NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 55, Number 3, December 2024, Page 404-411
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Biochemical and Electrolyte Parameters of Broilers Fed Ginger (*Zingiber officinale*) Based Meal

[1]Mba, I. K. and [1,2]Ukonu, C. E.
[1]Livestock Section, Farming Systems Research, Root for Health Unit,
National Root Crops Research Institute, Umudike
[2]Root for Health Unit, National Root Crops Research Institute, Umudike
Corresponding email: erastusdavid4real @gmail.com

Abstract
This study was carried out to evaluate the biochemical and electrolyte parameters of birds fed ginger-based meal as a replacement diet on the growth performance of broiler chicken. One hundred and twenty-day-old broiler chicks (arbo acre) were used, and were divided randomly into five treatments (T_1 - T_5) comprising twenty-four birds each and further replicated three times. Each treatment was fed one of 5 formulated diets containing ginger meal at levels of 0.0, 0.05, 0.10, 0.15, and 0.2g/100kg for eight weeks in a completely randomized design. At the termination of the experiment, three birds per treatment were selected and used for the evaluation of biochemical and electrolyte parameters. The result of this study showed that increased inclusion levels of ginger across the treatment group caused a significant ($p<0.05$) increase in total protein. There was no significant ($p>0.05$) difference in the AST, ALP, and creatinine in the ginger groups compared with the control. This implied that ginger at the various inclusion levels did not significantly ($p>0.05$) alter these enzyme biomarkers. It was observed that at the least inclusion level (0.05 g/100 kg), the cholesterol concentration was significantly increased ($p<0.05$) compared with other treatment groups and the control, whereas the highest inclusion 0.20 g/100 kg, significantly ($p<0.05$) reduced the mean value from 80.32±1.50 mg/dl to 70.62 ±0.51 mg/dl. This suggests that ginger at the highest inclusion (0.20 g/100 kg) used in this study significantly ($p<0.05$) lowered serum cholesterol concentration. Normal levels of blood protein, glucose, and other serum indices indicate adequate nutritional status and normal internal organ function. The serum electrolytes of the broiler chicken fed with varying degrees of ginger inclusions did not present significant variations in the serum chloride, phosphorus, sodium, and potassium concentrations across the treatments. It can be concluded from this study that ginger inclusion in the poultry diet, in addition to other nutritional and health benefits, keeps blood cholesterol within the normal range. Also, the dietary inclusion of ginger did not adversely affect the serum biochemistry and electrolyte values of the animals, hence, its inclusion in the diet is encouraged.
Keywords: Electrolyte, Ginger, biochemistry, and broiler chicken

Introduction

Normal electrolyte and serum biochemistry are important for *normostosis* (normal state and function) of the body cells, tissues, organs, and systems. It is also helpful for normal brain, kidney, and liver health and function. Tests conducted to determine the state of the cells, tissues, organs, and systems in the event of dietary inclusion of any substance are necessary for determining the health-friendliness of such substance. Hence, the biochemistry and electrolyte tests were employed to ascertain brain health and monitor the kidney, liver, and other vital organs state and functions. Couch et al (2017) stated that Serum biochemical parameters can be utilized to evaluate the physiological status of an animal and relate it to the animal's health. This study, therefore, evaluated the serum biochemistry and electrolyte parameters of ginger-based diets at various inclusion levels.

The main importance of poultry production is for the production of eggs, meat, etc., and the provision of protein to humans. Feeding constitutes up to 75%-80% of the total cost in monogastric production and is a major factor limiting the production of livestock. Mineral vitamin supplements may contribute up to 2-3% of the total cost of feed. The essence of animal protein will be compromised if livestock production is limited to synthetic minerals/vitamins, which are basically for vitality, acid-base balance, proper metabolism, muscle contraction and relaxation, etc. Synthetic vitamin-mineral supplements in the diet contain a synthetic antibiotic, which has a negative effect on the farm animal and beneficial microorganisms, and their residues in the carcass of animals could be detrimental to the human system, as well as contribute to the cost of production in animal husbandry.

Whereas, the interest of nutritionists, veterinarians, farmers, and other major players in recent years have directed towards the search for cheaper and organic mineral/vitamin additives that are locally available and nutritionally viable, non-toxic to the animals and beneficial to their microbes as well contribute to health benefits to the animal for optimum production, the determination of certain parameters like the biochemical and electrolyte parameters are part of the ways to determine that such dietary inclusions does not have deleterious effect on the animals.

Ginger (*Zingiber officinale*) is an important spice. It is a monocotyledonous herbaceous perennial plant that lives longer than two years, belonging to the family of *Zingiberaceae*, its flavouring type is classified as *Zingiber officinale,* which is the most popular hot spice in the world (Dhingra and Kumar, 2005). The main important compounds of ginger (*Zingiber officinale*) are Gingerol, combel, conceal, citral cultural, phellandrene, and resin. These compounds can stimulate the digestive enzymes and affect microbial activity (Dieumou *et al*., 2009). Also, it acts as an antioxidant and antimicrobial and has various pharmacological effects (Ali *et al.,* 2008). It has been reported that it enhances animals' nutrient digestion and absorption because of the positive effects on the gastric secretion of enterokinase and digestive enzyme activities (Platel *et al.*, 2009). Furthermore, ginger compounds have shown various pharmacological effects, including Immunomodulatory, Anti-lipidemic, Anti-inflammatory, Anti-hyperglycemic, and antiemetic effects (Ali *et al.*, 2008). However, information is lacking on the effect of ginger in comparison to synthetic vitamins/minerals premix.

This research investigated the serum biochemical and electrolyte response of

broiler chickens fed various ginger-based meals in comparison to a vitamin/mineral premix.

Materials and methods

Location of the study

The location of this study is situated in the rainforest zone of Nigeria and is characterized by annual rainfall of about 2167 mm, 8mm in 148 to 155 days. The average relative humidity during the rainy season is over 72%. It has an environmental temperature average of 22 °C to 30 °C. This study was conducted at the Poultry section of the Teaching and Research Farm of Michael Okpara University of Agriculture, Umudike, which is located at Latitude 5^{o} 28' N and Longitude 7^{o} 32' E lines at an altitude of 122 meters above sea level. (National Root Crops Research Institute-NRCRI, 2004).

Experimental birds and management

A total of 120 day-old broiler chicks were purchased locally within Nigeria for this experiment. Before the arrival, the experimental pens and equipment were washed and disinfected. On arrival at the experimental farm, they were allocated to experimental pens for brooding for 4 weeks, then they were allotted to five (5) dietary treatments: T_1, T_2 T_3, T_4, and T_5. The birds were fed diets with ginger-based meal at 0.00, 0.05, 0.10, 0.15, and 0.20 g/100kg, which was supplemented with the synthetic vitamin-mineral premix at 0.25, 0.20, 0.15, 0.10, and 0.05 kg/100 kg, respectively.

Routine vaccination and medication, heat, and water provision were provided across the groups. Each treatment diets were replicated 3 times.

Processing of ginger meal

The ginger was purchased fresh from the National Root Crops Research Institution (NRCRI), Umudike, and its rings and husks were peeled off using a knife. The peeled ginger was washed and dried (air and sun-dried). The essence of air drying was basically to preserve the aromatic compounds, vitamins, and mineral nutrients, and later pulverize and sieve the meal that was incorporated into the diets.

Biochemical Analyses

The blood was collected across the treatments with a sterile 21G needle and 5 ml syringes by venipuncture and emptied into a plain (without EDTA) blood sample bottle. The blood samples were allowed to clot, after which the serum was transferred to another well-labeled plain sample bottle for the biochemical tests. The tests were done according to Saeed *et al* (2011).

Mineral determination

The mineral content of the sample was determined by the dry ash extraction meth, od after which specific mineral elements were determined. A 2g portion of the sample was burnt to ashes in a muffle furnace, and the resulting ash was dissolved in 100 mL of dilute hydrochloric acid and then diluted to 100 mL with distilled water in a volumetric flask. The digest obtained was used for the various elemental analyses.

Determination of phosphorus

Phosphorus in the sample will be determined by Vanadomolybdate (Yellow) spectrometry as described by Hobson *et al* (1997). Then, 1 ml of extract from each sample was dispensed into a test tube. Similarly, the same volumes of standard phosphorus solution as well as water were put into other test tubes to serve as standard and blank, respectively. The content of each test tube was mixed with an equal volume of the *Vanado-molybate colou*r reagent. They were left to stand for 15 minutes at room temperature before their absorbance was measured in a Jenway spectrophotometer at a wavelength of 420nm. Measurement was given with the blank at zero phosphorus content and was calculated with the formula;

$$P = \left(\frac{mg}{100g}\right) x \frac{100\ x\ Au\ x\ C\ x\ Vf}{W\ x\ As\ x\ Va}$$

Where;

P = Phosphorus concentration in the sample (mg/100g of the sample).
Au = Absorbance of the unknown sample.
C = Concentration of the phosphorus in the standard solution (usually determined from the standard curve).
Vf = Total final volume of the extract after the reagent has been added.
W = Weight of the sample analyzed (in grams).
As = Absorbance of the standard phosphorus solution.
Va = Volume of the extract titrated (volume of the sample extract taken for analysis).

Determination of Potassium and Sodium

Potassium (K) and sodium (Na) were determined by flame photometry. Standard potassium and sodium were prepared separately, and each was diluted to a concentration of 2,4,6,8 and 10 ppm, and then filtered. The filtrate was used to carry out the test, and the values obtained were used as the result. The formula below was used to determine the concentration of potassium and sodium separately:

$$\text{Na or K (mg/100g)} = \left(\frac{100}{w}\right) x \frac{100\ x\ Vt\ x\ X\ x\ D}{W\ x\ 11\ x\ 101}$$

Where;
W = weight of sample used,
Vt= Total extract volume since 1ml will be siphoned into the instrument,
X = concentration from the standard graph, and
D = dilution factor, where applicable
11 and 101 = Constants

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA) and the results presented as Mean ±SEM (standard error of the mean). Means will be separated using Duncan's multiple range Test (DMRT).

Results and Discussion

The result (Table 2) showed that increased inclusion levels of ginger across the treatment group caused a significant ($p<0.05$) increase in total protein. The result of the albumin showed that the mean values obtained from T_2, T_4, and T_5 did not significantly ($p>0.05$) vary compared with the control group (T_1), but birds in T_3 recorded the highest significant ($p<0.05$) serum albumin compared with T_2 and T_1. There was a significant ($p<0.05$) increase in the mean values of urea and glucose in the ginger groups compared with the control. There was no significant ($p>0.05$) difference in the AST, ALP, and creatinine in the ginger groups compared with the control. This implied that the ginger at the various inclusion levels did not significantly ($p>0.05$) alter these enzyme biomarkers. Also, apart from the birds in the T group, the T_4 group, which recorded the least significant ALT mean value, other ginger inclusion groups (T_2, T_3, and T_5) showed no significant ($p>0.05$) alteration in their mean values compared with the control (T_1). It was observed that at the least inclusion level (0.05 g/100 kg), the cholesterol concentration was significantly increased ($p<0.05$) compared with other treatment groups and the control, whereas the highest inclusion 0.20 g/100 kg, significantly ($p<0.05$) reduced the mean value from 80.32±1.50 mg/dl to 70.62 ±0.51 mg/dl. This suggests that ginger at the highest inclusion (0.20 g/100 kg) used in this study significantly ($p<0.05$) lowered serum cholesterol concentration. This result aligns with Saeid *et al* (2010), who observed that aqueous extract of ginger at low and high concentrations significantly reduced the level of cholesterol in the blood of broilers. Also, Bhandari and Grover (1998) and Akhani *et al.* (2004) reported that ginger ginger-supplemented diet significantly decreased serum cholesterol.

Normal levels of blood protein, glucose, and other serum indices indicate adequate nutritional status and normal systemic protein function, while hypertriglyceridemia, low HDL levels, and high VLDL triglyceride usually result in metabolic syndrome (Ademola, 2009; Hong *et al.,* 2012). The result of the serum TP,

globulin, glucose, total cholesterol, creatinine, urea, AST, and ALT, is in total agreement with Alizadeh-Navaei *et al.* (2008); Zhang *et al* (2009); partially with George *et al.* (2015); Youseff *et al.* (2017), and in disagreement with Oorbanpour *et al.* (2018) who reported no significant effect on all the serum parameters even at 0.25% of ginger supplemented diet.

The serum electrolyte of the broiler chicken fed with varying degrees of ginger inclusions presented in Table 3 did not present significant variations in the serum chloride, phosphorus, sodium, and potassium concentrations across the treatments. The chloride concentration did not differ significantly across the groups. This observation agrees with the findings of Al-Homidan (2005) but differs from the findings of Onu (2010), Valiollahi *et al.* (2013), Amanduruonye *et al.* (2018), and is in partial agreement with Ademola *et al.* (2009), Tekeli *et al.* (2011), and Safa and Eltazi (2014).

Conclusion

This study was carried out to evaluate the biochemical and electrolyte parameters of birds fed a ginger-based meal as a replacement diet on the growth performance of broiler chicken. One hundred and twenty-day-old broiler chicks (arbo acre) were used, and divided randomly into five treatments (T_1 - T_5) comprising twenty-four birds each and further replicated three times. Each treatment was fed one of 5 formulated diets containing ginger meal at levels of 0.0, 0.05, 0.10, 0.15, and 0.2g/100kg for eight weeks in a completely randomized design. At the termination of the experiment, three birds per treatment were selected and used for the evaluation of biochemical and electrolyte parameters. The result of this study showed that increased inclusion levels of ginger across the treatment group caused a significant ($p<0.05$) increase in total protein. There was no significant ($p>0.05$) difference in the AST, ALP, and creatinine in the ginger groups compared with the control. This implied that ginger at the various inclusion levels did not significantly ($p>0.05$) alter these enzyme biomarkers. It was observed that at the least inclusion level (0.05 g/100 kg), the cholesterol concentration was significantly increased ($p<0.05$) compared with other treatment groups and the control, whereas the highest inclusion 0.20 g/100 kg, significantly ($p<0.05$) reduced the mean value from 80.32±1.50 mg/dl to 70.62 ±0.51 mg/dl. This suggests that ginger at the highest inclusion (0.20 g/100 kg) used in this study significantly ($p<0.05$) lowered serum cholesterol concentration. Normal levels of blood protein, glucose, and other serum parameters indicate adequate nutritional status and normal internal organ function. The serum electrolytes of the broiler chicken fed with varying degrees of ginger inclusions did not present significant variations in the serum chloride, phosphorus, sodium, and potassium concentrations across the treatments. It can be concluded from this study that ginger inclusion in the poultry diet, in addition to other nutritional and health benefits, keeps blood cholesterol within the normal range. Also, the dietary inclusion of ginger did not adversely affect the serum biochemistry and electrolyte values of the animals, hence, its inclusion in the diet is encouraged.

References

Ademola, S. G. Farinu, G. O. and Babatunde, G. M. (2009). Serum Lipid, Growth and Hematological Parameters of Broilers Fed Garlic, Ginger and Their Mixture. *World Journal of Agricultural Sciences*, 5 (1), 99-104.

Akhani, S. P., Vishwakarma, S. L. and Goyal, R. K. (2004). Anti-diabetic Activity of Zingiber officinale in Streptozotocin-induced type I diabetic rats. *Journal Pharmacy and Pharma*cology, 56, 101-105.

Al-Homidan, A.A. (2005). Efficacy of using different sources and levels of *Allium sativum* and *Zingiber officinale* on broiler chicks' performance. *Saudi Journal of Biological Sciences*, 12, 96– 102.

Ali, B.H., Blunden, G., Tanira, M. O. and Nemmar, A. (2008). Some Phytochemical, Pharmacological and Toxicological Properties of Ginger (*Zingiber officinale Roscoe*): A Review of Recent Research. *Food and Chemical Toxicology*, 46, 409-420.

Alizadeh-Navaei, R., Roozbeh, F., Saravi, M., Pouramir, M., Jalali, F. and Moghadamnia, A. (2008). An investigation of the effect of ginger on lipid levels. A double-blind controlled clinical trial. *Saudi Medical Journal,* 29(9), 1280–1284.

Amaduruonye, W., Ikwunze, K. and Oguike, M. A. (2018). Impact of ginger (*Zingiber officinale*) on Intestinal, caecal microbial loads and growth performance of broilers. *Nigerian Journal of Animal Science,* 20 (1), 123-133.

Bhandari, U. and Grover, J. K. (1998). Effect of ethanolic extract of ginger on hyperglycemic rats. *International Journal of Diabetes*, 6, 95-96.

Couch, C.E., Movius, M.A., Jolles, A.E., Gorman, M.E., Rigas, J.D., and Beechler, B.R. (2017). Serum biochemistry panels in African buffalo: Defining reference intervals and assessing variability across season, age, and sex. PLoS ONE 12(5): e0176830.

Dhingra, S. and Kumar, P. N. (2005). *Export of spices from India.* The Agriculture and Industrial Survey, New Delhi, pp. 136.

Dieumou, F.E., Teguia, A., Kuiate, J.R., Tamokou, J.D., Fonge, N.B. and Dongmo, M.C. (2009). Effects of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) essential oils on growth performance and gut microbial population of broiler chickens. *Livestock and Research for Rural Development*, 21, 25-34.

George, J.W. (2001). The usefulness and limitations of hand-held refractometers in veterinary medicine: A historical and technical review. *Journal of Veterinary Clinical Pathology,* 30(4): 201 - 210.

George, O. S., Kaegon, S. G. and Igbokwe, A. A. (2013). Effects of graded levels of ginger (Zingiber officinale) meal as feed additive on growth performance characteristics of broiler chicks. *International Journal of Science and Research,* 4, 805-808.

Hong, J.C., Steiner, T., Aufy, A. and Lien, T.F. (2012). Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota, and carcass traits in broilers. *Livestock Science*, 144, 253–262.

Lawrie, A.S., McDonald, S.J., Purdy, G., Mackie, I.J. and Machin, S.J. (1998). Prothrombin time-derived fibrinogen determination on Sysmex CA-6000. *Journal of Clinical Pathology*, 51(6): 462 – 466.

National Root Crops Research Institute-NRCRI (2004). Agro-meteorological Unit, National Root Crop Research Institute, Umudike, Umuahia, Nigeria.

Onu, P. N. (2010). Evaluation of two herbal spices as feed additives for finisher broilers. *Biotechnology in Animal Husbandry*, 26, 383-392.

Platel, K. and Srinivasan, K. (2000) Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. *Nahrung/Food*, 44, 42–46.

Saeed, N., Ahmad, O., and Fatemeh, N. (2011). Haematological and Serum Biochemical Analyses in Experimental Caprine Besnoitiosis. *Korean Journal of Parasitology*, 49(2),133-138.

Saeid, J. M., Arkan, B. M. and Al-Baddy, M. A. (2010). Effect of aqueous extract of ginger (*Zingiber officinale*) on blood biochemistry parameters of broiler. *International Journal of Poultry Science,* 9, 944-947.

Safa, M. A. and Eltazi, F. (2014). Effect of Using Ginger Powder as a Natural Feed Additive on Performance and Carcass Quality of Broiler Chicks. *Assiut Veterinary Medicine Journal*, 60 (141), 1-6.

Tekeli, A., Kutlu, H.R. and Celik, L. (2011) Effect of *Zingiber. Offincinale* and Propolis Extracts on the Performance, Carcass, and Some Blood Parameters of Broiler Chicks. *Current Research in Poultry Science,* 1, 12-23.

Valiollahi, M.R., Rahimian, Y., Miri, Y. and Rafiee, A. (2013): Effect use ginger (*Zingiber officinale*), black pepper (*Piper nigrum*) powders on performance, some blood parameters, and antibody titer against Newcastle vaccine on broiler chicks. *Scholarly Journal of Agriculture Science,* 3(12), 535-540.

Youssef, A. A., Hassan, S.S., Abd-El-Hamid, A. E. and Radwan, M.W. (2017). Response of Broiler Chickens to Dietary Supplementation of Ginger (*Zingiber Officinale*) Continuously or Intermittently in Comparison With Prebiotics. *Egyptian Poultry Science Journal*, 37 (II),523-543.

Zhang, G. F., Yang, Z. B., Wang, Y.,Yang, W. R., Jiang, S. Z. and Gai, G. S. (2009). Effects of ginger root (*Zingiber officinale*) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. *Poultry Science*, 88, 2159-2166.

Table 1: Composition of diets containing varying levels of Ginger meal (GM)

Ingredients	**T_1**	**T_2**	**T_3**	**T_4**	**T5**
Maize	51.5	51.5	51.5	51.5	51.5
Soya bean meal	35.00	35.00	35.00	35.00	35.00
PKM	8.5	8.5	8.5	8.5	8.5
Rice Bran	1	1	1	1	1
Ginger meal	0.00	0.05	0.10	0.15	0.20
Vitamin mineral Premix	0.25	0.20	0.15	0.10	0.05
Bone meal	3.00	3.00	3.00	3.00	3.00
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100
Calculated composition					
Crude protein %	22.25	22.25	22.25	22.25	22.25
ME (Kcal/g)	2840	2832	2826	2816	2810
Ca	1.7	1.7	1.7	1.7	1.7
Ph	0.6	0.6	0.6	0.6	0.6
Total	100	100	100	100	100

Note: Vitamins and trace mineral declaration: 2.5kg of premix contains

Vitamin A (Iμ) 15,000.00; Vitamin D3 (IU) 13,000; Thiamine (mg) 2; Riboflavin (mg) 6; Niacine (mg) 40; Cobalanine (g) 0.05; Pyridoxine (mg) 4; Choline chloride (g) 0.05; Biotin (mg) 0.08; Manganese (g) 0.096; Iron (g) 0.024; Zinc (g) 0.06. Copper (g) 0.06g; Iodine (g) 0.014, Cobalt (g) 0.024, Selenium (mg) 0.24, Antioxidant (g) 0.125

Table 2: Serum biochemistry of broiler chickens fed diets supplemented with ginger

Parameters	T_1	T_2	T_3	T_4	T_5
TP (g/dl)	3.09±0.02^{c}	3.85±0.03^{b}	4.13±0.02^{a}	3.87±0.09^{b}	4.17±0.03^{a}
Albumin (g/dl)	2.00±0.08^{bc}	1.92±0.06^{c}	2.32±0.14^{a}	2.11±0.03^{abc}	2.28±0.11^{ab}
Globulin (g/dl)	1.09±0.10^{b}	1.93±0.09^{a}	1.81±0.14^{a}	1.76±0.12^{a}	1.89±0.12^{a}
AST (U/L)	35.33±1.76	32.33±0.33	33.33±1.76	30.67±2.19	33.33±0.88
ALT (U/L)	26.33±1.86^{a}	25.67±2.19^{ab}	24.00±0.58^{ab}	20.00±2.89^{c}	24.33±0.33^{ab}
ALP (U/L)	55.33±1.76	49.33±0.88	52.00±3.51	51.67±0.88	50.67±0.88
Urea (mg/dL)	155.33±2.91^{b}	201.00±5.51^{a}	202.00±2.08^{a}	206.67±1.76^{a}	199.67±4.98^{a}
Creatinine(mg/dL)	0.40±0.01	0.42±0.04	0.42±0.07	0.47±0.30	0.47±0.02
Glucose (mg/dL)	155.33±2.91^{b}	201.00±5.51^{a}	202.00±2.08^{a}	206.67±1.76^{a}	199.67±4.9
Cholesterol(mg/dL)	80.32±1.50^{c}	105.91±1.22^{a}	86.9**6**±1.92^{b}	85.69±1.09^{b}	70.62±0.51^{d}

Note: Values are presented as Mean ± SEM. Means with different superscripts across rows are significantly different at p<0.05.TP: Total Protein; AST: Aspartate aminotransferase; ALT: Alanine aminotransfElectrolytes Alanine phosphatase

Table 3: Electrolyte of broiler chickens fed diets with ginger replacement

Treatments	Ph	Na^{+}(mEq/ml)	K^{+} (mEq/ml)	Cl^{-} (mEq/ml)
T_1	6.66±0.12^{a}	79.07±1.30^{a}	2.68±0.15^{a}	43.17±2.12^{ab}
T_2	6.68±0.11^{a}	73.93±2.84^{a}	2.79±0.02^{a}	41.67±1.95^{b}
T_3	6.59±0.10^{a}	76.50±1.51^{a}	2.63±0.15^{a}	46.57±0.34^{a}
T_4	6.52±0.04^{a}	74.93±0.82^{a}	2.75±0.09^{a}	47.13±2.05^{a}
T_5	6.67±0.10^{a}	80.17±7.30^{a}	2.78±0.17^{a}	47.07±0.90^{a}

Note: Values are pressuperscriptn± SEM; Means with the same superscript along the column are significantly different at P<0.05