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Original Research

Effect of diet modification with iron-on haematological and biochemical parameters of non-anaemic puppies

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ABSTRACT

Dietary iron intake is vital for proper growth and development in puppies. The aim of this study was to examine the effects of diet modification with iron on haematological and biochemical parameters of puppies. A total of ten apparently healthy puppies weighing between 4.3 – 6.5 kg were used for this study. They were assigned to two groups of five dogs each. Group I was iron-supplemented, while group II served as non-iron supplemented control. Puppies in group I were fed diet modified with iron supplementation, while the group II puppies were fed with puppy food without supplementation. Result of this study revealed significant changes in haematological and biochemical parameters in the iron-supplemented compared to the control. Haemoglobin levels, erythrocyte counts, and the mean corpuscular volume showed significant ($P \leq 0.05$) increase. Serum iron was elevated significantly ($p \leq 0.05$) in the iron-supplemented compared to the control group. However, the lipid profile, kidney function markers, oxidative stress biomarkers as well as the activities of the liver enzymes were comparable between the two groups ($p > 0.05$). The findings of this research suggest that iron modified diets improved haematological parameters and iron status in puppies.

Keywords: Iron supplementation, Iron deficiency anaemia, nutrition

INTRODUCTION

Iron plays important roles in variety of physiological processes such as oxygen transport, energy metabolism, immune function and also necessary for proper growth and development of young animals (Elawamy *et al*., 2020). Although, it is among the most abundant metals in the earth's crust, its deficiency still remains the most common cause of anaemia worldwide and poses a global health concern (Kassebaum *et al*., 2014). Physiologically, iron is bound to haemoglobin, and myoglobin, or stored as ferritin and hemosiderin, or attached to plasma protein carrier; transferrin (Gupta *et al*., 2014). In the body system, iron is tightly controlled to prevent the detrimental effect of excessive accumulation within the hepatocytes which could lead to hemochromatosis (iron overload) that is characterized by liver fibrosis and hepatic cirrhosis (Ferreira *et al*., 2014). On the other hand, deficiency of iron could deplete its store leading to iron deficiency anaemia and other iron related metabolic disorders (Wang & Pantopoulos, 2011; Naigamwalla *et al*., 2011).

In Africa, the deficiency of the micronutrient is very common and it affects all ages of animals and humans irrespective of their socio-economic status. This menace has remained significant resulting in detrimental effects on bodily functions (Bain *et al*., 2013). In Nigeria, the most important mineral deficiencies are iron deficiency, iodine deficiency, and zinc deficiency; which are commonly observed in animals fed grains cultivated on micronutrient deficient soils; linked to decades of soil degradation and inappropriate utilization of fertilizer (Kihara *et al*., 2017; Kihara *et al*., 2020). To curb this menace, array of National Micronutrient Deficiency Control (MNDC) guidelines including diet supplementation and modification of feed are currently being implemented with diet modification one of the widely acceptable because it is believed to be simple, effective and cheap (Allen *et al*., 2006; Anjorin *et al*., 2019).

In monogastric animals, iron deficiency occurs due to low intake (Dodd *et al*., 2021). The daily recommended iron intake for adult dogs is 0.5 milligrams of iron per kilogram

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of body weight while the recommended iron content in adult food is 80 milligram of iron per kilogram of dry matter. This requirement is expected to be higher in growing puppies to aid their fast growth rate (Dzanis *et al*.,1994). In suckling animals, low concentration of iron in breast milk have been implicated as the major cause of deficiency (Cappellini *et al*., 2020). Inadequate consumption is not common in puppies fed on commercial dog foods (Dodd *et al*., 2021), however, iron deficiency has been reported in dogs fed on home-cooked meals or vegetarian diets without supplementation or fortification (Michel, 2006; Sadighi *et al*.,2008; Gross *et al*.,2020). While iron deficiency anaemia is a well-known condition, effects of modifying diets with iron on haematological and biochemical parameters in Nigeria indigenous puppies are yet to be investigated. This research work is therefore designed to evaluate the haematological and biochemical changes in puppies fed with iron modified diet.

MATERIALS AND METHODS

STUDY AREA

This study was conducted at the Veterinary Teaching Hospital, University of Ilorin, Nigeria. Ilorin is located on latitude $8^0$24' N and $83^0$6' N and longitude $4^0$10' E and $4^0$36' E and at 941.14 ft above sea level. The town has a tropical humid and dry savannah climate.

EXPERIMENTAL ANIMALS

Ten Nigerian indigenous dogs of both sexes aged 6 - 9 months with mean weight of 4.7 kg were used for this study. Approval to conduct this research was granted by the University of Ilorin Committee on Animal Use and Care (Ethical code- UREC/FUM/2020/079). All experiments were reviewed and carried out in conformity with the ethical guidelines and regulations regarding the use of animals in research (2010/63/EU; European Commission, 2010).

They were kept in metal cages in a fly- proof kennel. They were fed once daily with commercial dog food. Clean drinking water was provided *ad libitum*. The dogs were allowed to acclimatize for two weeks before the commencement of the study. The puppies were assigned into groups of five dogs each. Group I was fed with home-made food with iron supplementation at 100 milligram of iron per kilogram of dry matter for four weeks, while group II (control) were fed with home-made food without iron supplementation. Baseline measurement of haematological, biochemical and oxidative stress parameters was recorded for all puppies prior to dietary intervention. Subsequently, bi-weekly measurements were taken for a period of 4-weeks to monitor changes in the parameters.

BLOOD SAMPLE COLLECTION

Blood sample (5 ml) was collected from the cephalic vein of each dog using 23G needle and syringe. The blood sample was divided into two parts; 1ml was dispensed into a tube containing ethylene diamine tetra acetic acid (EDTA) as an anticoagulant; the remaining 4ml was dispensed into a tube without anticoagulant for the preparation of sera for biochemical analyses. The blood samples for serum biochemical tests were allowed to clot at room temperature for 30 minutes and then centrifuged at 3000 g for 15 minutes; sera were carefully harvested into labelled vials and then stored at -20^{o}C until analysed.

LABORATORY ANALYSES

Haemoglobin concentration was estimated by Sahli's method, red blood cell count was enumerated by hemocytometry method, mean corpuscular volume was calculated as described by Esievo (2017). Iron levels and total iron-binding capacity (TIBC) were determined using Ferrozine based colorimetric assay while transferrin saturation was calculated as follows: (iron ÷ TIBC) and expressed as percentage. The concentrations of total protein, blood urea nitrogen, creatinine, total bilirubin, cholesterol levels and the activities of liver enzymes were determined by spectrophotometry method using Agappe kits (India) (Aktas *et al*., 2011). Malondialdehyde (MDA) levels, as well as the superoxide dismutase, catalase, and glutathione peroxidase activities were measured using calorimetric assay kit (Cayman Chemical Company, USA) (İnal *et al*., 2001). All measurements were conducted by trained personnel to ensure accuracy and reliability.

STATISTICAL ANALYSIS

Data obtained were analyzed using student's T-test to determine the significance of the observed changes in haematological and biochemical parameters between control and experimental puppies. Multiple comparison test was employed in order to detect which measurement results caused the differences. Significance was accepted for values of $p < 0.05$ with a 95% confidence interval.

RESULTS

Haemoglobin concentration (11.83±0.92, 13.46±1.10) erythrocyte count (8.64±1.02, 9.05±1.09) and mean corpuscular volume (80.70±2.94, 81.60±3.13) on weeks 2 and 4 showed significant increase in the iron-supplemented group ($p \leq 0.05$) when compared with the control (haemoglobin concentration 10.40±0.90, 10.71±0.88; erythrocyte count 6.04±0.83, 5.98±0.95; mean corpuscular volume 64.80±3.19, 67.20±3.30) respectively. Serum iron levels (24.68±1.15) on week 4 were also significantly higher ($p \leq 0.05$) in the iron-supplemented as compared to 17.84±1.05 of the control group. However, other iron

markers, the activities of liver enzymes, the kidney function markers or oxidative stress biomarkers did not vary (P>0.05). between the groups (Table 1).

blood. So, the markedly elevated haemoglobin concentration, RBCs count and MCV observed in the group fed diet

Table 1: Haematological and biochemical parameters of puppies fed iron modified diet

Variable		Control group (N =5)		Experimental group (N = 5)			*P*-value
	Baseline	Week 2	Week 4	Baseline	Week 2	Week 4	
Hb (g/l)	10.52±094	10.40±0.90	10.71±0.88	10.19±0.95	11.83±0.92	13.46±1.10	0.001
RBC(× 10^{12}/L)	5.83±1.00	6.04±0.83	5.98±0.95	6.00±0.94	8.64±1.02	9.05±1.09	0.001
MCV (fL)	65.00±2.01	64.80±3.19	67.20±3.30	65.40±2.01	80.70±2.94	81.60±3.13	0.001
SI (umol/L)	17.63±0.73	17.50±0.67	17.84±1.05	16.86±0.94	19.93±0.89	24.68±1.15	0.05
TS (%)	26.13±2.39	29.88±4.30	32.63±4.77	27.88±1.37	31.25±3.43	33.38±4.17	0.7128
TIBC (ug/dl)	264.9±17.86	242.90±32.59	265.40±11.94	275.9±21.66	279.4±20.11	280.6±13.95	0.1146
MDA (uM)	1.804±0.26	1.963±0.26	1.85±0.24	1.763±0.18	1.84±0.23	1.85±0.24	0.9518
GPx (U/ml)	27.06±1.69	27.96±1.48	33.20±3.96	30.89±2.73	28.43±2.42	31.41±3.79	0.6118
SOD (U/ml)	33.13±4.32	34.01±5.44	34.68±4.67	32.38±3.20	33.50±4.58	35.16±5.62	0.9988
CAT (U/ml)	44.93±2.49	42.88±2.57	47.63±2.24	41.25±2.90	46.13±2.33	46.00±1.71	0.2917
TP (g/dL)	55.54±5.61	60.01±2.44	61.17±2.18	57.57±12.53	66.14±14.62	64.57±7.85	0.384
Alb (g/dL)	26.43±1.21	28.26±1.46	28.29±1.26	30.43±6.70	29.43±7.04	25.29±3.99	0.281
BUN (mg/dL)	13.48±1.20	12.82±2.05	14.29±1.55	13.71±2.89	11.86±2.85	12.29±2.56	0.766
Cr (mg/dL)	1.057±0.17	0.87±0.15	0.96±0.20	0.81±0.34	1.13±0.44	0.90±0.36	0.305
TB (mg/dL)	0.14±0.05	0.19±0.80	0.14±0.05	0.19±0.10	0.16±0.07	0.14±0.06	0.867
TC (mg/dL)	69.86±6.64	70.01±9.63	76.68±7.96	76.71±16.97	82.57±15.27	85.14±21.23	0.674
AST (U/L)	12.67±1.68	13.29±0.84	12.99±1.31	12.86±2.34	13.50±2.50	11.71±4.10	0.445
ALT (U/L)	11.71±4.50	11.43±3.05	13.00±3.89	13.57±3.38	11.86±5.82	11.57±3.95	0.289
ALP (U/L)	43.14±21.17	39.28±31.20	44.08±15.11	41.00±28.37	42.57±30.19	46.71±14.77	0.949

Key: Hb = Haemoglobin concentration, RBC = Red blood cell count, MCV = Mean corpuscular volume, SI = Serum iron, TS = Transferrin saturation, TIBC = Total iron binding capacity, MDA = Malondialdehyde, GPx = Glutathione peroxidase, SOD = Superoxide dismutase, CAT = Catalase, TP = Total protein, Alb = Albumin, BUN = Blood urea Nitrogen, Cr = Creatinine, TB = Total bilirubin, TC = Total cholesterol, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase.

DISCUSSIONS

Haemoglobin (the protein responsible for transportation of oxygen) was suggested by WHO to be the most definitive indicator of iron profile in humans (and animals) as compared with other commonly used haematological indices (Adelugba, 2012; Camaschella, 2017; Mattiello *et al.*, 2020). Indices such as the mean corpuscular volume (MCV) is a measure of the mean size of red blood cells (RBCs), while the measurement of total RBCs only provides information on the average number of RBCs present in a given volume of

modified with iron suggest that diet-modification replenishes the body's iron stores thereby providing building-block for erythrogenesis. This finding agrees with those of previous workers (Pasricha *et al.*, 2012; Cargo-Froom *et al.*, 2019). The biochemical measures of body's iron store include serum iron (SI), total binding iron capacity (TIBC), transferrin saturation (TS), and ferritin (Grant *et al.*, 2021). In circulation, transferrin is the majorly involved in the transportation of SI and it could then be indirectly measured in the laboratory by its TIBC meanwhile, TS is expressed as the ratio of SI to TIBC, with a saturation percentage (%) of

less than 20% being indicative of iron deficiency anaemia (IDA) (Elawamy *et al.,* 2020). In this present study, SI concentrations were elevated in the experimental group indicating that the body iron store were efficiently maintained in puppies fed with an iron-modified diet. However, TIBC and TS values were within laboratory limits in all the puppies. This observation suggests that neither iron deficiency anaemia nor iron overload was observable in the experimental puppies during the period of study.

The body's iron balance is believed to be tightly regulated to prevent the toxic effects that could be associated with excess circulating iron (iron overload) which could lead to increased production of reactive oxygen species (ROS) which are harmful and detrimental to normal tissue functions. Mediator of iron haemostasis is the hepatic hormone; hepcidin (Camaschella, 2020). Invariably, an increase in ROS will lead to decrease in antioxidant concentration and subsequent oxidative distress (Qi *et al*., 2020; Bacou *et al*., 2021). The comparable serum MDA level in control and supplemented group is an indication that the observable increase in SI was not associated with overload and/or toxic changes such as high lipid peroxidation that could result in oxidative damage, cell necrosis or apoptosis (Zaka-Ur-Rab *et al*., 2016). A similar finding has been reported in rats fed with iron supplemented diet (Skypnik *et al*., 2021). Conversely, a human study on oral dietary iron supplementation reported a marked reduction in MDA (Kurtoglu *et al*., 2003). Interestingly, the comparable activities of SOD, CAT, and GPx reported in this study are indications that the modified diet did not induce oxidative stress and/or deplete the antioxidant system. This report is in agreement with previous findings (Todorova *et al*., 2005; Xu *et al*., 2008), but disagrees with the results of Abtahi *et al*. (2014) and Usman *et al*. (2019). Specie variation might be responsible for those differences.

The findings of this study that include unaltered total cholesterol, total bilirubin, kidney metabolites as well as the activities of the liver enzymes are pointer to the fact that the dietary modification did not predispose the puppies to an increased risk of cardiovascular diseases, gall bladder dysfunction, impaired bilirubin metabolism, hepatic and renal pathology (Esievo, 2017). Therefore, it is concluded that diet modification with iron can positively improve the haematological parameters of puppies. The elevation in serum iron seen in puppies fed with modified diet supports the role of iron in maintaining erythropoiesis.

REFERENCES

Adelugba, A. O. (2012). *The Assessment of Reticulocyte and Erythrocyte Haemoglobin Contents, and Their Use in the Evaluation of Iron Status in Hospitalised Patients* (Doctoral dissertation, University of Portsmouth).

Allen, L., De Benoist, B., Dary, O., & Hurrell, R. (2006). *Guidelines on food fortification with micronutrients*. WHO/FAO.

Aktas, S., Boyvat, F., Sevmis, S., Moray, G., Karakayali, H., and Haberal, M. (2011). Analysis of vascular complications after renal transplantation. In *Transplantation proceedings*, 43(2), 557-561. Elsevier.

Anjorin, O., Okpala, O., & Adeyemi, O. (2019). Coordinating Nigeria's micronutrient deficiency control programs is necessary to prevent deficiencies and toxicity risks. *Annals of the New York Academy of Sciences*, 1446(1), 153-169.

Bacou, E., Walk, C., Rider, S., Litta, G., & Perez-Calvo, E. (2021). Dietary Oxidative Distress: A Review of Nutritional Challenges as Models for Poultry, Swine, and Fish. *Antioxidants*, 10(4), 525-533.

Bain, L. E., Awah, P. K., Geraldine, N., Kindong, N. P., Siga, Y., Bernard, N., & Tanjeko, A. T. (2013). Malnutrition in Sub–Saharan Africa: burden, causes, and prospects. *Pan African Medical Journal*, 15(1), 1-9.

Camaschella, C. (2017). New insights into iron deficiency and iron-deficiency anemia. *Blood Reviews*; 31, 225–33.

Camaschella, C. (2019). Iron deficiency. *Blood*; 133, 30–9.

Cappellini, M. D., Musallam, K. M., & Taher, A. T. (2020). Iron deficiency anemia revisited. *Journal of internal medicine*, 287(2), 153-170.

Cargo-Froom, C. L., Shoveller, A. K., Minikhiem, D., Kuhlman, G., & Boebel, K. (2019). Diets containing naturally occurring iron or naturally occurring iron plus supplemental ferrous sulfate both maintain haematological status in adult dogs. *Journal of Food Nutrition and Metabolism*, 2(2), 2-8.

Dodd, S. A., Shoveller, A. K., Fascetti, A. J., Yu, Z. Z., Ma, D. W., & Verbrugghe, A. (2021). A comparison of key essential nutrients in commercial plant-based pet foods sold in Canada to American and European canine and feline dietary recommendations. *Animals*, 11(8), 2348.

Dzanis, D.A. (1994). The Association of American Feed Control Officials dog and cat food nutrient profiles: Substantiation of nutritional adequacy of complete and balanced pet foods in the United States. *Journal of Nutrition*, 124(12), 2535S–2539S.

Elawamy, H. A., Elmhdwi, M. F., Abdulla, S. A., Jaber, N. H., & Algathafy, K. G. (2020). Iron deficiency in non-anemic individuals: a retrospective analysis of Libyan population. *GSC Biological and Pharmaceutical Sciences*, *11*(1), 171-179.

Esievo, K.A.N. (2017). *Veterinary Clinical Pathology*. Spectrum Books Ltd, Ibadan, 1st Edition. pp. 143-144.

Ferreira, R. R., Gopegui, R. R., Araujo, M. M. R., & de Matos, A. J. (2014). Effects of repeated blood donations on iron status and hematologic variables of canine blood donors. *Journal of the American Veterinary Medical Association*, *244*(11), 1298-1303.

Grant, E. S., Clucas, D. B., McColl, G., Hall, L. T., & Simpson, D. A. (2021). Re-examining ferritin-bound

iron: current and developing clinical tools. *Clinical Chemistry and Laboratory Medicine*, 59(3), 459-471.

Gross, K. L., Wedekind, K. J., Cowell, C. S., Schoenherr, W. D., Jewell, E. E., Zicker, S. C. & Frey, R. A. (2000). Nutrients. *In: Small Animal Clinical Nutrition*. Hand MS, Thatcher CD, Remillard RL, Roudebush P.(eds.). Marceline-Missouri.

Hennigar, S. R. (2019). Ironing out the Relation between Iron Supplementation and Exercise Performance in the Absence of Anemia. *The Journal of Nutrition*, 149(2), 177-178.

İnal, M. E., Kanbak, G., & Sunal, E. (2001). Antioxidant enzyme activities and malondialdehyde levels related to aging. *Clinica chimica acta*, *305*(1-2), 75-80.

Kassebaum, N. J., Jasrasaria, R., Naghavi, M., Wulf, S. K., Johns, N., Lozano, R., & Murray, C. J. (2014). A systematic analysis of global anemia burden from 1990 to 2010. *Blood, The Journal of the American Society of Hematology*, 123(5), 615-624.

Kihara, J., Bolo, P., Kinyua, M., Rurinda, J., & Piikki, K. (2020). Micronutrient deficiencies in African soils and the human nutritional nexus: opportunities with staple crops. *Environmental Geochemistry and Health*, 1-19.

Kihara, J., Sileshi, G. W., Nziguheba, G., Kinyua, M., Zingore, S., & Sommer, R. (2017). The application of secondary nutrients and micronutrients increases crop yields in sub-Saharan Africa. *Agronomy for Sustainable Development*, 37(4), 1-14.

Kurtoglu, E., Ugur, A., Baltaci, A. K., & Undar, L. (2003). Effect of iron supplementation on oxidative stress and antioxidant status in iron-deficiency anemia. *Biological Trace Element Research*, 96(1), 117-123.

Mattiello, V., Schmugge, M., Hengartner, H., von der Weid, N., & Renella, R. (2020). Diagnosis and management of iron deficiency in children with or without anemia: consensus recommendations of the SPOG Pediatric Hematology Working Group. *European Journal of Pediatrics*, 179(4), 527-545.

Michel, K. E. (2006). Unconventional diets for dogs and cats. *Veterinary Clinics: Small Animal Practice*, 36(6), 1269-1281.

Naigamwalla, D. Z., Webb, J. A., & Giger, U. (2012). Iron deficiency anemia. *The Canadian Veterinary Journal*, 53(3), 250.

Pasricha, S. R., De-Regil, L. M., Garcia-Casal, M. N., Burford, B. J., Gwirtz, J. A., & Peña-Rosas, J. P. (2012). Fortification of maize flour with iron for preventing anaemia and iron deficiency in populations. *Cochrane Collaboration*, John Wiley and Sons, New York.

Qi, X., Zhang, Y., Guo, H., Hai, Y., Luo, Y., & Yue, T. (2020). Mechanism and intervention measures of iron side effects on the intestine. *Critical reviews in food science and nutrition*, *60*(12), 2113-2125.

Sadighi, J., Sheikholeslam, R., Mohammad, K., Pouraram, H., Abdollahi, Z., Samadpour, K. & Naghavi, M. (2008). Flour fortification with iron: a mid-term evaluation. *Public Health*, 122(3), 313-321.

Skrypnik, K., Bogdański, P., Sobieska, M., Schmidt, M., & Suliburska, J. (2021). Influence of multistrain probiotic and iron supplementation on iron status in rats. *Journal of Trace Elements in Medicine and Biology*, 68,126849.

Todorova, I., Simeonova, G., Kyuchukova, D., Dinev, D., & Gadjeva, V. (2005). Reference values of oxidative stress parameters (MDA, SOD, CAT) in dogs and cats. *Comparative Clinical Pathology*, 13(4), 190-194.

Wang, J., & Pantopoulos, K. (2011). Regulation of cellular iron metabolism. *Biochemical Journal*, 434(3), 365-381.

Xu, J., Knutson, M. D., Carter, C. S., & Leeuwenburgh, C. (2008). Iron accumulation with age, oxidative stress and functional decline. *PloS One*, 3(8), e2865.

Zaka-Ur-Rab, Z., Adnan, M., Ahmad, S. M., & Islam, N. (2016). Effect of oral iron on markers of oxidative stress and antioxidant status in children with iron deficiency anaemia. *Journal of Clinical and Diagnostic Research*, 10(10), SC13.