ORAL ADMINISTRATION OF AQUEOUS CRUDE EXTRACTS OF *A*VOCADO (*PERSEA AMERICANA* SEED POWDER) AND TAMARIND (*TAMARINDUS INDICA* PULP) AMELIORATES COLD-STRESS IN BROILER CHICKENS

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ABSTRACT
Low environmental temperature alters behaviour and physiologic responses leading to low productivity in poultry. This study was designed to evaluate the effect of orally administered aqueous crude extracts of Persea americana and Tamarindus indica in broiler chickens during cold season. Twenty-eight, 3-week old Arbor acres mixed sex broiler chickens were used. Seven broiler chickens were assigned each to Cg (control), TA (20% avocado aqueous extract), TB (20% aqueous tamarind extract) and TC (20% aqueous avocado extract + 20% aqueous tamarind extract). The treatments were administered as sole drinking water while the control received plain water. Thermal environmental data during the study period were measured, using a dry and wet bulb thermometer and relative humidity using a table, while the temperature humidity index was calculated. Cloacal temperature and blood samples were collected on days 28, 35 and 42 of the experimental periods. The Results show that the experimental period was thermally stressful (cold stress). Mean cloacal temperatures of broiler chickens in all the treatment groups were significantly (P < 0.05) higher (41.0 - 42.3°C) than those in control group (41.0 – 41.4°C) on days 28, 35 and 42. The heterophil/Lymphocyte ratio, pack cell volume, heamoglobin, total protein, total white blood cell, total red blood cell, weight gained were higher (P > 0.05) in treatment groups compared to control group. It was concluded that aqueous extracts of Persea americana and Tamarindus indica singly or in combination ameliorated cold-stress and improved broiler performance during cold-dry season.
Keywords: Cold stress, Cloacal temperature, Broiler chickens, *Persea americana, Tamarindus indica,* heterophil/lymphocyte ratio

INTRODUCTION
High or low ambient temperatures, high relative humidity hinder thermoregulatory processes, hence, increased body temperature fluctuation, which is often accompanied by reduced cloacal temperature (Refinetti *et al.,* 2007). Cold-stress affects haematological parameters in broiler chickens (Ewuola *et al.,* 2004), decreased number of lymphocytes, with a resultant increased in H/L ratio under cold-stress. The H/L ratio is a stress indicator in various animals including chickens (Campo *et al.,*2008). Nutritional manipulation as well as inclusion of feed additives such as vitamins, antioxidant, probiotics and prebiotic may reduce the negative impact of cold stress on chicken (Minka and Ayo, 2014). *Persea americana* (Avocado Seed) contains antioxidants which are linked with reduced risk of non-communicable disease and prevention of liver damage (Mfonobong *et al.,* 2013; Folashade *et al.,* 2016). *Tamarindus indica* (Tsamiya in Hausa) contains phenol and antioxidants, which decreases lipid peroxidation in-vitro (Tsuda *et al.,*1994), and heterophil/lymphocyte ratio (Aengwanich and Suttajit, 2010). To date, there is paucity of information in the available literature on the effect of cold-stress on broiler chickens. We hypothesize, aqueous extracts of avocado powder or tamarind pulp may ameliorate cold-stress, thereby, improve broiler chicken production during the cold-dry season.

MATERIALS AND METHODS
The study was conducted at the poultry pen, College of Agriculture and Animal Science, DAC, Ahmadu Bello University, Mando Road, Kaduna (latitude 11°N-10′E, longitude 07° 38/E) located in the Northern Guinea Savannah zone of Nigeria from December to January, 2022. Study protocol approval was obtained from the College of Agriculture and Animal Science, Ahmadu Bello University, Zaria, with number CAAS/ABU/2022/056.

Avocado seeds and tamarind pulp were sourced from fruit vendors at Kawo local market, Kaduna North Local Government Area, Kaduna state.

Preparation and aqueous extraction of avocado seed powder.

The preparation and extraction of avocado seed was according to the process as described by Folashade *et al.* (2016). Briefly, the seeds were removed from the flesh and washed with clean water. The seeds were chopped thinly and air dried under room temperature. The dried slices were ground into powder using a mortar and pestle, sieved and stored in polyethylene bags. 20g of the air-dried avocado seed powder was mixed with 100 mL (0.2 g/ mL) of room temperature water in a large conical flask and kept for 72 hours, it was agitated every 12 hours. The mixture was filtered and the process was repeated with the residue and the second filtrate was subsequently pooled with the first extract.

Preparation and aqueous extraction of tamarind pulp.

The preparation and extraction of tamarind pulp was according to the process as described by Aengwanich *et al.* (2010). Briefly, the pulps were carefully cleaned and wash with clean water. They were allowed to dry under room temperature. 20g of tamarind was weighed into 100 mL (0.2g/mL) of clean water in a large conical flask. The mixture was kept at room temperature for 72 hours and agitated every 12 hours, and allowed to settle. The supernatant was then filtered, and the procedure repeated. The filtrate were then pooled together.

A total of 28, 21-day-old, clinically healthy Arbor Acres hybrid broiler chickens were used for the study. They were managed on deep litter in four partitions with dimensions 1,200 X 2,000cm. Acclimatization period of 7 days and assigned into groups, in a completely randomized study design (CRD). The treatment groups were, T_A, T_B, T_C and Control (C_g) groups, and they received the corresponding aqueous extracts. T_A (20 % avocado powder aqueous extract), T_B (20 % aqueous tamarind pulp extract) and T_C (20 % aqueous avocado powder extract + 20 % aqueous tamarind pulp extract). The control received plain water, *ad-libitum*. The broiler chickens were fed with a commercial broiler finisher diet, ad-*libitum* (Chikun Feeds®, Olam Feeds and Flour Mills, Kaduna, Nigeria).

Experimental activities

Ambient temperature (AT) and relative humidity (RH) were determined using a dry and wet bulb thermometer, and relative humidity was determined using a table. The temperature humidity index (THI) was calculated (Tao and Xin, 2003). THI = 0.85(tdb) + 0.15 (twb); THI =Temperature humidity index, twb = wet-bulb temperature. tbd = dry-bulb temperature.

The cloacal temperature was recorded on days 28, 35 and 42 using digital thermometer.

Blood samples were collected from wing vein (Samour, 2016). On days 28, 35 and 42 of the study periods, two (2 mL) of blood was drawn and transferred into EDTA (ethylene diamine tetra acetic acid) blood tubes from 5 broiler chickens in each of the groups. The blood samples were labelled appropriately for haematology.

Statistical Analyses

The generated data are expressed as mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA). Values which show differences were compared using the Tukey Post-hoc test. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The AT range (17 - 18 °C) was below the recommended value of 18 – 28 °C for broiler chickens (Donkoh, 1989), while the RH (91.3 - 91.5 %) exceeded the normal range of 60 – 80 % (Hulzebosch, 2006) during the study period. The THI range (17.8 – 18.9 °C) were below established values for broiler chickens (20.08 °C) (Sinkalu *et al.,* 2015). The thermal environmental Results depict that the study period to be thermally stressful (cold-stress) to the broiler chickens. Broiler chickens in the treatment groups maintained a significantly ($P < 0.05$) higher CT compared to the control group, during the study period (Fig. 1). The result in the current study was similar to an earlier report on vitamin C and taurine, which exhibited thermogenic activity in broiler chickens (Minka and Ayo, 2014; Makeri *et al.,* 2023), respectively.

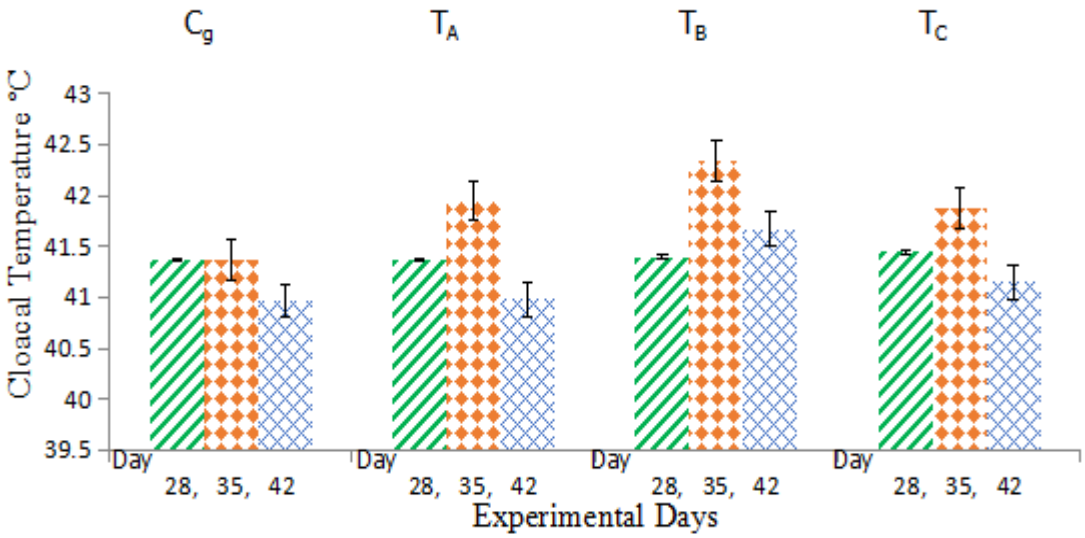

Figure 1: Graph showing the effect of aqueous extract of *Persea americana* (Avocado seed) and *Tamarindus indica* (Tamarind) on cloacal temperature during cold-dry season.
Key: = TA (Avocado extract) = TB (Tamarind extract) = TC (Avocado and Tamarind extract)

The suspected thermogenic effect observed with the administration of aqueous extracts in the current study require further investigation. The PCV, HGB, TP, TWBC, TRBC and H/L ratio in the study were higher (P > 0.05) in the treatment groups compared to control group (Table 1). The increased PVC and HGB agrees with the reports by Julian *et al.* (1989) and Borges *et al*. (2009), who reported that cold stress elevates haematocrit values and reduction in stress elevates HGB. Increase in HGB and TWBC in the treatment groups, might also be due to the hepato-stimulatory and hepato-protective effects of the extracts, resulting in the formation of more HGB in bone marrow (Sharma *et al.,*2003). The TP values were higher in treatment compared to control groups, however, this contradicts the finding by Daneshyar *et al.* (2009), they reported that TP of cold temperature treated birds were lower than control group birds. All treatment groups exhibited higher H/L ratio compared to control group, this agrees with the finding of Campo *et al.* (2008), they reported that cold stress decreased the number of lymphocytes, with increased H/L ratio. However, in the current study, the general increase in the H/L ratio in the treatment group could be as a result of the increased erythropoiesis (Sharma *et al.,* 2003). The decreased H/L ratio with age may be attributed to decreased response to stress with prolonged exposure (Moberg and Mench, 2000). The weight gain values were slightly higher (P > 0.05) in all the treatment compared to the control groups.

Table 1: Shows the effect of aqueous extracts of avocado seed powder and tamarind pulp on haematological parameters of broiler chickens on days 28, 35 and 42 of study during cold-dry Season (Mean ± SEM, n = 7).

Key	Day 28				Day 35				Day 42			
	C_g	T_A	T_B	T_C	C_g	T_A	T_B	T_C	C_g	T_A	T_B	T_C
PVC	27.8±1.52	31.60±1.02	29.80±1.4	31.00±1.3	28.20±1.7	29.2±0.7	29.20±0.6	31.20±1.2	23.6±2.2	27.8±1.01	29.00±2.	26.80±1.4
HGB	9.28±0.5	10.54±0.34	9.94±0.47	10.32±0.4	9.42±0.5	9.70±0.2	9.72±0.2	10.14±0.4	6.52±0.2	9.26±0.34	9.66±0.8	8.93±0.5
TP	5.88±0.04	6.08±0.04	6.08±0.04	6.16±0.13	6.12±0.22	6.20±0.1	6.22±0.2	6.16±0.17	5.80±0.3	6.52±0.14	6.12±0.1	5.96±0.7
TWBC	13.22±1.7	10.72±0.43	9.66±1.77	12.72±1.8	10.72±0.4	12.12±2	12.72±1.8	9.68±1.78	8.96±1.5	11.48±1.6	13.6±1.4	13.46±2.1
TRBC	7.20±0.47	6.58±0.18	6.26±0.26	7.30±0.60	6.56±0.24	7.68±0.3	7.04±0.5	6.32±0.21	7.40±0.3	6.76±0.26	6.92±0.5	7.38±0.6
HET	19.8±1.7	23.0±3.4	22.4±2.7	24.4±3.1	23.2±3.1	19.8±1.7	24.4±3.1	22.4±2.7	18.8±4.1	22±3.2	19.8±1.7	17±2.9
LYM	78.2±1.6	71.2±4.0	74.4±3.4	73.0±2.4	72.4±4.1	77.4±2.0	74±2.6	76.2±3.7	75.8±3.7	74.6±3.3	71.2±4.0	77±3.4
H/L	0.25	0.32	0.30	0.33	0.30	0.25	0.32	0.29	0.24	0.29	0.27	0.22

[abc]Mean values with different superscripts are significantly diffrent (P > 0.05); SEM: Standard error of the mean
T_A = Avocado extract, T_B = Tamarind extract, T_C = Combination of Avocado and Tamarind extract, PCV = Pack cell volume, HGB = Haemoglobin, TP = Total Protein, TWBC = Total White Blood Cell, TRBC = Total Red Blood Cell, H/L = Heterophil/Lymphocyte Ratio

CONCLUSION

The study showed that the administration of aqueous extracts of *Persia americana* and *Tamarindus indica* improved thermoregulation and blood picture, therefore, could be used to improve productivity of broiler chickens during period of cold stress.

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