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HAEMATOLOGICAL INDICES AND SERUM BIOCHEMISTRY OF BROILER CHICKEN FINISHER FED DIETS CONTAINING DRIED AVOCADO PEAR SEED (*PERSEA AMERICANA*) MEAL

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Abstract: The general acceptability of plants with proven records of medicinal values in the tropics in recent times has necessitated the use of dried avocado pear in the broiler diet and its possible effects on haematological variables and biochemical indices. The study focused on haematological and biochemical responses of broilers to varying levels of the inclusion of dried avocado pear seeds. The inclusion levels were 0% of the dried avocado pear seed meal as a control, 2.5%, 3.5%, 4.5% and 5.5%. The experiment lasted for eight weeks: four weeks for the starter phase (for the acclimatization of the birds and the maturation of their GIT) and four weeks for the test trials (the finisher phase). The result showed significant differences ($P<0.05$) in the haematological parameters, which implied that the inclusion of the dried avocado pear seeds in broilers' diets had a positive influence on platelets, lymphocytes, monocytes, eosinophils and heterophils. The presence of lymphocytes indicated the adequate production of antibodies, which could prevent opportunistic infections, as was observed during the experimental trials with birds without the test ingredients. The dried avocado pear seed had a positive influence on aspartate aminotransferase (AST), albumin (ALB), total protein (TP), triglyceride and high-density lipoprotein (HDL). It could be concluded from this study that avocado pear seed has significant effects on improving the haematological and serum characteristics of broiler birds, with the highest values recorded in T4 (4.5%) and T5 (5.5%), respectively.

Key words: broiler birds, haematology, high-density lipoprotein, total protein, dried avocado pear seed.

Introduction

Poultry production has been identified as the fastest means of bridging the protein deficiency gap, especially in developing countries. This is because poultry remains one of the most efficient converters of food to animal protein as it is

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known to have a short gestation interval and can multiply quickly. Broiler birds have been widely reported to be a good converter of feed to meat without any religious barriers (Haveenstein et al., 1994). Poultry has achieved its pride of place due to its superior economic importance occasioned by egg production (Laseinde, 2007). Poultry meat and eggs are beginning to substantially contribute to ameliorating animal protein insufficiency in many African countries. However, not much can be said of poultry without a mention of feed.

A greater percentage of the total feed produced in Nigeria is majorly poultry feed, according to Agbede (2019). The sharp increase in the demand for poultry products in recent times, especially in Nigeria, has been attributable to the growth in numbers of fast-food restaurants whose major menu source in urban centres is chicken (Akinnusotu et al*.,* 2018). Feeds and feeding play a very important role in poultry farming as it determines the profit of the business. The poultry industry in Nigeria is faced with the challenges of limited/non-availability of conventional feed ingredients (Agbede, 2019). Hence, a search for alternative feedstuffs that could reduce the cost of feed production becomes a relevant factor. The use of unconventional feed sources like crop residues and agro-industrial by-products has been seen as an alternative solution to the problem of feed crises in poultry production (Agbede, 2019; Igbasan, 2019). A suggestion has been made by Akinsanmi et al. (2020) on the need for partial or complete replacement of costly feed ingredients with less costly non-conventional ones.

Conventional feed ingredients such as maize and soybean have become inadequate for the production of animal products, especially from poultry, as a result of an undue competition between man and livestock over the available feed ingredients, which have resulted in the high cost of feed. This scenario is common in developing nations. However, soybean meal (SBM) and groundnut cake, which are the commonly used plant protein sources in the poultry diet, have become extremely expensive in the last decade, especially in developing countries. Therefore, the search for alternative plant protein sources, which are cheap and locally available, has become an urgent subject to poultry nutritionists (El-Hussieny et al., 2001).

Olorede et al., (1999) reported that feed alone accounted for about 70% of the total cost of poultry production. However, the introduction of avocado seed in poultry diets suggests a future hope for some neglected plant products in the global economy. Aside from the useful protein profiles attributed to avocado seed, Dembitsky et al. (2011) explicitly described the importance of the plant product as a provider of folic acid and appreciable amounts of calcium, potassium, magnesium, sodium, phosphorus, sulfur and silicon, as well as vitamins E, B1, B2 and D. Avocado commonly referred to as the African pear (*Dacryodes edulis*) is a well-known plant in West Africa. The fruits are edible, and the bark, leaves, stem and roots are used for local medicine against diseases associated with the vital organs of the human body (Neuwinger, 2000; Jirovetz et al., 2003). The numerous potentials of avocado seed remain unexploited, however, the industrial use of the seed, if fully utilized, can bring about a drastic reduction in the prices of conventional raw materials such as groundnut cake, soybean meal and other oil seeds used for the production of livestock feed. The determination of blood components by laboratory tests is an important procedure to assist in the diagnosis of various poultry diseases and disorders.

Thus, it is important to know these variations when assessing clinical blood parameters in birds. Also, Genilson et al. (2020) argue that, in Brazil, there is insufficient information at both reference levels for haematological and biochemical parameters for broilers, which makes this study more relevant, and it is essential to draw a blood profile from birds in different experimental situations.

Materials and Methods

Experimental site

The research was carried out at the Poultry Unit of the Teaching and Research Farm, Osun State University, College of Agriculture, Ejigbo Campus. The farm is located on latitude 7º54'N and longitude 4º18'E and 4º54'E at an altitude of 426 m above sea level (Wikipedia-Ejigbo, 2012). Ejigbo is located in the middle portion of 35 km to the north-east of Iwo, 30 km from Ogbomosho in the north and about 24 km east to Ede.

Plant sample collection
The source of avocado pear seeds

Ripe avocado pear pods were obtained from various towns within Osun State. The pods were cut open to gain access to the seeds for further processing. The seeds were chopped into smaller pieces to make the drying process faster and ensure that the seeds were completely dried. After chopping, the seeds were dried in a room away from direct sunlight in order to keep the nutrient profile intact and also until a constant weight was achieved and there was a total reduction in moisture content. The dried seeds were ground (pulverized) using a hammer mill, after which they were incorporated into the feed at various inclusion levels.

Experimental birds
Preparation before arrival

Disinfection, screening and fumigation of the brooding pen had been done before the broiler chicks were brought in, which served as a biosecurity measure.

The nylon screening was removed after 48 hours of fumigation to allow the escape of any residual gases (fumigants).

The arrival of birds

On arrival, the chicks were graded, i.e. unboxed, counted, isolated from weak ones while the active ones were kept in the already prepared pen. Also, the birds were given a solution of glucose and vitamins to serve as an anti-stress agent.

Experimental procedure

Two hundred day-old broiler chicks were used for the experiment. These birds were obtained from a reputable farm in Osogbo, Osun State. The chicks were brooded for fourteen (14) days on a deep litter system using 200-watt electricity bulbs as a source of heat. From 0 to 4 weeks, the chicks were group-fed for acclimatization and the development of the GIT prior to the inclusion of the test ingredient at the finisher phase. The feeding trial was between days 28–56. The chicks, which had already been randomly selected and assigned to five treatments, were replicated three times, ten (10) birds were allocated to each replicate, making a total of 30 birds per treatment. Vaccination and medication programs were administered based on the standard procedure laid down at the University Teaching and Research Farm. Both routine and occasional management practices were thoroughly carried out with strict hygiene measures.

The feed compositions at both starter and finisher phases are shown in Table 2 below. Water was supplied *ad-libitum* throughout the experiment, and avocado pear seeds at varying levels (0%, 2.5%, 3.5%, 4.5%, and 5.5%) were added to their diet at the finisher phase, respectively.

Experimental treatments and experimental design

The following dietary treatments were employed, with the experimental birds being completely randomized in their respective pens. The test ingredient (avocado pear seed) was incorporated into the basal diet. The birds were allotted to the treatments, as illustrated below:

Treatment 1 – Control (0% of avocado pear seed);
Treatment 2 – (2.5% of avocado pear seed);
Treatment 3 – (3.5% of avocado pear seed);
Treatment 4 – (4.5% of avocado pear seed);
Treatment 5 – (5.5% of avocado pear seed).

Table 1. Normal physiological ranges of some biochemical and haematological components for broilers.

Parameters	Reference range
Haematological components	
Haemoglobin (g/dl)	10.2–15.1
Red blood cells (×10^6uL)	2.5–3.5
PVC %	22–35
WBC (×10^4u)	1.2–3.0
Heterophils (%)	1.5–4.0
Basophils (%)	Rare
Platelet (×10^6μl^{-1})	1.5–3.2
Lymphocyte (%)	45–70
Monocytes (%)	5.0–10
Eosinophils (%)	1.6–6.0
Biochemical components	
Total protein (mg/dl)	3–4.9
Cholesterol (mg/dl)	129–297

Source: Jain (1993) as cited by Aeanwanich et al. (2004).

Table 2. The gross composition of the experimental diets at both starter and finisher phases.

	Finisher diet					
Ingredients	0%	2.5%	3.5%	4.5%	5.5%	Starter diet
Maize	50.00	47.50	47.50	47.50	47.50	50.00
Wheat offal	15.50	14.50	15.00	12.00	10.00	8.90
GNC	15.00	15.00	15.50	16.00	16.00	10.00
SBM	15.00	16.00	15.00	15.50	16.50	24.50
Fish meal	0.00	0.00	0.00	0.00	0.00	3.00
DCP	0.10	0.10	0.10	0.10	0.10	0.20
Bone meal	3.00	3.00	3.00	3.00	3.00	2.50
Methionine	0.35	0.35	0.35	0.35	0.35	0.25
Lysine	0.35	0.35	0.35	0.35	0.35	0.00
Broiler premix	0.30	0.30	0.30	0.30	0.30	0.25
Salt	0.40	0.40	0.40	0.40	0.40	0.40
APS	0.00	2.50	3.50	4.50	5.50	0.00
Total	100.0	100.0	100.0	100.0	100.0	100.0

Sample collection for serum and haematological parameters

At the termination of the experiment (8 weeks), the feed was withdrawn 12 hours prior to blood collection. Two birds were selected from each replicate, making a total of six birds per treatment. Then, 5 ml of blood was drawn from each bird through the jugular vein using a hypodermic needle and syringe; blood samples from each bird were released into pre-labelled sample bottles containing ethylene diamine tetra-acetic acid (EDTA) as an anti-coagulant. The collected blood samples were used for the determination of haematological parameters. The PCV was estimated by spinning about 75ul of each blood sample in heparinized capillary tubes in a haematocrit micro centrifuge for 5 minutes, while the total red blood cell count (RBC) was determined using normal saline as the diluting fluid. The haemoglobin concentration (HBC) was estimated using the cyanomethaemoglobin method. Similarly, the erythrocyte sedimentation rate (ESR) of the blood was determined as described by Lamb (1981).

Some blood samples were also collected in test tubes without EDTA for serum analysis. The blood contained in the bottles without the anti-coagulant was allowed to stand in the test tube rack in the laboratory in a slanting position for about 6 hours. The serum separated from each blood sample was then decanted after centrifugation at 2,000 rpm for four minutes. The serum was kept deep frozen prior to further studies. The sera were analyzed for serum biochemical indices. The total protein was determined by the Biuret method of Reinhold (1953) using a commercial kit (Randox Laboratories Ltd, UK), while albumin value was obtained by the bromocresol green method according to Doumas and Bigg (1971). The globulin and albumin/globulin ratio were determined according to the method of Coles (1986). The free cholesterol was determined by Nonane extraction and enzymatic colorimetric methods, respectively, using commercial test kits (Quimica clinica applicada, S.A), while the serum enzymes alanine amino-transferase (ALT) and aspartate amino-transferase (AST) were obtained using the Randox laboratories Ltd, UK test kits and read in the spectrophotometer.

Statistical analysis

All data on haematological variables and serum biochemistry were subjected to one-way analysis of variance (ANOVA) using the statistical analysis system (SAS Version 9.1, 2008). Where significant differences were separated, Duncan's multiple range tests of the same statistical package (Duncan, 1975) were used.

Results and Discussion

Haematological parameters

The haematological parameters (Table 3) showed significant ($P<0.05$) differences in all the blood components at different levels of avocado pear seed meal inclusion, except in PCV, Hb, WBC, RBC and BA. The value of the platelet decreased as the inclusion level increased up to T3 ($3.5\times10^6\mu l^{-1}$). Except in T4, which had an increase of about $1.44\times10^6\mu l^{-1}$ as its value against T3. The PCV results across the whole treatments showed they supported values elicited in Table 1. However, there were no significant differences ($P>0.05$) between T4, T2 and T3.

The highest platelet value was recorded in birds under T1 (0%) with the value of $1.79 \times 10^6 \mu l^{-1}$, while the least was found in T5. LYM obtained in this study recorded the highest values in T3. However, T2 and T3 had 64.50% and 69.50%, respectively, as their values. However, there was no significant (P>0.05) difference between T1 and T5 and between T2 and T4, with T5 being the least. The HET content showed T5 had the highest value, followed by T1 and T2, and the least values were found in T4 and T3. There was a reduction in the value of HET up to T4 (4.5%). However, there were similarities between T1, T2 and T4. The least value was found in T3. Values obtained in MON showed that T4 and T5 were the highest in the monocyte content of the blood, followed by T2 and T1, and the least in T3. However, there was no significant difference (P>0.05) between T1 and T2 in the monocyte content. Values obtained in EOS showed that T1 had the highest value, followed by T2 and T3, and the least value was found in T5. Also, there were similarities between T2, T3 and T4. Basophil had the highest value in T1, T2 and T5, which had no significant difference (P>0.05) between them, with T3 and T4 having the lowest value. The results from the study on basophils supported the findings of Jain (1993) as cited by Aeangwanich et al. (2004) in Table 1.

Table 3. The effect of avocado pear seed at varying inclusion levels on haematological indices of broiler birds.

Parameters	T1 (0%)	T2 (2.5%)	T3 (3.5%)	T4 (4.5%)	T5 (5.5%)	±SEM
PCV %	25.00	25.00	24.50	27.00	26.00	0.90
HBC (g/dL)	8.33	8.47	8.07	9.27	8.95	0.38
WBC ($\times 10^4$u)	14975.00	16900.00	17675.00	18125.00	14325.00	1200.35
Platelet	179000.00^{a}	159500.00^{ab}	133000.00^{ab}	144000.00^{ab}	108500.0^{b}	15512.36
LYM %	61.00^{b}	64.50^{ab}	69.50^{a}	66.00^{ab}	60.00^{b}	2.29
HET %	31.00^{ab}	27.50^{ab}	24.00^{b}	26.50^{ab}	33.00^{a}	2.46
MON %	3.00^{bc}	3.50^{bc}	2.50^{c}	4.00^{ab}	4.50^{a}	0.43
EOS %	4.50^{a}	4.00^{ab}	4.00^{ab}	3.50^{ab}	2.50^{a}	0.56
RBC ($\times 10^6$uL)	2.17	2.71	2.28	2.97	2.73	0.36
BA %	0.50	0.50	0.00	0.00	0.50	0.22

^{abc} means in the same row with different superscripts are significantly different (P<0.05). PCV = packed cell volume, RBC = red blood cell, HBC = haemoglobin count, WBC = white blood cell, LYM = lymphocyte, HET = heterophils, MON = monocytes, EOS = eosinophils and BA = basophils.

Serum biochemistry

The main effect of avocado pear seed on the serum indices of broiler chicken is shown in Table 4. There were no significant differences (P>0.05) between ALT, CHOL and ALP. There were differences (P<0.05) in the result obtained for AST across the treatments. T1 and T5 had the highest value and T3 had the lowest value. The result decreases from T1, T5, T4 and T3, respectively, with T3 having the lowest value. The similarity was noticed between T2 and T4 and also between T1 and T5. T2 had the highest value for TP, followed by T2 and T3, respectively, with T1 and T4 having the lowest value. The similarity was noticed between T1 and T4, also between T2, T3 and T5. T5 had the highest value for the albumin content of the blood, followed by T3 and T2, respectively, with T1 having the least value. There was no significant difference (P > 0.05) between T2 and T3. T2 had the highest value (0.93 g/dL) for globulin, followed by T3 and T1, respectively (0.78 and 0.76 g/dL). The lowest value was recorded in T4 (0.48g/dL). There were similarities between T1, T3 and T5. TG was affected (P< 0.05) by the inclusion of avocado pear seed. T5 had the highest value and the lowest value followed by T2, and the similarity was noticed between T1 and T4 and also between T2 and T3 with SEM (4.12). The HDL value was found to be the highest in T3 and the lowest in T4. The similarity was noticed between T1 and T5, and also between T2 and T3 with SEM (1.24).

Table 4. The effect of dried avocado pear seed at various inclusion levels on serum characteristics of broiler birds.

Parameters	T1 (0%)	T2 (2.5%)	T3 (3.5%)	T4 (4.5%)	T5 (5.5%)	±SEM
AST(IU/l)	178.52[a]	152.09[ab]	117.97[b]	150.52[ab]	177.74[a]	11.75
ALT(IU/l)	20.79	22.67	13.03	14.98	22.25	3.24
Cholesterol (mg/dl)	114.83	132.07	111.91	82.07	97.59	16.79
Total protein (g/dl)	2.60[b]	3.25[a]	3.08[a]	2.63[b]	3.16[a]	0.10
ALB (g/dl)	1.84[c]	2.32[ab]	2.30[ab]	2.15[b]	2.48[a]	0.08
GLOB (g/dl)	0.76[ab]	0.93[a]	0.78[ab]	0.48[b]	0.68[ab]	0.10
TRG (mg/dl)	116.37[b]	56.04[c]	66.22[c]	115.07[b]	130.97[a]	4.12
HDL (mg/dl)	36.86[ab]	37.74[a]	38.45[a]	33.39[b]	36.56[ab]	1.24
ALP (IU/l)	436.07	446.95	492.37	464.13	506.11	3.67

[abc] means in the same row with different superscripts are significantly different (P<0.05). T1–T5 = treatments, TRG = triglyceride, LDL = low-density lipoprotein, HDL = high-density lipoprotein, AST = aspartate amino-transferase, ALT = alanine amino-transferase; CHOL = cholesterol, TP = total protein, GLO = globulin, ALB = albumin, ALP = alkaline phosphatase.

Blood represents a means of assessing the clinical and nutritional health status of the animal in feeding trials (Aletor and Egberongbe, 1992) and according to Togun and Oseni (2005), haemoglobin indices such as RBC, WBC, PCV and Hb have been found useful for disease prognosis and for therapeutic as well as feed stress monitoring. The result from this study could help determine the health status of the birds as it reveals the numbers and morphological status of the cellular components that make up the blood, which is often described as factors necessary for the diagnoses and monitoring of diseases according to the Merck manual (2012).

Lymphocytes, PCV, eosinophils, WBC and platelet values obtained in this study were within the normal ranges reported in the literature for chickens (Aeangwanich et al., 2004). The result indicated that avocado pear seed meal did not have any negative effects on blood formation. The platelet values obtained ranged from 1.09 to $1.79\times10^6\mu l^{-1}$. The lowest values were recorded in T3, and the highest was recorded in T1. The values obtained were within the range (1.5–$3.2\times10^6\mu l^{-1}$), as buttressed by Aeangwanich et al. (2004). The values recorded for the PCV for the experimental birds ranged from 24.50 to 27.00%. Birds fed 4.5% APS meal recorded the highest value (27.00%), which was higher than the control diet, and this again was corroborated by Akinduro et al. (2017), wherein in a related study, it was revealed that certain medicinal herbs have the tendency to improve haematological parameters in animals.

However, there was no significant difference (P>0.05) in the values of PCV obtained. The PCV values obtained were within the range (22–35%) reported by Aeangwanich et al. (2004). The WBC values recorded for the experimental birds ranged from 1.43 to $1.81\times10^4\mu l$. Birds fed the 4.5% APS meal recorded the highest value ($1.81\times10^4\mu$), which was higher than those on the control diet, which could help explain the immunity potential of the dried avocado seed, even though there was no significant difference (P>0.05) in the values of WBC obtained. The WBC values obtained were within the range (1.2–$3.0\times10^4\mu$) as reported by Aeangwanich et al. (2004).

The values recorded on the lymphocyte level for the experimental birds ranged from 60.00 to 69.50%. Birds fed the 3.5% APS meal recorded the highest value (69.50%), which was significantly ($P < 0.05$) higher than those in the control diet and the treatments. The lymphocyte levels obtained for the birds fed the T3 diet being generally higher than for those fed the other diets imply that the APS fed at 3.5% increased the lymphocyte level in the animal's blood, thereby increasing the level of their immunity, as lymphocyte is a type of white blood cell that functions as part of the immune system and responds to foreign invaders in the animal body. The lymphocyte values obtained were within the range (45–70%), as reported by Aeangwanich et al. (2004).

The values recorded in the monocyte level for the experimental birds ranged from 2.50 to 4.50%. The highest value was obtained in T5 with 4.50%, and the lowest value was obtained at T3 with the value of 2.5%. The result contradicted the report by Aeangwanich et al. (2004), whose value ranges from 5.0% as the lowest value compared to 10.00% as the highest value. This means that the APS decreases the level of monocyte in their blood, which brought about a decline in the adaptive immunity of the animals. The values obtained for eosinophils ranged from 2.5% to 4.5%, where T2 and T3 had the same values of 4.00%, and T5 had the lowest value of 2.5%. The result was within the range (1.6–6.0%), as reported by Aeangwanich et al. (2004).

The serum biochemical indices showed AST, blood albumin and blood were not adversely affected by feeding broiler with avocado pear seed. Most blood proteins are formed in the liver, and plasma protein synthesis is usually reduced in severe liver damage or prolonged protein deficiency (Duke, 1975), a situation which has been explicitly defined by the results of this study. According to Aro et al. (2012), the concentration of biochemical substances in the serum changes in abnormal conditions and, therefore, aids in disease diagnosis. Usually, total proteins, albumin, urea, creatinine, and cholesterol analyses are used for the renal function test, while sodium, potassium, chloride, bicarbonate, alkaline phosphate, aspartate amino-transferase (AST), alkaline amino-transferase (ALT) and bilirubin analyses are used to assess the functions of the liver.

Conclusion

It could be concluded from the study that dried avocado pear seeds have a significant effect on the haematological content of broilers, with the highest value recorded in T4 (4.5%) and T5 (5.5%). The study also revealed that T2 (2.5%) had a significant effect on the serum characteristics of broiler birds, hence the improved quality and general wellbeing noticed during the trial exercise. It was also discovered that T2 had high records of TP, ALB, GLOB, ALT, HDL, and ALP amongst all other treatments, which could have been a result of the inclusion of the test ingredient.

Also, the lymphocyte value obtained showed that the APS assisted in the production of antibodies for proper immune function.

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HEMATOLOŠKI INDEKSI I SERUMSKA BIOHEMIJA TOVNIH BROJLERA HRANJENIH OBROKOM SA BRAŠNOM SEMENA AVOKADA (*PERSEA AMERICANA*)

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R e z i m e

Upotreba biljaka sa dokazanim lekovitim svojstvima u tropskim oblastima u poslednje vreme zahtevala je upotrebu osušenog avokada u ishrani brojlera i njegove moguće uticaje na hematološke varijable i biohemijske indekse. Studija se fokusirala na hematološke i biohemijske odgovore brojlera na različite količine osušenog semena avokada. Udeo ovog hraniva u obroku je bio 0% osušenog semena avokada kao kontrole, 2,5%, 3,5%, 4,5% i 5,5%. Ogled je trajao osam nedelja: četiri nedelje za početnu fazu (za aklimatizaciju ptica i sazrevanje njihovog gastrointestinalnog trakta) i četiri nedelje za probna ispitivanja (završna faza). Rezultat je pokazao značajne razlike (P<0,05) kod hematoloških parametara, što implicira da je uključivanje osušenog semena avokada u ishranu brojlera imalo pozitivan uticaj na trombocite, limfocite, monocite, eozinofile i heterofile. Prisustvo limfocita ukazuje na adekvatnu proizvodnju antitela, što bi moglo sprečiti oportunističke infekcije, kao što je primećeno tokom oglednih ispitivanja sa pticama bez ispitivanih sastojaka. Osušeno seme avokada imalo je pozitivan uticaj na aspartat aminotransferazu (AST), albumin (ALB), ukupne proteine (TP), trigliceride i lipoproteine visoke gustine (HDL). Iz ove studije se moglo zaključiti da seme avokada značajno utiče na poboljšanje hematoloških i serumskih karakteristika brojlera, pri čemu su najveće vrednosti zabeležene kod T4 (4,5%) odnosno T5 (5,5%).

Ključne reči: brojleri, hematologija, lipoprotein visoke gustine, ukupni protein, osušeno seme avokada.

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