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Haematological and Serum Biochemical Responses of Ovambo Chickens Fed Provitamin A Biofortified Maize

■Author(s)

Odunitan-Wayas F[I]
Kolanisi U[I]
Chimonyo M[I]

[I] University of KwaZulu-Natal, Pietermaritzburg Campus School of Agricultural, Earth and Environmental Sciences, Private Bag X01, Scottsville, 3209, South Africa.

■Mail Address

Corresponding author e-mail address
Michael Chimonyo
Department of Animal and Poultry Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu- Natal,Private Bag X01, Scottsville, 3209, South Africa.
Phone: +27767309002
Email: chimonyo@ukzn.ac.za

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ABSTRACT

The current study was conducted to investigate the influence of provitamin A biofortified maize (PABM) diet, sex and age of birds on the haematological and serum biochemistry parameters of indigenous chickens. A total of ninety-six 13 week old male and female Ovambo chickens were reared and fed on either white maize (WM) a low vitamin A diet or a PABM based diet for eight weeks. Each diet was replicated four times. Packed cell volume (PCV), haemoglobin (Hb), erythrocyte concentration (RBC), leucocytes concentration (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured. Albumin (ALB), globulin (GLOB), total proteins (TP), triglycerides (TRI), cholesterol (CHOLES), uric acid (UA) and creatinine (CREAT) concentrations were assessed. The activity of alanine transaminase (ALT) alkaline phosphate (ALP) and aspartate transaminanse (AST) were also assayed. The PABM diet increased (p<0.05) PCV and WBC of female birds. All mean values for the haematological parameters regardless of the age, sex and diets were within the normal range. Age had an effect (p<0.05) on ALT, TP, GLOB, TRI and UA. Sex of the birds significantly influenced the TP, GLOB, ALB, CREAT and TRI levels. There was significant interaction of diet and age of bird on ALP, ALT, and GLOB concentrations. The cholesterol level in 18 weeks old male birds fed on the WM diet was above the normal range. In conclusion, feeding PABM diet to female and male indigenous chickens will not negatively impact on the health status of indigenous chickens.

INTRODUCTION

Indigenous chickens in Africa are increasingly being preferred for consumption by consumers due to their unique organoleptic properties such as taste, flavour, darker cooked meat colour, chewy texture and low chemical contamination when compared to commercial chickens (Chumngoen & Tan, 2015; Magala *et al.*, 2012). Diseases and scarcity of nutritious feed are however, some of the major limiting factors of indigenous chicken development and production (Desta & Wakeyo, 2013). As the growth rate of indigenous chickens is low and feeds are costly, there is minimal need to feed them with high levels of proteins and energy to boost their growth rate and performance as the commercial birds. However, it is important to derive means of improving the general well-being and immune response of indigenous chickens for better development which will improve production without unnecessary higher cost implication.

Vitamin A is a micro-nutrient that is required for immunity, vision, growth and development (Bárdos *et al.*, 2011). It can either be in the form of preformed vitamin A or provitamin A carotenoid. Provitamin A carotenoids have the ability to convert to vitamin A when ingested

(Pixley *et al.*, 2013). They do not have the risk of vitamin A toxicity as the preformed vitamin A because the cleavage of provitamin A carotenoids to retinal which is converted to retinol is a highly regulated step that is dependent on the body's requirement (Penniston & Tanumihardjo, 2006). Because of the functions of vitamin A, vitamin A supplement is included in commercial poultry's diet (Desta & Wakeyo, 2013). Vitamin A supplement s are however not included in the diet of indigenous chickens. Most of the diet of indigenous chickens are got from scavenging and supplementary feed which constitute mainly leftovers from households, whole or crushed maize grains, millets and sorghum (Desta & Wakeyo, 2013). It is important to include vitamin A in the diet of indigenous chickens because of its functions.

Maize as a major ingredient in the diet of poultry accounts for up to 70% of their diets (Summers, 2001). The common types of maize used to feed chickens are the white and yellow maize, both low in vitamin A (Aganga *et al.*, 2003; Pillay *et al.*, 2011).White maize is the commonly grown maize for consumption in southern Africa, and indigenous chickens are often given this maize either as whole grain or crushed (Smale *et al.*, 2013). Provitamin A biofortified maize (PABM) has been biofortified with high provitamin A carotenoids, especially β- carotene. The PABM has all the nutrients present in yellow and white maize, plus the added benefits of having higher provitamin A carotenoids concentration and the potential of being drought tolerant (Aluru *et al.*, 2008; Liu *et al.*, 2015). The PABM can be easily incorporated into the diet of chickens as it can substitute white maize or yellow maize. It can also be included in their diet in any quantity without any risk of vitamin A toxicity implications. The replacement of white maize by PABM has been established to improve the skin and meat muscle colour of the indigenous chicken with age and sex as contributory factors (Odunitan-Wayas *et al.*, 2016). Skin and meat colour are physically used to assess the freshness, quality and health status of chickens (Kennedy *et al.*, 2005). Haematological and serum biochemical responses are used to assess the clinical and physiological responsiveness and well-being of chickens (Sharma *et al.*, 2015). There are however little or no information on the effect of PABM on the blood composition of the chickens. The replacement of white maize with PABM is likely to boost the immunity and health status of chickens based on the functions of vitamin A. To validate these assumptions, it is important to assess the influence of PABM on the blood composition of the indigenous chickens to determine the extent of its influence on boosting their immunity and health status which will consequently influence their growth and development.

One of the common indigenous chicken breeds in southern Africa is the Ovambo (Van Marle-Koster & Nel, 2000). The Ovambo is aggressive and agile and has mostly dark red, brown and black plumage which aids to camouflage the bird to avoid predators, making it popular for rearing among rural poultry farmers (Van Marle-Köster & Casey, 2001).Ovambo chickens have the highest dressed carcass mass among the South African indigenous breeds (Van Marle-Koster & Webb, 2000). These superiorities make the Ovambo breed highly desirable for meat purpose. Due to the increasing consumer's demand of the indigenous chicken despite their slow growth and high mortality rates, it is necessary to improve their production for better survival and performance. This will also consequently contribute to the livelihood of the rural poor who rear indigenous chickens. The health status of chickens is paramount in the performance of chickens (Yang *et al.*, 2009). Haematological and serum biochemical parameters are influenced by feed, medication, toxic compounds, infections, age and sex of the birds (dos Santos Schmidt *et al.*, 2009; Huff *et al.*, 2008). The intention of the study was to test whether provitamin A carotenoids in PABM could have an effect on the indigenous chicken's metabolism due to its functions. The objective of the study was to compare the haematological and biochemical responses of female and male Ovambo chickens to PABM at different ages. The hypothesis is that provitamin A biofortified maize, age and sex of the Ovambo chickens have no effect on their blood composition.

MATERIALS AND METHODS

Study site and the ethical aspects of the study

The management and care of the chickens were in accordance with internationally accepted standards for the welfare and ethics of chickens. Management and use adopted for the study was approved by the University of KwaZulu-Natal Animal Ethics Research Committee (019/14/Animal). The study was conducted between September 2014 and February 2015 at Cedarain KwaZulu-Natal, South Africa. The site is in the upland savannah zone on 29.53 ^{o}S and 30.27 ^{o}E. The average environmental temperature was 21.3^{o}C and the relative humidity an average of 63.2 %.

Birds, diets, diet analysis and management

A total of 200 unsexed Ovambo chicks that were hatched from parent stock by the Agricultural Research Council (ARC), Irene, Pretoria in South Africa were raised together in a well-ventilated floor area of 2m by 2.5m in width and breadth respectively under a deep litter management system. The floor was adequately covered with 12cm layer of wood shavings. A commercial standard broiler starter meal was given *ad libitum* to the chickens from day 1 to day 49 and commercial standard grower diet was given from day 50 to day 84. Water was offered *ad libitum* in 4L plastic founts and feed was provided in tube feeders made of standard gutter materials. Light and heat were provided continuously using infra-red lamps. The birds were vaccinated against Newcastle disease at 14 days of age. Gumboro vaccine was also given at 6 weeks of age. The vaccinations were administered orally in the drinking water of the chickens.

At 12 weeks (84 days), 48 male (average body weight= 1.5 ± 0.3kg) and 48 female birds (average body weight = 1.1 ± 0.4 kg) were selected. The birds were acclimatized to their experimental pen environment for 7 days prior to the commencement of the experimental trial at 13 weeks of age. During this period, the birds were fed on a commercial standard grower diet. The pens were placed in open sided houses with cement floor on a 15cm deep wood shaving litter. Each pen was 230 cm long, 143cm wide and 120 cm high. An experimental unit, represented by a pen, contained six randomly selected birds of the same sex. Eight pens were randomly assigned for each diet, four pens for male birds and four pens for the female birds. A minimum of 15h light was provided daily throughout the experimental period. No antibiotic or growth promotant was administered. Water and feed were given *ad libitum*. Water was provided in 4L plastic founts and the feed was given in 10L plastic hanging feeders. The wood shavings were changed fortnightly or whenever there was water spillage. At 13 weeks at age, the birds were introduced to the experimental dietary treatments. The initial average individual weights of the male and female birds at 13 weeks were 1.6 ± 0.33 and 1.2 ± 0.42 kg respectively.

Two dietary treatments were used. The control diet was formulated with 100 % white maize (WM) and the PABM-based diet was formulated with 100 % HP326-6 maize variety (Table 1). The PABM maize was obtained from Makhathini Research Station, Jozini, KwaZulu-Natal where it was planted. The aim of biofortification of maize with provitamin A carotenoids was to increase the concentration of β- carotene in the endosperm of the maize.

Table 1 – Feed composition of experimental diets.

Ingredients	(Control-WM) kg	PABM(kg)
Provitamin A biofortified maize	0.0	417.7
White maize	417.7	0
Soya meal	175.4	175.4
Vegetable oil	23.8	23.8
Limestone	12.3	12.3
Declaim phosphate	6.9	6.9
Salt	1.9	1.9
DL-Methionine	1.2	1.2
L-Lysine	0.1	0.1
Vit.-min. premix (excluding vit A)	3.2	3.2
Nutrient composition		
Metabolizable energy (MJ/kg)	12.56	13.01
Crude protein (g/kg)	199	198
Ash (g/kg)	110	97.3
Calcium (g/kg)	10	11
Phosphorus (g/kg)	7.4	8.1
Provitamin A carotenoids(mg/kg)	0.1	0.5

One kg of feed contained the following:; cholecalciferol,60 mg; all-rac-_ tocopheryl acetate, 30 mg; menadione, 3 mg; thiamine, 22 mg; riboflavin, 8 mg; pyridoxine, 5 mg; cyanocobalamin, 11 mg; folic acid, 1.5mg; biotin, 150 mg; calcium pantothenate, 25 mg; nicotinic acid, 65 mg; Mn, 60 mg; Zn, 40 mg; I, 0.33 mg; Fe, 80 mg; Cu, 8 mg; Se, 0.15 mg; ethoxyquin, 150

Feed Analysis

The fat content of WM and PABM dry milled maize flour was determined using Soxhlet extraction method (AOAC, 1984). To determine the crude protein, the total nitrogen content was determined by Kjeldahl nitrogen analysis according to AOAC (1995). The percentage ash content was calculated as: % ash = weight of ash x 100/ weight of sample (AOAC, 1980). The gross energy values were estimated by multiplying the crude protein, fat and carbohydrate by their water values. Calcium and phosphorus were determined by atomic absorption spectrophotometer method and colorimetrically respectively according to AOAC (1984). Carotenoid analysis was carried out using a Hewlett Packard 1100 HPLC (Agilent Technologies Incorporated, Loveland, CO, USA) consisting of a binary pump, autosampler, column thermostat, diode array detector and ChemStation software (Revision B.03 02, Agilent Technologies Incorporated, Loveland, CO, USA).

Data collection

At 18 and 21 weeks (5 and 8 weeks of feeding on experimental diets), 16 birds from each dietary treatment, that is, eight female and eight male birds, two birds from each replicate were randomly selected, totalling 32 birds (16 female and 16 male birds) from both dietary treatment. From each of the selected birds, two sets of blood samples were collected via the jugular vein. One set of blood samples was collected into 5ml purple top vacutainer tubes containing an anticoagulant, ethylene diaminetetra-acetic acid (EDTA) for determining the haematological parameters. The other set of blood samples was collected into 5ml vacutainer tubes that did not contain any anti-coagulant. The coagulated blood samples were centrifuged for 15 minutes at 3000 rpm to collect the serum before they were stored at -20^{0}C pending analyses.

Haematological parameters

The packed cell volume (PCV) was measured by a microhaematocrit capillary tube using a Hemocrit reader. Erythrocyte concentration (RBC), haemoglobin (Hb) and leucocytes concentration (WBC) counts were measured using an automated cell counter within 24 hours after collection of blood. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were also calculated (Jain, 1986). The formulae used were:

MCV in femrolitres (fL) = 10 x PCV (%)/RBC counts (millions/µl).

MCH in pg/cell= haemoglobin (g/ 100 ml)/ RBC counts (millions/µl).

MCHC in g/dl= haemoglobin (g/ 100 ml) x 100/PCV (%)

Biochemical parameters

The albumin, serum lipid profile, total protein, uric acid, creatinine and activities of the liver enzymes, alanine transaminase, aspartate transaminase and alkaline phosphatase were measured at the School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal, Westville Campus, Durban, South Africa using an automated chemistry analyser (LabmasPlenno, Labtest, Lagoa-Santa Brazil). Globulin was calculated as the difference between total proteins and albumin.

Statistical Analyses

The data were analyzed using the PROC MIXED of SAS (Statistical Analysis System, version 9.2) with tests of means (PROC LSMEANS) for significant variables. The model used included diet, age and sex as fixed variables. Statistical significance was considered at the 5% level of probability.

The model used was: $Y_{ijkl}=\mu + D_i + S_j + A_{k+} (D \times S)_{ij} + (D \times A)_{ik} + (D \times S \times A)_{ijk+} E_{ijkl}$

Where: $Y_{ijkl=}$ response variable (haematological and serum biochemistry parameters)

μ= the overall mean

D_i= effect of the ith diet with i = PABM and WM diet;

S_j= effect of the jth sex with j= male and female birds

A_k= effect of the kth age with k= 18 and 21 weeks;

$(D \times S)_{ij}$= interaction of the ith diet and the jth sex;

$(D \times A)_{ik=}$ interaction of the ith diet and the kth age of bird;

$(D \times S \times A)_{ijk}$ = interaction of the ith diet, jth sex of bird and the kth age of bird;

E_{ijkl}= random error term assumed to be normally and independently distributed with mean 0 and variance equal to ð$^{2.}$

RESULTS

Effect of diet, sex and age on haematological parameters of Ovambo chickens

The effects of diet, sex and age on haematological parameters of Ovambo chickens are reported in Table 2. Female birds fed on the PABM diet had a significantly higher RBC than the female birds fed on the WM diet. The male birds had higher PCV than the female birds, but it was only significant in the WM fed birds. The PABM fed birds had significantly higher WBC than the WM fed birds. The WBC of the female birds was higher than the male birds. At 21 weeks of age, the PABM fed females had a lower MCV than the WM fed female birds, while the PABM fed male birds had a higher MCV than the WM fed male birds. At 18 weeks of age, the PABM fed female birds had a lower MCH than the WM fed female birds. The PABM fed birds at 21 weeks of age had higher MCHC then the PABM fed birds at 18 weeks of age. The PABM fed male birds at 21 weeks of age had higher MCHC than the WM fed birds at 21 weeks of age (Table 2).

Effect of diet, sex and age on serum biochemistry of Ovambo chickens

The effects of the diet, sex and age on serum biochemistry of Ovambo chickens are shown in Table 3. The PABM fed male birds at 21 weeks of age had a significantly higher ALB than the WM fed male birds of the same age. The male birds had higher ALB than the female birds, but it was only significant in the WM fed male birds and female birds at 18 weeks. At 18 weeks of age, the ALP of the PABM fed male birds was lower (p<0.05) than the WM fed male birds (Table 3). The WM fed female had a higher AST than the PABM

Table 2 – Effect of diet, sex and age of bird on the haematological parameters of Ovambo chickens.

	18 weeks				21 weeks				
	WM		PABM		WM		PABM		
	Male	Female	Male	Female	Male	Female	Male	Female	SEM
RBC (x106µ)	2.79^{ab}	2.57^{b}	2.85^{ab}	2.88^{a}	2.84^{ab}	2.45^{b}	2.77^{ab}	2.86^{a}	0.11
Hb (g/dl)	11.28^{b}	10.16^{a}	11.45^{b}	10.80^{ab}	11.00^{ab}	10.23^{a}	11.48^{b}	11.01^{ab}	0.36
PCV (%)	38.00^{a}	33.75^{b}	38.25^{a}	36.50^{ab}	38.20^{a}	34.34^{b}	37.67^{a}	35.94^{ab}	1.07
WBC (g/dl)	15.95^{a}	20.65^{b}	23.20^{bc}	26.20^{c}	15.15^{a}	20.50^{b}	22.16^{bc}	23.45^{bc}	1.29
MCV (fL)	136.50^{b}	131.61^{ab}	134.96^{b}	127.41^{a}	131.20^{b}	133.02^{ab}	135.99^{b}	125.66^{a}	2.17
MCH (pg/cell)	40.43^{b}	39.61^{b}	40.37^{b}	29.64^{a}	39.21^{b}	40.18^{b}	41.44^{b}	38.50^{b}	0.57
MCHC (g/dl)	29.64^{a}	30.11^{a}	29.94^{a}	29.58^{a}	29.10^{a}	30.00^{a}	30.48^{b}	30.63a	0.29

Superscript letters indicate differences (*p*<0.05) in rows.

WM: white maize (control), PABM: provitamin A biofortified maize, RBC: red blood cell, Hb: haemoglobin, PCV: packed cell volume; WBC: white blood cell, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration. SEM: standard error of mean.

female at 21 weeks of age. The PABM fed female birds had a higher ALT than the WM fed female birds at 21 weeks of age. White maize fed male birds had a significantly higher ALT than the PABM fed male birds at 18 weeks of age (Table 3).

Cholesterol concentrations in the WM fed male birds were higher than the PABM male fed birds at 18 and 21 weeks of age, however it was only significant between the male birds at 18 weeks of age. The WM fed male birds at 21 weeks of age had a significantly lower creatinine concentration than the WM fed females of the same age. The female birds at 21 weeks of age had higher TP than the male birds of the same age. The PABM fed females at 21 weeks of age had higher (*p*<0.05) globulin than the WM fed females of the same age. The female birds at 21 weeks of age had a significantly higher (*p*<0.05) globulin amount than male birds of the same age (Table3). The triglycerides of the female birds regardless of the diet and age were higher than the male birds; however, it was only significant between the female and male birds at 21 weeks of age. The triglycerides of the female birds at 21 weeks of age were also significantly higher than the female birds at 18 weeks of age. The PABM fed male birds at 21 weeks of age had a significantly lower uric acid concentration than the PABM fed female birds and the WM male birds of the same age (Table 3).

Table 3 – Effect of diet, sex and age of bird on the serum biochemistry of Ovambo chickens

	18 weeks				21 weeks				
	WM		PABM		WM		PABM		
	Male	Female	Male	Female	Male	Female	Male	Female	SEM
ALB (mg/dl)	2.08^{bc}	2.12^{bc}	2.06^{bc}	2.17^{bc}	1.50^{a}	2.36^{c}	1.97^{b}	2.15^{bc}	0.11
ALP (U/L)	986.50^{c}	703.75^{ab}	558.50^{a}	647.00^{ab}	686.00^{ab}	791.00^{bc}	714.25^{ab}	713.50^{ab}	76.43
ALT (U/L)	6.25^{abd}	3.00^{ac}	1.13^{c}	4.25^{ac}	3.50^{ace}	4.50^{ad}	6.50^{de}	8.75^{bd}	1.19
AST (U/L)	276.38^{a}	241.88^{a}	325.00^{a}	276.13^{a}	263.75^{a}	855.00^{b}	279.50^{a}	240.25^{a}	146.26
CHOLES (mg/dl)	230.63^{d}	113.63^{ac}	115.50^{ac}	106.25^{ac}	119.25^{ac}	141.50^{ac}	100.75^{a}	129.00^{ac}	46.08
CREAT(ml)	1.69^{ab}	0.62^{a}	1.40^{a}	0.77^{a}	1.02^{a}	5.54^{b}	1.00^{a}	3.43^{ab}	1.42
TP (g/dl)	4.14^{b}	4.17^{b}	4.10^{b}	4.24^{b}	3.55^{a}	4.85^{c}	4.00^{ab}	5.22^{c}	0.17
TRI (mg/dl)	66.25^{a}	96.63^{a}	65.75^{a}	81.88^{a}	56.25^{a}	512.25^{c}	51.75^{a}	441.50^{c}	120.97
Uric acid (mg/dl)	10.92^{a}	10.94^{a}	9.17^{a}	10.71^{a}	8.29^{a}	8.96^{a}	5.26^{b}	9.06^{a}	0.96
Globulin	2.06^{a}	2.04^{a}	2.04^{a}	2.06^{a}	2.05^{a}	2.49^{c}	2.03^{a}	3.06^{b}	0.10

Values with different superscripts in the same row differ (*p*<0.05). WM: white maize, PABM: provitamin A biofortified maize, ALB: albumin, ALP: alkaline phosphate, ALT: alanine transaminase, AST: aspartate transaminase, CHO: cholesterol, CREAT: Creatinine TP: total protein, TRI: triglycerides.

DISCUSSION

The normal ranges of the haematological parameters in chickens are RBC: 2.5-3.5 x10^6 µl, PCV: 22-35 %, Hb: 7-13 g/dl and WBC: 12-30 x 10^3 µl (Bounous & Stedman, 2000). The MCV is used to calculate the average erythrocyte size, the MCH to measure haemoglobin amount per blood cell and the MCHC to know the amount of haemoglobin relative to the size of the cell per red blood cell. Their normal ranges are MCV: 90-140 fL, MCH: 33-47 pg/cell and MCHC: 26-35 g/dl (Bounous & Stedman, 2000). All the values obtained for the haematological parameters in this study were within their normal range.

The function of RBC is to transport oxygen from the lungs to tissues and remove carbon dioxide from the tissues to the lung in the body via haemoglobin. The RBC range of chickens is affected by sex and diet of birds (Kaminski *et al.*, 2014). The findings in the current study indicate that the effect of PABM on RBC is more evident in female birds is comparable with earlier study (Kaminski *et al.*, 2014). The RBC and Hb concentrations are influenced by reproductive hormones. The significantly higher PCV of the male birds fed WM diet than the WM fed female birds is supported by earlier findings on indigenous chickens (Elagib & Ahmed, 2011; Sharmin & Myenuddin, 2004). The higher amount of Hb and MCV in the male birds could be because of the androgen hormone present in the male birds, as increased level of PCV in male birds correspond with the time of androgen production (Adedibu *et al.*, 2014; Cecil & Bakst, 1991). The increase in PCV can be used to envisage sexual maturity and the start of semen production. It would seem that the PABM reduced the effect of sex on PCV as the PCV of the PABM male and female birds was not significant. In general, haematological parameters of indigenous birds are influenced by several factors that include the sex and diet (Elagib & Ahmed, 2011).

The WBC aids to protect the body from pathogen and carotenoids build up immunity (Osman *et al.*, 2004; Saladin, 2003). The PABM diet increased the WBC of the birds in the current study. This is similar to earlier report that supplementation of vitamin A increases WBC concentration in chickens (Akbari *et al.*, 2008).Other reports also indicate that vitamin A and carotenoids increase the immune response in chickens and also reduce common avian infections such as coccidiosis and lesions that cause significant losses to poultry farmers (Díaz-Gómez *et al.*, 2015; Sepehri Moghaddam & Emadi, 2014). Carotenoids have been documented to have antioxidant activities that reduces stress and help the birds to fight infections better (Nogareda *et al.*, 2016). Higher level of WBC in female birds than the male birds is similar to earlier findings (Addass *et al.*, 2012; Cucco *et al.*, 2007; Sharmin & Myenuddin, 2004). Lower WBC in male birds could be due to the presence of testosterone in the male's plasma which could suppress immune response (Müller *et al.*, 2003). In the current study, it is evident that the PABM diet boosted immune response.

Total protein is made up of ALB and globulin. Globulin is calculated as the difference between TP and ALB. The normal ranges of the TP and ALB in bird's blood are 3.0-4.9 mg/dl and 1.17-2.74 g/dl, respectively (Meluzzi *et al.*, 1992). All the birds regardless of their diet, age at slaughter and sex were within the normal range of TP and ALB. Albumin, a serum protein is synthesized in the liver. It is responsible for transporting insoluble substance in the blood and aids to maintain oncontic pressure (Fischbach & Dunning, 2009). A higher concentration of ALB usually denotes dehydration while a lower concentration may be due to the liver not functioning adequately due to factors such as malnutrition and infection (Esubonteng, 2011). Total proteins in the female birds were higher than the male birds. This could be attributed to oestrogen induced increase in globulin in preparation of the female bird's body for egg laying (Simaraks *et al.*, 2004).

Liver enzymes, namely the alanine transaminase albumin (ALT), alkaline phosphatase (ALP) and aspartate transaminase (AST) are important in the determination of the proper functioning of the liver (Ambrosy *et al.*, 2015). These enzymes are present in negligible concentration. An increase in the concentration of these enzymes may be because of damaged or diseased cells which denote the status of the liver function. The high concentration of AST in the WM fed female birds at 21 weeks of age could be an indication of damage to the liver. The normal range of the concentration of liver enzymes are: AST:70-220 U/L, ALP: 568-8831 U/L (Meluzzi *et al.*, 1992). Vitamin A deficiency increases the levels of AST and ALT (Roodenburg *et al.*, 1996).

Creatinine is used to determine the status of the kidney. The functions of the kidney include excretion of waste products resulting from protein metabolism and muscle contraction (Ileke *et al.*, 2014). Creatinine is excreted by the kidney as a by-product of creatinephosphate metabolism which is produced as a result of energy production by the skeletal muscles (Esubonteng, 2011). The high level of creatinine in the female birds at 21 weeks can be attributed to the metabolic changes as a result of sexual maturity (Menon *et al.*, 2013). A high amount of creatinine could also indicate that the kidney is not functioning optimally thus, the high level in the WM fed female birds at 21 weeks of age compared to the PABM fed females indicates a lower than optimal functioning of the kidney. Vitamin A and its active metabolites have been reported to affect the development of kidney which is associated with the proper functioning of kidney resulting in the high level of creatinine (Gilbert, 2002).

High protein intake, increased protein metabolism, stress and dehydration influence the concentration of uric acid in the blood as it is produced as a result of protein metabolism (Chernecky & Berger, 2008). The normal range of uric acid is 1.9-12.5 mg/dl (Clinical diagnostic division, 1990). Age, sex and diet of birds influence the amount of uric acid. A high level of uric acid (hyperuricemia) is usually evident in female birds due to ovulatory activities (Ibrahim *et al.*, 2012). The amount of uric acid in female birds was higher than the male birds at both stages of slaughter; however, it was only significant at 21 weeks of age in the PABM fed birds. This can be attributed to the fact that, at 18 weeks of age, the female birds have not started laying eggs. The average maturity age for egg laying is 21 weeks (Van Marle-Koster & Nel, 2000).

The concentration of cholesterol of the WM fed male birds at 18 weeks was above the normal range,87-192 mg/m (Meluzzi *et al.*, 1992) of cholesterol in birds. Cholesterol is synthesized from fats consumed and endogenously synthesized within the cells. A high level of cholesterol is an indication of a high risk to cardiovascular disease. The findings of the current study agrees with reports that β- carotene diet reduces serum cholesterol (e Silva *et al.*, 2013). Other earlier report agree with the finding of this study that carotenoids diet reduces total cholesterol in chickens (Rao & Shen, 2002). Furthermore, the absence or presence of cholesterolaemic effects of dietary components in an animal depends on various factors such as breed, sex and age, and also on the composition of the feed (Toghyani *et al.*, 2010).

Triglycerides (TRI) are synthesized in the liver from fatty acids, and from protein and glucose when they are above the body's current needs and then stored in adipose tissue (Esubonteng, 2011). The TRI of the WM fed female birds at 21 weeks of age was above the normal range (45.7-172) mg/ml (Meluzzi *et al.*, 1992) and higher than the PABM fed female birds and the male birds Serum biochemical constituents are positively correlated with the quality of the diet (Adeyemi *et al.*, 2000; Etuk *et al.*, 2014).

CONCLUSIONS

The use of PABM as a major feed ingredient and source of vitamin A for female and male Ovambo chickens within the ages of 13 to 21 weeks will positively impact on the health status of the Ovambo chicken. The sex of the birds is a contributory factor to the impact of the PABM on the haematological parameters. The PABM improved the immune response of the birds, especially in the female birds and the cholesterol levels of the birds. These will consequently improve the general performance of Ovambo chickens. Consequently, white maize commonly fed to Ovambo chickens in southern Africa can be replaced with PABM without any detrimental effects to the well-being of the chickens. Consumption of the PABM diet will result in healthier Ovambo chickens with better resistance to avian diseases and infections that cause significant economic losses to the poultry farmers. Commercial poultry farmers could also adopt the use of PABM diet to reduce cost in terms of purchasing vitamin A supplementation, medications and vaccines to combat infections.

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