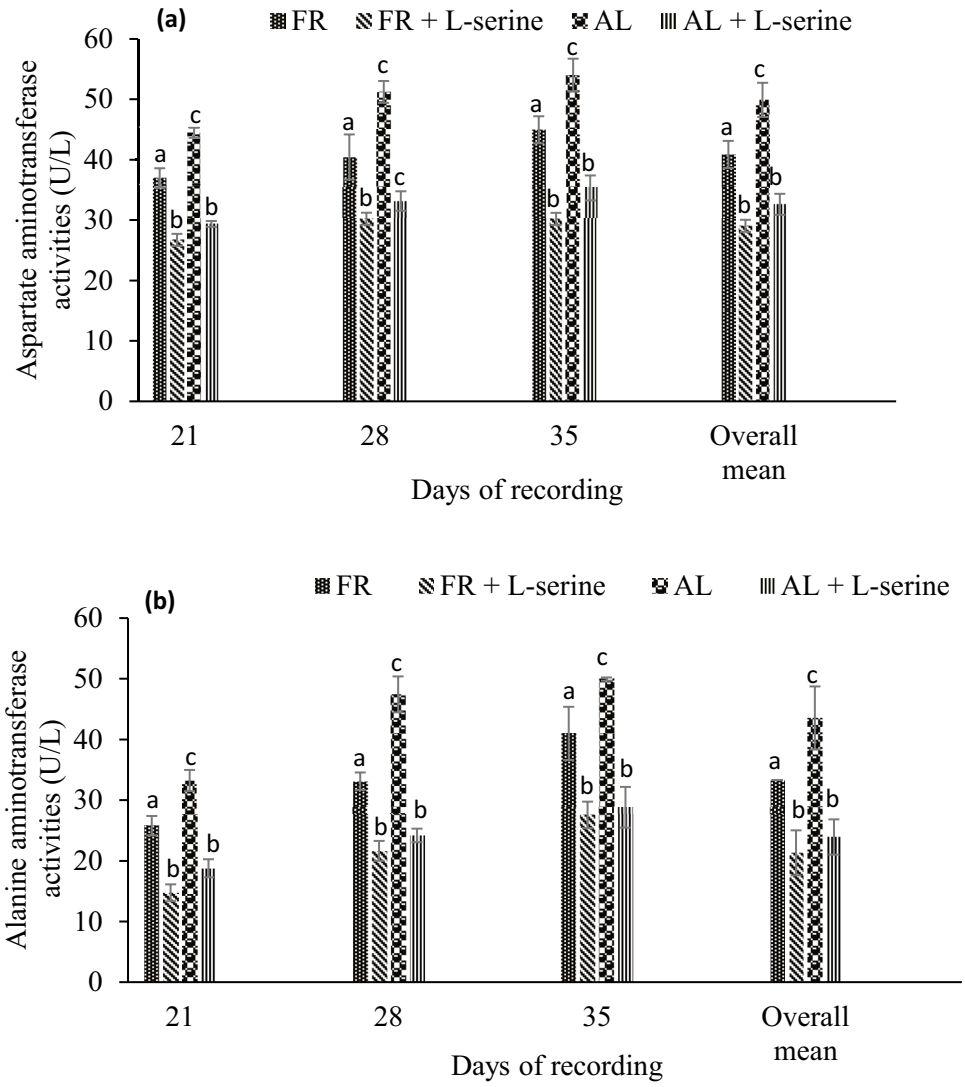

Fig. 3 **a-b** Effect of L-serine on activities of aspartate aminotransferase and alanine aminotransferase in broiler chickens subjected to feed restriction during the hot-dry season. Bars with different superscript letters (**a**, **b**, **c**) are significantly ($P < 0.05$) different. FR, feed restriction; AL, fed *ad libitum*; FR + L-serine, feed restriction + L-serine; AL + L-serine, fed *ad libitum* + L-serine; $n = 7$

MCHC across all groups. The result agrees with the finding of Egbuniwe et al. (2018a), who reported insignificant increase in MCHC in heat-stressed broiler chickens, administered antioxidants, betaine and ascorbic acid. All values of the erythrocytic parameters were within normal ranges (Bounous and Stedman 2000), indicating that changes in the parameters in broiler chickens were not influenced significantly by exposure to 20%-feed restriction and environmental heat stress. The finding requires further investigations, involving longer duration and in different seasons.

The result shows that L-serine administration enhanced the total leucocyte count in FR + L-serine and AL + L-serine broiler chickens. The finding demonstrates that L-serine was beneficial in increasing total leucocyte count and, consequently, it may enhance the immune response in broiler chickens exposed to environmental stresses, including heat stress and nutritional stress. The result agrees with the finding of Abudabos et al. (2018), who demonstrated increased total leucocyte count in heat-stressed broiler chickens, administered with ascorbic acid. Heterophil count was, however, highest only in AL group compared to L-serine-supplemented groups, indicating that L-serine decreased the heterophil count, and may mitigate the negative effects of heat stress in broiler chickens. The increase in lymphocyte count recorded in FR + L-serine and AL + L-serine broiler chickens compared to other groups shows that L-serine enhanced the lymphocyte count in the peripheral circulation, and, consequently, may increase the immune response. The increase in lymphocyte was responsible for the high count of the total leucocyte count in all the L-serine-treated groups; thus, the H/L ratio was least in both FR + L-serine and AL + L-serine broiler chickens. It has been shown that a decrease in H/L ratio is an evidence of improved response of the body against stress situations (Scanes 2016; Wang et al. 2020). However, it has been demonstrated that heat stress induces the release of corticosterone that increases the H/L counts in circulation by preventing the endothelial passage; thus, the cells spend more time in the circulation (Spiers et al. 2015; Xu

et al. 2018). Overall, the mean H/L in FR + L-serine broiler chickens was least, indicating that early feed restriction was less stressful and may decrease the high metabolic rate that occurs in heat-stressed broilers. The decrease in the metabolic rate reduces the impact of heat stress, which, consequently, increases the survival rate of broiler chickens (Syafwan et al. 2011). The finding of the current study agrees with that of Hassan and Asim (2020), who reported increased percentage of lymphocyte and reduced H/L ratio in heat-stressed broiler chickens, administered with an antioxidant, vitamin C, combined with acetylsalicylic acid. Thus, L-serine was demonstrated to be a potential agent that may enhance the immune response in broiler chickens against environmental heat stress and, particularly, nutritional stress.

The result shows that L-serine administration facilitated the increase in the concentrations of total protein in FR + L-serine and AL + L-serine-treated groups. Similar results were obtained for albumin and globulin, which suggest the involvement of L-serine in the synthesis of protein in the treated groups. Thus, L-serine, being an amino acid, may interact with other essential amino acids to enhance the synthesis of proteins (Metcalf et al. 2018). The increase in the albumin and globulin concentrations recorded in the present study shows that L-serine may enhance the humoral immune response of the broiler chickens to combat the negative effects of environmental heat stress and, particularly, feed restriction. The concentrations of albumin and globulin, though relatively increased, were within the normal ranges of 3.28 ± 0.06 g/dL for albumin (Attia et al. 2021) and 4.99 ± 0.06 g/dL for globulin (Omodewu and Tiamiyu 2021) in broiler chickens. The findings also agree with those of Zhou et al. (2021), who obtained increased total protein in laying hens administered with serine; and Hosseini-Vashan et al. (2020), who demonstrated increased total protein in heat-stressed broiler chickens, administered with grape (*Vitis vinifera*) pomace, exerting antioxidant effects. Furthermore, the present study has shown that 20%-feed restriction is an adaptive physiological mechanism in broiler chickens, aimed at ameliorating the negative impact of environmental heat stress on the chickens. The findings have demonstrated that feed restriction was beneficial to broiler chickens exposed to environmental heat stress. L-serine administration was beneficial by increasing serum protein concentration, which may enhance the body resistance to heat stress and nutritional stress of feed restriction in broiler chickens.

The serum activities of AST and ALT show that some catabolic processes occurred in the broiler chickens (Ahmed-Farid et al. 2021), with an increase in age, particularly in AL group followed by FR group. The administration of L-serine, apparently, retarded the processes as evidenced by the lower activities recorded in the two groups administered with L-serine. Thus, L-serine administration may delay the negative catabolic processes associated with both environmental heat and nutritional stresses, acting upon the broiler chickens simultaneously during the hot-dry season in the zone. The result agrees with the similar finding of Zhou et al. (2018), who reported the antioxidant role of serine in decreasing the ALT and AST activities in high-fat-induced oxidative stress in mice. Taken together, the findings of the present study show that L-serine may enhance homeostasis in broiler chickens subjected to unfavourable heat stress conditions and feed restriction that occur during the hot-dry season. Furthermore, the results show that the adverse effects of feed restriction, which may naturally occur in broiler chickens whenever there is deficiency or scarcity of feeds (Chander and Kannadhasan 2021), may be ameliorated by the administration of L-serine.

Conclusion

It was concluded that L-serine and/or feed restriction reduced the negative effects of environmental heat stress by increasing erythrocyte and total leucocyte counts, protein concentration and decreasing heterophil/lymphocyte ratio as well as the activities of AST and ALT. L-serine and/or feed restriction may be beneficial in improving the health and productivity of broiler chickens under natural environmental heat-stressed conditions.

Declarations

Ethical approval This experiment received ethical approval from the Ahmadu Bello University Committee on Animal Use and Care, with the reference number ABUCAUC/2021/028.

Conflict of interest The authors declare no competing interests.

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