

Original Research Article

Effect of fermentation with rumen content on the feeding value of boiled iron tree (*Prosopis africana*) seedcoat on haematology and serum biochemistry of broiler chickens

ABSTRACT

Aims: The study was conducted to evaluate the effect of inclusion of *Prosopis* seed coat fermented with rumen content at different levels as energy source on the performance of broiler chickens.

Study design: Data collected were subjected to analysis of variance in a completely randomized design.

Place and Duration of Study: The study was conducted at the Livestock Teaching and Research Farm of the University of Agriculture Makurdi, Benue State, Nigeria. Between November 2016 and January 2017.

Methodology: The study was at two phases; the starter and finisher phase, in each phase, there were five dietary groups and fermented *Prosopis africana* seed coat meal (FPASCM) was included at 0, 10, 15, 20 and 25% to replace maize. The total of two hundred and sixty (260), day-old Zartech broiler chicks were randomly distributed to five dietary treatments with four replicates of 52 chickens per treatment and each replicate had 13 birds. The birds were raised on deep litter and fed ad-libitum and drinking water provided daily and performance was monitored.

Results: The results of haematological parameters of broiler chickens fed varying levels of fermented *Prosopis africana* seed coat are presented in table 3. There were no significant ($P > 0.05$) difference between treatments for parameters measured except for PCV and haemoglobin. The PCV values ranged from 25.00 - 28.25% and haemoglobin values ranged from 8.35 - 9.40g/dl. It is observed that most of the haematology indices parameters of the birds fell within the normal range values. The results of serum biochemistry assay of broiler chicken fed graded levels of seed coat diet are presented in table 4. Results revealed that serum glucose 45.85-107.22 mg/dl was significant ($P < 0.05$) among the dietary treatments. Birds in treatment 5 (25%) FPSCM had the highest value while those on treatment 3 (15%) FPASCM recorded the least value.

Conclusion: Based on the observation from this study, it was concluded that FPASCM could be included in the diets of broiler chickens up to 25% for optimum growth performance of birds as majority of the haematological and serum biochemical parameter falls within the normal reference ranges across the treatments.

Keywords: Broiler chickens, Haematology, FPACM and Serum Biochemistry.

1. INTRODUCTION

The footprint of animal protein in the diet of the average Nigerian is very low. The repercussions of this include stunted physical and mental growth of children and a stunted productivity of the populace. This, unfortunately, is common to the developing world, Nigeria inclusive. The production and productivity of poultry in the tropics has been limited by high prices of animal feeds, which result from high costs of the conventional protein and energy feed ingredients such as maize, soya bean, wheat, and guinea corn [3]. The high cost of these conventional ingredients partly emanates from the competitive demand from the human food industry. Since feed constitute 60-80% of the total cost of production, it is very important to identify and bring into use feed resources that are not subject to competition from the human food industry. This will increase the profitability of livestock production, by reducing feed cost, leading to higher productivity, lower animal product prices and higher animal protein consumption by the general populace.

Prosopisafricana is a leguminous tree and one of the 44 species of the genus *Prosopis*. It is abundant in the derived savanna and Sahel regions of tropical Africa [24]. In Nigeria *Prosopis* seeds are used for making food seasoning, called *Okpehe*(Idoma), *Gbaaye*(Tiv), and *Kiriya*(Hausa) in the middle belt and northern parts of Nigeria, with the empty fruit pods and seed coats forming the major waste products of the process. A lot of researchers have exploited these waste products to the advantage of livestock production.

The study is designed to investigate the effect of fermentation on boiled iron tree(*Prosopisafricana*) seed coat with rumen content as energy source on haematological and serum biochemical indices of broiler chickens.

Prosopis seed coat is a waste material that is thrown away in a large quantity producing area like Benue state of Nigeria, this can be used extensively, and will therefore, be much cheaper than ingredients such as maize, millet, guinea corn. A few researchers, [2], [9] have used *prosopis* seed coat meal on chickens and quails without deleterious effect. If it could be utilized as source of nutrients by broiler chicken, the result will be, cheaper feeds, lower animal product prices and an improvement in the consumption of table meat by the poorer segments of the human population. The result of this will be higher developmental and productivity indices for the people.

2. MATERIAL AND METHODS

Nutrients composition of the experimental broiler starter diet

The result of the proximate composition of starter diets is presented in Table 1. The result revealed that crude protein ranged from 21.56-22.13%. The crude protein level was within the recommended level for broiler starter by Aduku [16]. The result of crude fibre ranged from 3.12-4.32%. It was within the required values recommended for broiler starter. The value for Ether extract 3.26-4.15 was within the range recommended level by Aduku [16].

Table 1: Energy and Proximate Composition of the Experimental Broiler Starter Diets

Nutrient	FPASCM T ₁ (0%)	T ₂ (10%)	T ₃ (15%)	T ₄ (20%)	T ₅ (25%)
Crude protein (%)	22.13	21.89	22.04	21.56	22.13
Crude fibre (%)	3.12	3.56	3.71	4.23	5.13
Ether extract (%)	4.15	3.86	3.49	3.26	3.19
Ash (%)	3.81	4.01	4.10	4.61	5.02
NFE (%)	66.79	66.68	66.66	66.34	64.53
ME (Kcal/kg)	3526.43	3490.12	3464.95	3417.18	3368.34

NFE= Nitrogen Free Extract

ME= Metabolizable energy was calculated using the method of Ponzenga [10], ME

(Kcal/kg)= 37 x% CP +81.1x% EE +35.5x% NFE

FPASCM= Fermented *Prosopisafricana* Seed Coat Meal.

Nutrients composition of the experimental broiler finisher diet

The result of the proximate composition of broiler finisher diet is presented in Table 2. The result revealed that the crude protein (CP) ranged from 18.05-19.68%. The crude protein was within the recommended level for finisher broiler [16]. The crude fibre (CF) ranged from 2.67-4.24 %. The result of CF was within the required range recommended for finisher diet [16]. Ether extract (EE) ranged from 2.54-4.02. It was observed that the EE reduced as inclusion progresses.

Table 2: Energy and Proximate Composition of the Experimental Broiler Finisher Diets

Nutrient	FPASCM T ₁ (0%)	T ₂ (10%)	T ₃ (15%)	T ₄ (20%)	T ₅ (25%)
Crude protein (%)	19.68	19.05	18.05	18.65	18.50
Crude fibre (%)	2.67	3.23	3.04	3.47	4.24
Ether extract (%)	4.02	3.85	3.51	3.20	2.54
Ash (%)	3.31	3.84	3.69	4.05	4.33
NFE (%)	70.32	70.03	71.71	70.63	70.39
ME (Kcal/kg)	3550.54	3503.16	3498.22	3456.94	3389.34

NFE= Nitrogen Free Extract

ME= Metabolizable energy was calculated using the method of Ponzenga [10], ME (Kcal/kg)= 37 x% CP +81.1x% EE +35.5x% NFE

FPASCM= Fermented *Prosopis africana* Seed Coat Meal

Blood Profile of Birds Fed Fermented *Prosopis africana* Seed Coat Meal

The results of haematological parameters of broiler chickens fed varying levels of fermented *Prosopis africana* seed coat are presented in Table 3. There were no significant ($P=0.05$) difference between treatments for parameters measured except for PCV and haemoglobin. The PCV values ranged from 25.00 -28.25% and haemoglobin values ranged from 8.35 -9.40 g/dl. It is observed that most of the haematology indices parameters of the birds fell within the normal range values.

Table 3: Haematological Indices of Broiler Chickens Fed Diets Containing Varying Levels of *Prosopis africana* Seed Coat Meal.

Parameter	FPASCM T ₁ (0%)	T ₂ (10%)	T ₃ (15%)	T ₄ (20%)	T ₅ (25%)	P-Value
Haemoglobin (g/dL)	9.18 ^a	9.40 ^a	8.98 ^a	8.35 ^{ab}	8.93 ^b	.05*
Red blood cell (×10 ¹² /L)	2.50	2.30	2.35	2.13	1.73	.31
White blood cell (×10 ⁹ /L)	1.90	1.75	1.75	1.98	2.13	.65
Packed cell volume (%)	27.50 ^a	28.00 ^a	27.00 ^a	25.00 ^b	28.25 ^a	.001*
Mean corpuscular volume (FL)	112.45	112.45	117.63	123.53	124.70	.94
Mean corpuscular haemoglobin (Pg)	37.50	40.03	39.10	34.70	41.63	.56
Mean corpuscular haemoglobin concentration (g/dL)	33.33	33.50	35.65	33.40	33.38	.51

*: Significant

^{a, b, c}: means in the same row with different superscripts are significantly (P<0.05) different

NS: Not significant

FPASCM= Fermented *Prosopis africana* Seed Coat Meal

Serum biochemistry of birds fed fermented *Prosopis africana* seed coat Meal

The results of serum biochemistry assay of broiler chicken fed graded levels of seed coat diet are presented in Table 4.

Results revealed that serum glucose 45.85-107.22 mg/dl was significant (P<.05) among the dietary treatments. Birds in treatment 5 (25%) FPSCM had the highest value while those on treatment 3 (15%) FPASCM recorded the least value.

Table 4: Serum Biochemical Parameters of Birds fed Diets containing Fermented *Prosopis africana* Seed Coat Meal

Parameters	FPASCM T ₁ (0%)	T ₂ (10%)	T ₃ (15%)	T ₄ (20%)	T ₅ (25%)	P- Value
Albumin (g/dl)	1.45	1.57	1.60	1.75	1.58	.38
Globulin (g/dl)	1.88	0.43	1.55	1.18	0.75	.49
Total protein (g/dl)	3.33	2.00	3.15	2.93	2.33	.55
Glucose (mg/dl)	82.95 ^b	95.08 ^{ab}	45.85	91.65 ^b	107.22 ^a	.01*
Cholesterol (mg/dl)	112.83	117.65	166.57	86.15	128.93	.28
Creatinine (mg/dl)	1.35	1.50	1.43	1.40	1.65	.98
Urea (mg/dl)	50.55	62.50	55.45	50.10	58.00	.79

*: Significant

NS: Not significant

FPASCM =Fermented *Prosopis africana* Seed Coat Meal

3. RESULTS AND DISCUSSION

The results of haematological parameters of broiler chicken fed varying levels of fermented *Prosopis africana* seed coat are presented in Table 3. There were no significant ($P>0.05$) difference between treatments for parameters measured except for Packed cell volume and Haemoglobin. The Haemoglobin was significantly ($P=0.05$) affected by the dietary treatments it ranged between 8.35-9.40g/dL.

The Red blood cell was not significantly ($P=0.05$) affected by the dietary treatments it was between $1.73-2.50 \times 10^{12}/L$.

The White blood cell was not significant ($P=0.05$), it ranged between $1.75-2.13 \times 10^9/L$.

The Packed cell volume was significantly ($P<0.001$) affected by the dietary treatment, it ranged between 25.00-28.25%.

Mean corpuscular volume of the birds ranged between 112.45-124.70fL was not significantly ($P>0.05$) affected by the dietary treatment.

Mean corpuscular haemoglobin ranged from 34.70-41.63Pg was not significantly ($P=0.05$) affected by the dietary treatment.

The mean corpuscular haemoglobin concentration was between 33.33-35.65g/dL.

Serum biochemistry

The results of serum biochemistry assay of broiler chicken fed graded levels of seed coat diet are presented in Table 4. Revealed that the serum glucose was significant among the dietary treatment, it ranged 45.85 - 107.22mg/dl. Treatment 5 (25%) inclusion had the highest and the lowest was from treatment 3 (15%) inclusion rate.

The albumin was between 1.45-1.75g/dl not significantly ($P=0.05$) affected by the dietary treatments.

The globulin ranged between 0.43-1.88g/dl not significant. Total protein was 2.00-3.33g/dl not significant.

Glucose ranged between 45.85-107.22 mg/dl was significantly ($P=0.05$) affected by the dietary treatments. Cholesterol level ranged from 86.15-166.57mg/dl not significant.

The creatinine level ranged between 1.35-1.65mg/dl was not significant.

Urea level of the birds ranged between 50.10-62.50mg/dl was not significant.

Discussion

The haematological profile of broiler chicken fed PASC based diets in this study Table 3 showed that the PCV and haemoglobin were significantly ($P=0.05$) influenced by the treatment. The RBC, WBC, MCV, MCH and MCHC did not vary among the treatment groups. The PCV values of 25.00 to 28.25% recorded in this study fall within the reference range of 22.0-35.0% as reported by Banerjee [17], 24.90-45.20% reported by Mitruka and Rawnsley [26] 26.70%-45.20% .reported by Hewitt et al. [22] and 22-55% by Terry and Campbell [29]. According to these authors, a PCV of less than 22% indicates anaemia and one greater than 55% indicates dehydration. RBC values in this study ranged from $1.73-2.50 \times 10^{12}/L$. This values fall with the range $1.50 \times 10^{12} - 3.80 \times 10^{12}/L$ reported by McDonald et al. [25] for normal chicken. $2.33-2.66 \times 10^{12}/L$ reported by Terry and Campbell [29] who reported that many factors influences the red blood cell count in birds which include species, age of the birds, sex, hormonal influences, hypoxia, environmental factors and nutritional status. The values of RBC obtained in this study are indication that there was no malnutrition among birds fed experimental diets. Haemoglobin (Hb) values observed in this study range from 6.93-9.40 g/dl which is within the rage of 7.40 g/dl to 13.10 for normal healthy birds [26]. 7.0-13.0 g/dl reported by Banerjee [17]and 88.9-13.5 g/dl reported by Terry and Campbell [29]. This result suggests that the inclusion of PASC with maize in broiler chicken diets did not affect nutrients availability to bring about protein and iron deficiency and hence causing anaemia. The WBC ranged from $1.75-2.13 \times 10^9/L$, this values is quite lower to the values of $15-30 \times 10^9/L$ reported by Ganti [21]. According to this author, a white blood cell less than $15 \times 10^9/L$ indicates leukopenia, and the one greater than $30 \times 10^9/L$ indicates leukocytosis. This implies that the birds of this present studies could be leukopenia or related problems but there was no symptoms or signs among birds. The major functions of the WBC and its differential counts are to fight infections, defend the body by phagocytosis against infection by foreign organisms and to produce or at least transport and distribute antibodies in immune response. The MCV values 112.45-124.70fl are comparable with the normal reference values 102-139.00fl reported by Mitruka and Rawnsley [26] MCV are important in determining the morphological characteristics of anaemic cells [18]. The MCH values recorded in this study varied from 34.70-41.63 which are comparable with the normal reference values of 31.90pg – 40.50pg and fall within 33.00-47.00pg as reported by Mitruka and Rawnsley [26] and Jain [23], respectively for healthy birds, 33-47pg reported by Rick [28]. According to Rick, the erythrocyte parameters are measurement used to characterize anaemia. The MCHC values in this study occurred between 3.33—35.65 which is within the normal reference values of 25.90 g/dl – 33.90 g/dl for normal chicken as reported by Mitruka and Rawnsley [26] and 26.0 g/dl – 35.0 g/dl reported by Jain [23]. The value of MCHC is a measure of the quantity of haemoglobin in each cell and also shows the ratio of weight of haemoglobin to the volume and haemoglobin content together, so that MCHC is mostly normal. MCHC value decreases only in severe iron deficiency [8]. These three mean corpuscular values are important to determining the morphological characteristics of anaemic cells. The values of erythrocyte indices obtained in this study appear to be normal, an indication that FPASC based diet were adequate. In protein, vitamins and minerals including irons. Adequate iron with good quality protein is essential for their formation and maintenance of RBC and prevention of anaemia. The non-significance of most of the parameters investigated in this study agreed with the report of Anonymous [30] who indicated that the non-significance of most haematological and corpuscular indices, erythrocytic and differential counts are indication that the experimental birds were not adversely affected by the dietary treatments.

Serum biochemical indices

The serum biochemical indices determine in this study Table 4 showed that only glucose was affected by the dietary treatments while the other indices were not influenced. This result is in harmony with the report of Romeo [15] who indicated that prosopis pod meal had no effect on biochemical indices of broiler chickens. The total protein level in this study varied from 2.33-3.33 g/dl reported Adeyemo [1]. Iyaji and Tewe [5] reported that the total serum protein level depends on both quality and quantity of protein supplied in the diets. Globulin values obtained in this study range from 0.43-1.88 g/dl which is lower than 33.35 g/dl as normal range reported by Ganti [21]. According to the author, an elevated globulin concentration (hyperglobulinaemia) indicates immune stimulation or chronic inflammation with increased globulin product. On the other hand, a low globulin concentration (hypoglobulinaemia) indicates low immunoglobulin concentration and can be caused by conditions such as haemorrhage, protein-losing gastro enteropathy, immune suppression, immunodeficiency syndrome and severe malnutrition. The serum cholesterol level of 86.15-166.57 g/dl was within the normal reference value (52.00-146 g/dl) [26]. Birds of this study show that there high level of cholesterol. This result is in line with the report of Idowu et al. [14] who observed that dietary fibre binds with fat and its associates and therefore reduce their assimilation and further deposition in the tissues, organs and products. Madubuike and Ekenyem [7] reported that elevated level of cholesterol is an indication of higher than desired level of saturated fats in the diets. It was observed that only glucose was influenced by dietary treatments. The range of values (45.85 – 107.22mg/dl) for glucose was lower than 200-230mg/dl recommended as the optimum glucose level in avian species [27]. However, birds did not show any sign of disease that may be associated with energy inadequacy. Malik *et al.* [8] have opined that the non-significance for most of these parameters is an indication of good liver functioning when high fibre diets are used in poultry nutrition. Aside some genetical factors observed in birds, PASCAM is an energy and fibre source in poultry diets, its inclusion in the diet in this study seems to enhance the haematological and biochemical characteristics of broiler chickens. This findings is similar to the result obtained by Schley and Field [12]; Kwariet *al.* [6] and Malik *et al.* [8] who observed that the different energy sources have no negative effect on serum biochemical indices of avian species. The albumin values ranged from 1.45- 1.75g/dl, it is within the range 1.17-2.74g/dl. A higher concentration of albumen usually denotes dehydration while a lower concentration may be due to the liver not functioning adequately due to factors such as malnutrition and infection. [13]. Albumin is a protein serum is synthesized in the liver, it is responsible for transporting insoluble substance in the blood and aids to maintain pressure [20]. Hassan et al. [4] suggest that non-significant values for albumin and Globulin in Decorticated fermented *Prosopisafricana* seed coat meal is a nutritional adequacy of the dietary protein for the treatment. The values for urea ranged from 50.10- 62.50 mg/dl is above the normal range 1.9-12.5 mg/dl reported by Clinical Diagnostic Division [19] for uric acid. A low urea suggests more efficient metabolism proper renal and hepatic function. Age, sex and diet of birds influence the amount of uric acid. A high level of uric acid (hyperuricaemia) is usually evident in female birds due to ovulatory activities. Creatinine values present in the experimental birds range from 1.35-1.65mg/dl, this is within the range 1.41 reported by Sandhu et al. [11] for broilers at 35 days. He stated that younger and older broiler chickens have low levels of blood creatinine. Creatinine is used to determine of the status of the kidney. The functions of the kidney include excretion of waste products resulting from protein metabolism and muscles contraction. High amount of creatinine could also indicate that the kidney is not functioning optimally.

4. Conclusion

Based on the conclusions drawn from this study, haematological indices were within the normal reference ranges across treatments group. It is suggested that FPASCAM could be included in the diets of broiler chickens up to 25% as there was no physiological disorder in the birds.

REFERENCES

Reference to a journal:

For Published paper:

1. Adeyemo GO. Effect of Cottonseed cake based diets on haematology and serum biochemistry of egg-type chickens. *International Journal of Poultry Science*.2008; 7(1): 23-27.
2. Adeyi MO, Abang FBP, and Attah S. Performance of broiler chickens fed diets containing graded levels of boiled *Prosopisafricanaseed* coat meal treated with multi-enzymes. *Journal of Science and Technology and Education*. 2021; 9(1):206-210.

3. Atawodi SE, Mari D, Atawodi JC and Yahaya Y. Assessment of Leukaemia leucocephalleaves as feed supplement in laying hens. *African Journal. Biotechnology*. 2008; 7: 317- 321.
4. Hassan D, Yusuf N, Musa M, Musa Azara I, Barde R, Ogah D, Yakubu A. and Ari M. Haematology and some serum parameters of broilers fed decorticated fermented *prosopisafricanaseed* meal. *Journal for Agricultural Science and Technology*. 2014; 6(2): 170-174.
5. Iyaji E A, and Tewe, OO. Serum total protein, urea and creatinine levels as indices of quality of cassava diets for pigs. *Journal ofTropical Veterinarian*. 1998; 8:11-15.
6. Kwari ID, Igwebuikue JU, Taiya H, Muhaamad AA, and Raji AO. Haematology and seriology of chickens fed maize, sorghum and millet, and their combination in the semi-arid zone of Nigeria. *International Journal of Science and Nature*. 2014;5(2): 319-322.
7. Madubuikue FN, and Ekenyem BU. Haematology and Serum Biochemistry characteristics of broiler chicks fed varying levels of *Ipomeaasarifolia leaf meal*. *International Journal of Poultry Science*; 2006. 5:9-12.
8. Malik AA, Kudu YS, and Gold RO. Haematology, Serum Biochemistry and Economic Characteristics of cockerels fed diets containing Varying levels of Water Hyacith [*Eichlorniacrassipes (mart.) solms-Laubach*] meal supplemented with maxigrain R enzyme. *Nigerian Journal of Animal Science*. 2018; 20 (2):241-249.
9. Odeh MO, Attah S, and Abang FB. Performance and Carcass Characteristics of Layer Chickens Fed Diets containing *Prosopisafricana* seed coat meal treated with polyenyme. *International Journal of Environment, Agriculture and Biotechnology*2019; 4(6):1750-1757.
10. Pauzenaga U. Feeding parent Stock. *Zoo-tech. International*. 1985; Pp. 22-25
11. Sandhu BS, Singh B, and BrarRS. Haematology and biochemical studies in broiler chickens fed ochratoxin and inoculated with inclusion body hepatitis virus, singly and in concurrence. *Veternary Research Communications*.1998; 22:335-346.
12. Schley PD, and Field CJ. The immune enhancing effects of dietary fibres and probiotics. *British Journal of Nutrition*. 2002; 87(2): 221-230.

For Accepted, unpublished papers.

13. Esubonteng PKA An assessment of the effect of moringaolifera leaf powder as a nutritional supplement in the diet. Kumasi: Kwame Nkruma University of Science and Technology. (2011). (*In press*).
14. Idowu OMO. Effect of unpeeled cassava based diets on performance and cholesterol level in laying hens. Ph.D. Thesis, Department of Animal Nutrition, University of Agricultural, Abeokuta, Ogun State, Nigeria. 2004; 186Pp. (*In press*).
15. Romeo AC. Effect of *prosopisafricana* Pod Meal fermented with rumen content on serum biochemical indices of broiler finishers. B. *Agriculture.*, Federal University of Agriculture, Makurdi, Benue State, Nigeria. 2016; 71Pp. (*In press*).

Reference to a book:

16. Aduku AO. *Animal Nutrition in the tropics: Feed and Feeding, Pasture Management, Monogastrics and Ruminants Nutrition*. 1st ed. University Press. ABU, Zaria, Nigeria; 2004.
17. Banerjee GC. *A Text book of Animal Husbandary*, 8thed.New Delhi, Oxford and IBH Publishing Co. PVT Ltd, Pp. 118-138; 2004.
18. Cambell TW. *Avian haematology and cytology*. Iowa State University Press/Ames, 248Pp; 1998.

19. Clinical Diagnostic Division. Veterinary reference guide: a summary of reference intervals for use with KODALEKTACHEM Products. Rochester (NY). Eastman Kodak Company. 1990
20. Fischbach FT, Dunning MB. A Manual of laboratory and diagnostic tests. Philadelphia: Lippincott; 2009.
21. Ganti, AS. Veterinary Clinical Pathology, CBS Publishers and Distributors. New Delhi/Bangalore, Pune. (India). 3rd ed. 200Pp; 2009.
22. Hewitt CD, Innes DJ, Sarory J. and Willis MR. Normal biochemical and haematology Values for New Zealand White rabbits. *Clinical Chemistry*, 35(8):1777-1779; 1989
23. Jain NC. *Schalm's Veterinary Haematology*, 4th Ed. Philadelphia, USA, Lea and Febiger, Pp.34-50; 1986.
24. Keay RWJ. Trees of Nigeria, Clarendon press, Oxford, UK; 1989.
25. McDonald P, Edwards RA, Greenhalgh JFD, and Morgan CA. Animal Nutrition, 5th edition (3rd report). *Longman Scientific and Technical publishers* 607pp; 1998.
26. Mitruka BM, and Rawnsley HM. Clinical and biochemical haematological reference values in normal experimental animal. Manson publishing Company, New York, Pp. 231-245; 1977.
27. Olomu JM. Monogastric Animal Nutrition, Principle and Practices. Second Edition. Benin City, Nigeria. St. Jackson Publishers, 478Pp; 2011.
28. Rick, L. C. Veterinary Clinical Pathology Secrets, Elsevier ink. 1st ed. 150 Pp; 2004.
29. Terry, W. and Campbell, M. S. Avian haematology and cytology, 1st ed. Iowa State University press, Ames. Pp. 5-13; 1988.

Reference to Web-resource or Electronic articles.

30. Anonymous. Guide to the care and use of experimental animals, Vol. (1). Canadian Council of Animal Care, Ottawa, Ontario, Canada. 1980; Pp. 85-90. Accessed 30 January, 2017. Available: <http://www.sciepub.com>>reference.