

Haematological Parameters, Serum Biochemical Indices and Weight of Internal Organs in Three Chicken Strains in Response to Incremental Levels of *Moringa oleifera* Leaf Meal

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Key words: Gastrointestinal tract, haematology, indigenous chickens, internal organs, *Moringa oleifera* leaf meal, serum biochemistry

Abstract: Continuous supplementation of chicken diets with plant-based alternative feed resources such as *Moringa oleifera* has the potential to modify the bird's anatomy and physiology. A 90 days feeding trial was conducted to determine the effect of *M. oleifera* leaf meal supplementation on weight of internal organs, haematological parameters and serum biochemical indices in three chicken strains that are normally reared extensively in South Africa. Moringa leaves were harvested by hand, air-dried and milled into *M. Oleifera* Leaf Meal (MOLM). The leaf meal was chemically analysed and used to dilute a commercial broiler finisher diet at 0 (MOLM0), 25 (MOLM25), 50 (MOLM50) and 100 (MOLM100) g kg⁻¹ DM, producing four isoenergetic and isonitrogenous dietary treatments. The 216 Potchefstroom Koekoek (PK), Ovambo (OV) and Black Australorp (BA) chickens were raised on a commercial starter mash for 3 weeks. On the 4th week, experimental diets were offered until 13 weeks of age. At 13 weeks of age blood samples were taken from 6 chickens (3 males and 3 females) per treatment and used for biochemical and haematological analysis. Higher inclusion levels of MOLM resulted in longer small intestines and larger gizzards in both male and female chickens. Male BA chickens on MOLM0 diet had the least Red Blood Cell (RBC) and haematocrit counts compared to other diets. When offered MOLM50, female OV chicken strain had lower Aspartate Transaminase (AST) and alkaline phosphate (ALKP) (156.9 U L⁻¹) compared to BA and PK chicken strains. Incremental levels of MOLM resulted in higher Total Protein (TP) in female chickens. In male chickens low levels of Alanine Transaminase (ALT) were observed when

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Page No.: 1-11

Volume: 13, Issue 1, 2019

ISSN: 1815-9354

Research Journal of Agronomy

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offered MOLM50 (10.0 U L⁻¹) and MOLM100 (11.0 U L⁻¹). It was concluded that inclusion of MOLM

at levels up to 10 g kg⁻¹ had no adverse effect on the health and nutritional status of the three chicken strains.

INTRODUCTION

Extensively-reared chickens contribute >50% of the total eggs and meat consumed by people living in rural areas of South Africa. As such, the contribution of these chickens to food and nutrition security in these resource-poor communities is unequivocal. However, their productivity lags behind that of the genetically improved strains used to provide meat and eggs in commercial production enterprises. The result is that intensive production of indigenous chicken strains remains an unattractive option for many small scale farmers, since, the returns are relatively lower. The major stumbling block is the cost of commercial feeds which are required in larger quantities for indigenous chickens whose growth rates are significantly lower than in improved chicken strains. A possible solution is the use of non-conventional feedstuffs as alternatives or supplements to the commercial diets. Locally available, plant-based non-conventional feedstuffs represent a cheaper but not necessarily viable, alternative. One such plant being grown on a large scale in South Africa is *Moringa oleifera*.

The leaves of *M. oleifera* can be used to Make a Leaf Meal (MOLM) that may have potential as a low-cost feed supplement. *Moringa oleifera* has been reported to be a good source of vitamins and amino acids (Olugbemi *et al.*, 2010). The plant has also been reported to boost the immune system in broilers (Jayavardhanan *et al.*, 1994; Fuglier, 2005; Olugbemi *et al.*, 2010). In evaluating the nutritive value of non-conventional feed resources such as *M. oleifera*, it is also important to assess the anatomical, physiological and health effects that such feed resources may have on the target animal. Several factors such as nutrition, age, gender, breed, health and physiological status, may influence the normal blood values of various species (Jain, 1993). Esonu *et al.* (2001) reported that haematological constituents reflect the physiological responsiveness of an animal to its internal and external environments which include feed and feeding. Nickon, *et al.*, reported that *M. oleifera* extract has antibacterial properties and antifungal activities. The extract is also said to have hypotensive (Ara *et al.*, 2008), hypoglycemic and hypocholesterolemic (Dangi *et al.*, 2002; Ghasi *et al.*, 2000; Ara *et al.*, 2008), anti-inflammatory, anti-hepatotoxic and anti-helminthic properties (Nikken *et al.*, 2003a, b). *Moringa oleifera* leaf meal contains iron (23 mg 100 g⁻¹) which is necessary for many functions in the body including the formation of haemoglobin and myoglobin. The

anti-nutritional compounds present in *M. oleifera* leaf may have detrimental effect on blood parameters and liver function in chickens. Liver enzymes are found in the hepatocytes where they carry out different functions ranging from metabolism, detoxification, synthesis and regulation. Transaminases or amino transferases, Alanine Transferase (ALT), Aspartate Transferase (AST) and alkaline phosphatase are membrane bound enzymes whose concentration in blood indicates the health status of liver cells (Bruraimoh *et al.*, 2011).

For a comprehensive nutritional assessment of MOLM in chickens, it is imperative that its anatomical and physiological effects be evaluated. There is a dearth of information on hematological, electrolyte and serum biochemical parameters in domestic indigenous chicken strains. This study was therefore, designed to examine the haematological and serum biochemical indices of one improved (Black Australorp) and two indigenous (Potchefstroom Koekoek and Ovambo) chicken strains, supplemented with incremental levels of *M. oleifera* leaf meal.

MATERIALS AND METHODS

Study sites: This study was conducted at the North-West University Experimental Farm (Molelwane), Mafikeng (25.8°S and 25.5°E), South Africa. *Moringa oleifera* leaves were obtained from Patience Wellness Centre in Limpopo province (24.305°S and 29.565°E). The ambient temperature in this area ranges from 27-37°C during summer and between 11 and 17°C during winter. The annual rainfall ranges between 500 and 800 mm. The leaves were air-dried at a room temperature and then milled to pass through a 2 mm sieve.

Diet formulation: Four diets were constituted using *M. oleifera* leaf meal (MOLM) as follows: 100% broiler finisher mash (MOLM0); 97.5% broiler finisher mash and 2.5% MOLM (MOLM25); 5% MOLM and 95% broiler finisher mash (MOLM50) and 10% MOLM and 90% broiler finisher mash (MOLM100). The composition of MOLM and experimental diets are presented in Table 1 and 2 (Sebola *et al.*, 2015) for descriptive purposes. The experimental diet formulation was done at a commercial feed manufacturing company, NutriFeed (Mafikeng, South Africa). Experimental diets were formulated to be isonitrogenous and isoenergetic.

Experimental design: The 216 chickens from Potchefstroom Koekoek, Ovambo and Black Australorp strains were raised on a commercial starter mash (Nutri

Table 1: Gross composition of *Moringa oleifera* Leaf Meal (MOLM)-based experimental diets

Variables	Diet ¹			
	MOLM0	MOLM25	MOLM50	MOLM100
MOLM (g kg ⁻¹ diet)	0	25.0	50.0	100.0
Yellow maize	670.6	658.8	647.1	623.6
Prime gluten 60	50.0	50.0	50.0	50.0
Full fat soya meal	70.0	70.0	70.0	70.0
Soya bean meal	85.3	71.8	58.2	31.1
Sunflower oilcake	80.0	80.0	80.0	80.0
Limestone powder	12.3	9.7	7.1	1.8
Potassium carbonate	1.2	1.0	0.9	0.5
Mono calcium phosphate	9.8	9.9	10.0	10.3
Salt	3.2	3.17	3.15	3.11
Soya oil	7.8	10.6	13.5	19.1
Premix	6.8	6.8	6.8	6.7
Lysine	2.7	2.7	2.7	2.7
Methionine	0.3	0.5	0.7	1.0

¹Diet; MOLM0 = Broiler finisher without MOLM inclusion; MOLM25 = Broiler finisher diluted at 25 g kg⁻¹ MOLM; MOLM50 = Broiler finisher diluted at 50 g kg⁻¹ MOLM; MOLM100 = Broiler finisher diluted at 100 g kg⁻¹ MOLM

Feed, Mafikeng, South Africa) for four weeks. At four weeks of age, the chickens from each breed were randomly allocated to the 4 experimental diets. A 3 (chicken strains)×4 (diets) factorial treatment arrangement in a Complete Randomised Design (CRD) was used for this experiment. The experimental unit was a pen holding 6 birds which was replicated 3 times, resulting in a total of 36 floor pens with 6 birds (3 males and females) per replicate. All chickens were vaccinated against Marek's disease, Newcastle disease, infectious bursal disease (Gumboro) in the first 4 weeks.

Blood collection and analysis: At the end of the 13-week feeding trial, blood samples were collected from all 6 birds (3 males and 3 females) in each feeding trial replicate. Bleeding was done from a punctured wing vein with a 5 mL scalp vein needle set. About 2 mL of blood was collected from each bird into two sets of sterilised bottles, one containing Ethylene Diamine Tetra Acetic acid (EDTA) as the anti-coagulant. Haematological parameters (Haemoglobin (Hb), Red Blood Cells (RBC) White Blood Cells (WBC), Haematocrit (Hct), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH)) were determined using an automated Idexx Laser Cyte Haematology (IDEXX Laboratories, Inc) and the values were recorded in g/100 mL. Mean Corpuscular Haemoglobin Concentration (MCHC) was calculated as:

$$\text{MCHC} = \frac{\text{MCH}}{\text{MCV}}$$

where, MCH is mean corpuscular haemoglobin and MCV is the mean corpuscular volume. Clotted blood (collected in red top tubes) was centrifuged in a macro centrifuge to

generate serum for biochemical analysis. Total Protein (TP), urea, creatinine, albumin, serum cholesterol, aspartate transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphate (ALKP) were analysed using an automated Idexx Vet Test Chemistry Analyser (IDEXX Laboratories, Inc).

Internal organs: At 13 weeks of age, all chickens were electrically stunned and killed by manual exsanguination. Weights of the liver, gizzard (cleaned), heart, lungs and pancreas as well as the length of small intestines were determined using a sensitive weighing balance and measuring tape (cm), respectively.

Statistical analysis: Data were statistically analysed separately for male and female chickens, since, gender differences in terms of haematology and biochemical indices are well established in literature (Peters *et al.*, 2011; Addass *et al.*, 2012). Thus, for each gender, the experiment took the form of a 3 (chicken strains)×4 (experimental diets) factorial treatment arrangement in a completely randomized design. Variation in organ size, haematological and serum biochemical indices data was analysed using SAS software according to the following general linear model:

$$Y_{ijk} = \mu + S_i (i = 1-2) + D_j (j = 1-5) + (S \times D)_{ij} + E_{ijk}$$

Where:

- Y_{ijk} = Dependent variable (organ size, haematological and serum biochemical indices)
 μ = Overall mean
S_i = Effect of bird strain level i
D_j = Effect of experimental diet level j
(S×D)_{ij} = Interactive effect of bird strain and diet
E_{ijk} = Random error, assumed to be normally and independently distributed

The level of significance was set at p<0.05. For parameters where significant variation was detected, multiple comparisons of treatment means were carried out using the probability of difference (pdiff) option of the General Linear Models (GLM) procedures of SAS. Nonlinear regression analysis was used to determine the response relationship organ size, haematological and serum biochemical indices to incremental levels of MOLM.

RESULTS AND DISCUSSION

Haematological parameters in female chicken strains:

The interaction term 'diet×strain' significantly influenced (p<0.05) Haemoglobin (Hb), Red Blood Cells (RBC), White Blood Cells (WBC), Haematocrit (Hct), Mean

Table 2: Chemical analysis of diets on an 'as fed basis' and chemical composition of dried *Moringa oleifera* Leaf Meal (MOLM)

Variables	Diet ¹			
	MOLM0	MOLM25	MOLM50	MOLM100
MOLM	0	25.0	50.0	100.0
Dry matter	896.0	874.0	851.0	807.0
Crude protein	189.0	189.0	189.0	189.0
Ether Extract	52.0	57.0	61.0	69.0
Ash	49.0	47.0	45.0	42.0
ADF ²	36.0	42.0	47.0	57.0
NDF ³	96.0	100.1	106.0	116.0
Crude Fibre	36.0	35.0	34.0	33.0
ME ⁴ (Kcal kg ⁻¹)	3157.6	3157.4	3157.2	3156.8
Lysine	9.7	9.7	9.7	9.7
Methionine	4.0	4.2	4.3	4.5

¹Diet; MOLM0 = Broiler finisher without MOLM inclusion; MOLM25 = Broiler finisher diluted with 25 g MOLM kg⁻¹; MOLM50 = Broiler finisher diluted with 50 g MOLM kg⁻¹; MOLM100 = Broiler finisher diluted at 100 g MOLM kg⁻¹; ²ADF = Acid detergent fibre; ³NDF = Neutral detergent fibre; ⁴ME = Metabolizable energy

Table 3: Haematological parameters in 13-week Old Ovambo (OV), Potchefstroom Koekoek (PK) and Black Austrorip (BA) female chickens fed incremental levels of *Moringa oleifera* Leaf Meal (MOLM)

Parameters	Strain	Diet (g kg ⁻¹)				SE
		MOLM0	MOLM25	MOLM50	MOLM100	
RBC(10 ¹² /L)	OV	2.7 ^{ba}	2.8 ^{aB}	2.8 ^{aA}	2.8 ^{aA}	0.049
	PK	2.5 ^{bc}	2.9 ^{aA}	2.9 ^{aB}	3.2 ^{aA}	
	BA	2.7 ^{abB}	2.9 ^{aA}	2.5 ^{bb}	2.6 ^{BB}	
Haematocrit (%)	OV	27.6 ^A	26.9 ^B	26.3 ^B	27.0 ^B	0.498
	PK	25.2 ^{CB}	26.5 ^{bcB}	27.7 ^{aA}	29.9 ^{aA}	
	BA	25.9 ^{bb}	29.5 ^{aA}	26.4 ^{baB}	27.1 ^{bb}	
Haemoglobin (g L ⁻¹)	OV	8.4 ^b	6.5 ^{cc}	9.7 ^{ab}	10.2 ^a	0.576
	PK	8.9 ^b	11.1 ^{aA}	9.8 ^{ab}	9.9 ^{ab}	
	BA	9.2	8.5 ^B	9.1	8.2	
MCV(fl)	OV	99.3	98.0 ^A	99.0 ^B	98.0 ^{BC}	1.544
	PK	99.2 ^a	92.2 ^{bb}	92.3 ^{bc}	93.8 ^{bc}	
	BA	96.8 ^b	101.6 ^{aA}	104.2 ^{aA}	106.2 ^{aA}	
MCH (pg)	OV	36.7 ^{bb}	24.3 ^{bc}	37.9 ^{aB}	41.3 ^{aA}	0.990
	PK	43.8 ^{aA}	38.9 ^{aA}	32.7 ^{bc}	34.0 ^{bc}	
	BA	35.1 ^{bb}	38.3 ^{aA}	33.5 ^{bb}	34.8 ^{bb}	
MCHC (%)	OV	36.9 ^a	24.1 ^{bb}	36.8 ^a	30.9 ^a	2.055
	PK	35.3 ^b	34.1 ^{aA}	35.3 ^b	33.0 ^b	
	BA	35.5 ^a	28.6 ^{bb}	34.3 ^{ab}	30.2 ^{ab}	
WBC(10 ⁹ /L)	OV	10.8 ^{ba}	11.9 ^{aA}	11.6 ^{aA}	11.6 ^{aA}	0.185
	PK	6.8 ^{bc}	5.1 ^{cC}	6.8 ^{bc}	10.9 ^{ab}	
	BA	9.3 ^{ab}	6.5 ^{cb}	7.5 ^{bb}	6.9 ^{bc}	
Lymphocytes (%)	OV	65.6 ^{aA}	67.9 ^{aA}	47.0 ^b	54.4 ^{ba}	2.942
	PK	44.6 ^B	42.2 ^C	46.9	44.9 ^B	
	BA	51.2 ^B	53.5 ^B	51.5	54.6 ^A	
Neutrophils (%)	OV	15.8 ^{cb}	45.7 ^{aA}	32.8 ^{abAB}	22.5 ^{cb}	5.551
	PK	33.6 ^{abA}	46.0 ^{aA}	24.3 ^{bb}	25.3 ^{bb}	
	BA	23.5 ^{baB}	27.3 ^{bb}	45.1 ^{aA}	33.4 ^{abA}	
Monocytes (%)	OV	13.9 ^{aA}	11.3 ^{ba}	12.5 ^{ba}	10.6 ^{ba}	0.440
	PK	9.8 ^{ab}	10.2 ^{aA}	8.2 ^{bb}	7.0 ^{bb}	
	BA	8.0 ^{bc}	8.3 ^{bb}	7.7 ^{bb}	10.1 ^{aA}	
Eosinophils (%)	OV	4.5 ^{abB}	3.8 ^{ba}	3.8 ^{ba}	4.7 ^{aA}	0.249
	PK	3.6 ^{ac}	3.1 ^{abAB}	2.5 ^{bcB}	2.2 ^{cC}	
	BA	5.4 ^{aA}	2.8 ^{bb}	2.6 ^{bb}	3.2 ^{bb}	
Basophils (%)	OV	1.0 ^{ab}	0.6 ^{ba}	0.9 ^{aA}	1.0 ^{aA}	0.038
	PK	1.8 ^{aA}	0.7 ^{ba}	0.7 ^{bb}	0.7 ^{bb}	
	BA	1.8 ^{aA}	0.55 ^{bb}	0.6 ^{bc}	0.7 ^{ab}	

^{ab}In a row, lowercase superscripts compare strains within diet; ^{AB}In column, uppercase superscripts compare diets within strains (p<0.05); ¹Diet: MOLM0 = Broiler finisher without MOLM inclusion; MOLM25 = Broiler finisher diluted at 25 g kg⁻¹ MOLM; MOLM50 = Broiler finisher diluted at 50 g kg⁻¹ MOLM; MOLM100 = Broiler finisher diluted at 100 g kg⁻¹ MOLM

Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin (MCH), lymphocytes, neutrophils, monocytes, eosinophils and basophils of female chicken

strains (Table 2 and 3). In OV and PK strain, incremental level of MOLM had highest (p<0.05) RBC, Hb, MCH and MCHC compared to control diet. MOLM100 resulted in

Table 4: Haematological parameters in 13-week old Ovambo (OV), Potchefstroom Koekoek (PK) and Black Australorp (BA) male chickens fed incremental levels of *Moringa oleifera* Leaf Meal (MOLM)

Parameters	Diet (g kg ⁻¹)					SE
	Strain	MOLM0	MOLM25	MOLM50	MOLM100	
RBC(10 ¹² /L)	OV	2.89 ^A	2.79 ^A	2.92 ^A	3.05 ^A	0.119
	PK	2.89 ^A	2.72 ^A	2.90 ^A	2.90 ^A	
	BA	2.76 ^B	2.79 ^A	2.65 ^B	2.72 ^B	
Haematocrit (%)	OV	27.9	27.4	28.4 ^{AB}	28.2	0.985
	PK	27.4 ^{ab}	26.5 ^b	31.2 ^{aA}	27.7 ^{ab}	
	BA	26.7 ^a	26.5 ^a	25.8 ^{bB}	27.5 ^a	
Haemoglobin (g L ⁻¹)	OV	8.7 ^{bB}	10.4 ^a	8.4 ^{bB}	9.2 ^{abB}	0.637
	PK	8.5 ^B	9.0	8.7 ^B	9.4 ^B	
	BA	10.3 ^{aA}	9.0 ^a	10.6 ^{aA}	10.4 ^{aA}	
MCV(fl)	OV	95.5 ^{aAB}	98.2 ^{aA}	97.4 ^{aA}	92.5 ^{bB}	1.513
	PK	94.8 ^{bA}	97.4 ^{aA}	94.6 ^{bA}	95.6 ^{abA}	
	BA	96.7 ^{aAB}	94.3 ^{abB}	97.2 ^{aAB}	101.2 ^{aA}	
MCH (pg)	OV	29.4 ^{bB}	37.8 ^a	30.5 ^{bB}	36.6 ^{aA}	2.521
	PK	29.4 ^{bB}	36.3 ^a	36.8 ^{aA}	34.2 ^{abA}	
	BA	42.5 ^{aA}	35.2 ^b	34.5 ^{bAB}	35.3 ^{bA}	
MCHC (%)	OV	31.0 ^{aB}	37.9 ^{aA}	29.5 ^{bB}	32.8 ^{abB}	2.537
	PK	30.9 ^{aB}	33.9 ^{abB}	27.9 ^{bB}	33.8 ^{abB}	
	BA	38.5 ^{aA}	33.9 ^{bB}	40.9 ^{aA}	38.0 ^{aA}	
WBC(10 ⁹ /L)	OV	6.4 ^{cB}	7.3 ^{bcB}	8.9 ^{bB}	10.7 ^a	0.220
	PK	9.3 ^A	10.5 ^A	10.9 ^A	11.3	
	BA	9.8 ^A	10.6 ^A	10.9 ^A	11.3	
Lymphocytes (%)	OV	66.1 ^{abA}	70.5 ^{aA}	44.6 ^{cB}	61.7 ^{bA}	1.999
	PK	50.2 ^{aB}	49.2 ^{abB}	46.9 ^{bB}	44.9 ^{bB}	
	BA	52.4 ^{abB}	49.2 ^{bB}	54.1 ^{abA}	56.9 ^{aA}	
Neutrophils (%)	OV	12.7 ^{aB}	13.8 ^{acC}	11.1 ^{cB}	17.8 ^{bcC}	5.160
	PK	26.0 ^{bA}	20.1 ^{bB}	34.1 ^{abA}	40.6 ^{aA}	
	BA	27.1 ^{aA}	25.9 ^{aA}	32.8 ^{aA}	27.9 ^{abB}	
Monocytes (%)	OV	13.4 ^{aA}	11.5 ^{bA}	10.6 ^{bcA}	9.1 ^{cA}	0.564
	PK	10.4 ^{aB}	9.1 ^{aB}	9.8 ^{aA}	7.0 ^{bB}	
	BA	8.1 ^{bC}	9.1 ^{abB}	9.9 ^{aA}	9.3 ^{abA}	
Eosophils (%)	OV	0.6 ^{bB}	4.2 ^{aA}	0.7 ^{bB}	4.7 ^{aA}	0.242
	PK	1.9 ^{bA}	3.1 ^{aB}	2.4 ^{abA}	1.9 ^{bC}	
	BA	1.7 ^{bA}	2.3 ^{abC}	2.5 ^{aA}	2.8 ^{aA}	
Basophils (%)	OV	1.0 ^{aA}	0.9 ^{aA}	0.8 ^{bA}	0.7 ^{bA}	0.043
	PK	0.6 ^{acC}	0.6 ^{aB}	0.6 ^{aB}	0.4 ^{bB}	
	BA	0.9 ^{aB}	0.7 ^{bB}	0.7 ^{bAB}	0.8 ^{abA}	

^{ab}In a row, lowercase superscripts compare strains within diet; ^{AB}In column, uppercase superscripts compare diets within strains (p<0.05); ^aDiet: MOLM0 = Broiler finisher without MOLM inclusion; MOLM25 = Broiler finisher diluted at 25 g kg⁻¹ MOLM MOLM50 = Broiler finisher diluted at 50 g kg⁻¹ MOLM; MOLM100 = Broiler finisher diluted at 100 g kg⁻¹ MOLM

lower (p<0.05) RBC count compared to control diet. No variation (p>0.05) was observed in Hct, Hb, MCH and MCHC of BA chicken strains when offered incremental level of MOLM. In OV chicken strain, incremental level of MOLM had higher (p<0.05) WBC count than control diet (MOLM0). When offered incremental level of MOLM, OV and PK strain exhibited higher WBC count. No variation (p>0.05) observed in lymphocytes in BA and PK chicken strain across all diets, whilst OV strain had a lower (p<0.05) lymphocytes with incremental level of MOLM. In OV and PK strain, MOLM25 and MOLM50 had higher neutrophils than control diet, whilst BA strain had higher (p<0.05) neutrophils with MOLM incremental level. In PK and BA strains, incremental level of MOLM resulted in lower eosinophils compared MOLM0. Potchefstroom Koekoek strain had highest (p<0.05) RBC count (2.9×10¹²/L), Hbg (3.2 g L⁻¹) MCH (37.3 pg), MCHC (36.4%) but had lower eosinophils (2.84%) than OV and BA chicken strains. No variation (p>0.05) was

observed in Hct and neutrophils in all chicken strains. Black Australorp had lower lymphocytes (52.7%), eosinophils (3.47%), basophils (0.64%) and higher (p<0.05) MCV (102.2 fl) than PK and OV strains. Ovambo strain had higher WBC count (11.5×10⁹/L), monocytes (12.1%), eosinophils (4.2%) and basophils (0.89%) than PK and BA strains.

Haematological parameters in male chicken strains:

The interaction term 'diet×strain' did not (p>0.05) affect RBC, Hct, Hb and MCHC in male chickens but significantly influenced MCH, WBC, lymphocytes, neutrophils, monocytes, eosinophils and basophils. Incremental level of MOLM resulted in higher (p<0.05) MCH in OV and PK chicken strain, whilst BA strain had lower (p<0.05) MCH when offered incremental level of MOLM (Table 4 and 5). In OV strain, incremental level of MOLM exhibit higher WBC count than control diet (MOLM0). When offered MOLM incremental level, PK

Table 5: Serum biochemical indices in 13-week Old Ovambo (OV), Potchefstroom Koekoek (PK) and Black Australorp (BA) male chickens fed incremental levels of *Moringa oleifera* Leaf Meal (MOLM)

Parameters	Diet (g kg ⁻¹)					SE
	Strain	MOLM0	MOLM25	MOLM50	MOLM100	
Urea (mmol L ⁻¹)	OV	0.4 ^{bb}	0.45 ^{ab}	0.50 ^{aA}	0.40 ^{bb}	0.029
	PK	0.55 ^{aA}	0.45 ^{bc}	0.40 ^{bB}	0.50 ^{abA}	
	BA	0.6 ^{aA}	0.50 ^b	0.45 ^{bcAB}	0.40 ^{bB}	
Creatinine (μmol L ⁻¹)	OV	10.7 ^{dA}	14.5 ^{ab}	13.0 ^c	13.7 ^{ba}	0.127
	PK	11.0 ^{ba}	10.2 ^{cC}	13.0 ^a	12.7 ^{aC}	
	BA	10.0 ^{bB}	15.0 ^{aA}	13.0 ^b	13.3 ^{ba}	
Uric (μmol L ⁻¹)	OV	275.0 ^{aA}	269.5 ^{bb}	269.0 ^{ba}	257.0 ^{ca}	1.546
	PK	285.0 ^{aA}	277.7 ^{ba}	192.0 ^{dB}	207.0 ^{cC}	
	BA	278.0 ^{aaB}	195.0 ^{cC}	195.0 ^{bb}	229.0 ^{bb}	
Total protein (g L ⁻¹)	OV	41.0 ^A	35.5	40.5	36.0 ^B	1.936
	PK	41.0 ^A	35.5	40.5	36.0 ^B	
	BA	38.0 ^{bb}	40.0 ^{ab}	44.0 ^a	44.0 ^{aA}	
Albumin (g L ⁻¹)	OV	12.5 ^a	10.5 ^{ab}	10.0 ^{bb}	10.5 ^{abB}	0.69
	PK	12.5	10.5	12.5 ^A	10.5 ^B	
	BA	11.0 ^b	11.0 ^b	14.0 ^{aA}	14.0 ^{aA}	
Globulin (g L ⁻¹)	OV	28.5	25.5	25.5 ^B	26.0 ^B	1.137
	PK	28.5	25.5	27.5 ^{AB}	26.0 ^B	
	BA	27.0	28.0	30.0 ^A	30.0 ^A	
Cholesterol (mmol L ⁻¹)	OV	2.85	3.18	3.10	2.76	0.293
	PK	2.61	2.66	2.81	2.60	
	BA	2.66	3.0	2.88	3.32	
ALT (U L ⁻¹)	OV	12.4 ^{ab}	11.3 ^{bb}	10.85 ^{ca}	11.6 ^{ba}	0.113
	PK	13.0 ^{aA}	11.0 ^{bb}	10.0 ^{bB}	11.0 ^{bb}	
	BA	13.0 ^{aA}	12.4 ^{ba}	10.0 ^{dB}	11.0 ^{bB}	
AST (U L ⁻¹)	OV	132.0 ^{ab}	129.0 ^{bc}	125.0 ^{cB}	121.2 ^{dB}	0.256
	PK	134.8 ^{aA}	135.2 ^{aA}	130.0 ^{ba}	124.7 ^{ca}	
	BA	131.2 ^{ac}	130.4 ^{ab}	125.7 ^{bb}	120.5 ^{cB}	
ALKP (U L ⁻¹)	OV	185.2 ^{aA}	172.9 ^{bb}	152.7 ^{dB}	166.2 ^{cB}	0.471
	PK	183.2 ^{ab}	174.9 ^{ba}	155.6 ^{dA}	166.7 ^{cB}	
	BA	177.9 ^{ac}	169.7 ^{bc}	153.3 ^{cB}	170.5 ^{ba}	

^{ab}In a row, lowercase superscripts compare strains within diet; ^{AB}In column, uppercase superscripts compare diets within strains (p<0.05); ¹Diet: MOLM0 = Broiler finisher without MOLM inclusion; MOLM25 = Broiler finisher diluted at 25 g kg⁻¹ MOLM; MOLM50 = Broiler finisher diluted at 50 g kg⁻¹ MOLM; MOLM100 = Broiler finisher diluted at 100 g kg⁻¹ MOLM

and OV strains resulted in lower lymphocytes compared to MOLM0 whilst no variation (p>0.05) was observed in BA chicken strain. Lower (p<0.05) neutrophils were observed with MOLM incremental level in OV strain. Highest MOLM inclusion MOLM100 had higher eosinophils on both OV and BA strains compared to MOLM0. Black Australorp had lower (p<0.05) RBC (2.7×10¹²/L) and higher Hbg (10.1 fl), MCH (37.1 pg), MCHC (37.9%) and MCV (97.4 fl) compared to OV and PK chicken strains. Higher RBC count was observed in PK and OV strain (2.95 and 2.90×10¹²/L, respectively) than BA strain. Potchefstroom Koekoek had higher Hct (28.2), lymphocytes (47.8%) and basophils (0.54%). OV had lower WBC count than BA and OV strains. Overall, OV chickens had lower (p<0.05) neutrophils compared to BA and PK chickens.

Blood chemistry in female chicken strains: The interaction term 'diet×strain' did not (p>0.05) affect female total protein, cholesterol but significantly influenced all the other serum biochemical indices. Incremental level of MOLM resulted in lower (p<0.05) urea and uric acid in all chicken strains (Table 6).

Creatinine increased (p<0.05) with MOLM incremental level across all strains. In all strain, incremental level resulted in higher albumin and globulin level than MOLM0. When offered MOLM incremental level, all chicken strains resulted in lower (p<0.05) ALT, AST and ALKP concentration compared to MOLM0. Black Australorp strain had lower uric acid (279.0 μmol L⁻¹), AST (127.3 U L⁻¹) and higher (p<0.05) total protein (43.1 g L⁻¹), albumin (13.3 g L⁻¹) and globulin (29.88 g L⁻¹) than OV and PK chicken strains. Potchefstroom Koekoek had lower creatinine and ALKP than other strains. Ovambo strain had higher urea (0.49 mmol L⁻¹) and ALKP (143.8 U L⁻¹) level than other strains. No variation (p>0.05) in cholesterol was observed across all strains.

Blood chemistry parameters in male chicken strains: Diet x strain interaction did not (p>0.05) affect male globulin and cholesterol levels but significantly influenced all other serum biochemical indices. When offered MOLM incremental level BA strain resulted in lower (p<0.05) urea than MOLM0 whilst no variation (p>0.05) was observed in PK and OV chicken strains

Table 6: Serum biochemical indices in 13-week Old Ovambo (OV), Potchefstroom Koekoek (PK) and Black Australorp (BA) female chickens fed incremental levels of *Moringa Oleifera* Leaf Meal (MOLM)

Parameters	Diets (g kg ⁻¹)					SE
	Strain	MOLM0	MOLM25	MOLM50	MOLM100	
Urea (mmol L ⁻¹)	OV	0.50 ^{ab}	0.45 ^{ba}	0.50 ^{aA}	0.50 ^a	0.014
	PK	0.50 ^{aA}	0.40 ^{cB}	0.40 ^{cB}	0.50 ^a	
	BA	0.40 ^{bc}	0.50 ^{aA}	0.50 ^{aA}	0.50 ^a	
Creatinine (μmol L ⁻¹)	OV	10.0 ^c	12.0 ^{aA}	11.0 ^{bb}	12.0 ^a	0.144
	PK	10.0 ^c	12.0 ^{aA}	11.0 ^{bb}	12.0 ^a	
	BA	10.0 ^c	10.5 ^{bb}	12.0 ^{aA}	12.0 ^a	
Uric (μmol L ⁻¹)	OV	311.0 ^{aA}	279.7 ^{bb}	277.5 ^{ba}	276.0 ^{bb}	1.12
	PK	300.2 ^{bb}	306.0 ^{aA}	258.9 ^{dB}	290.4 ^{cA}	
	BA	310.0 ^{aA}	270.0 ^{cC}	257.0 ^{dB}	279.0 ^{bb}	
Total protein (g L ⁻¹)	OV	40.0	39.0 ^B	40.0 ^B	39.0 ^B	0.59
	PK	41.0	39.0 ^B	40.0 ^B	39.0 ^B	
	BA	41.50 ^b	41.0 ^{ba}	48.0 ^{aA}	42.0 ^{ba}	
Albumin (g L ⁻¹)	OV	11.5 ^b	12.0 ^b	13.0 ^{aA}	13.0 ^a	0.204
	PK	11.5 ^b	12.0 ^b	13.0 ^{aA}	13.0 ^a	
	BA	12.0 ^b	12.0 ^b	12.0 ^{bb}	13.0 ^a	
Globulin (g L ⁻¹)	OV	28.5 ^a	27.0 ^{bb}	27.0 ^{bb}	28.0 ^{ab}	0.479
	PK	28.5 ^a	27.0 ^{bb}	27.0 ^{bb}	28.0 ^{ab}	
	BA	29.5 ^b	29.0 ^{ba}	32.0 ^{aA}	31.0 ^a	
Cholesterol (mmol L ⁻¹)	OV	2.74	3.09 ^A	2.63	2.99	0.239
	PK	2.72	2.28 ^B	2.77	2.49	
	BA	2.25 ^b	2.54 ^{ab}	2.99 ^a	2.26 ^a	
ALT (U L ⁻¹)	OV	12.15 ^{ab}	11.4 ^b	10.26 ^{cAB}	10.0 ^{cB}	0.199
	PK	13.6 ^{aA}	11.0 ^b	10.65 ^{ba}	13.0 ^{aA}	
	BA	13.0 ^{aA}	11.0 ^b	10.0 ^{cB}	10.0 ^{cB}	
AST (U L ⁻¹)	OV	133.0 ^{ab}	130.9 ^{bb}	127.0 ^{dB}	130.5 ^{cA}	0.138
	PK	131.6 ^{bb}	134.4 ^{aA}	130.2 ^{cA}	129.9 ^{cB}	
	BA	134.0 ^{aA}	129.0 ^{bc}	125.0 ^{cC}	121.0 ^{dc}	
ALKP (U L ⁻¹)	OV	177.8 ^{aC}	170.2 ^{ba}	156.9 ^{cB}	169.1 ^{ba}	0.867
	PK	190.0 ^{aA}	143.0 ^{bb}	117.0 ^{dc}	125.0 ^{cC}	
	BA	180.4 ^{ab}	170.9 ^{ba}	160.8 ^{cA}	156.6 ^{dB}	

^{ab}In a row, lowercase superscripts compare diets within strain (p<0.05); ^{ABC}In a column, uppercase superscripts compare strains within diet (p<0.05);

¹Diet: MOLM0 = Broiler finisher without MOLM inclusion; MOLM25 = Broiler finisher diluted at 25 g kg⁻¹ MOLM; MOLM50 = Broiler finisher diluted at 50 g kg⁻¹ MOLM; MOLM100 = Broiler finisher diluted at 100 g kg⁻¹ MOLM

(Table 5). In all strain, incremental level MOLM resulted in higher creatinine content than MOLM0. Lower (P<0.05) uric acid level was observed with MOLM incremental level across all chicken strains. In BA strain, MOLM incremental level resulted in higher total protein and albumin level compared to MOLM0. When offered MOLM incremental level, all chicken strains resulted in lower (P<0.05) ALT, AST and ALKP concentration compared to MOLM0. Black Australorp and PK strain had higher total protein (27.0 and 28.9 g L⁻¹), albumin (41.5 and 38.3 g L⁻¹) and globulin (12.5 and 11.5 g L⁻¹), respectively than PK strain. No variation (p>0.05) in creatinine, AST and ALT across all strains. Lower (p<0.05) level of urea was observed in OV strain than other strains.

Internal organs: In male chickens, the interaction term 'diet × strain' significantly influenced (p<0.05) length of small intestines (Fig. 1a) and gizzard weight (Fig. 1b) but not the size of the heart, liver and pancreas. Black Australorp (128.5 cm), OV (118.5 cm) and PK (111.5 cm) chickens fed MOLM0 had the shortest (p<0.05) intestinal length compared to other diets. Higher levels of MOLM

resulted in longer intestinal length in all chicken strains. Diet had no effect (p>0.05) on heart, liver and pancreas weights of three chicken strains. Diet MOLM25 resulted in lower gizzard weights in BA (30.5 g) and PK (32.5 g) chicken strains compared to other diets. Male chickens offered MOLM100 had the longest small intestines (144 cm) and highest gizzard weight (42.8 g). Similarly, in female chickens, birds offered MOLM100 had the longest small intestines (130.8 cm) and the highest gizzard weights (40.7 g). In female chickens, the interaction term 'diet×strain' significantly influenced (p<0.05) liver weight only. Diet significantly affected (p<0.05) the size of the heart, liver, gizzard (Fig. 2a) and small intestine (Fig. 2b) in female chickens. Diet had no effect (p>0.05) on pancreas weights of BA, OV female chickens (Bach Knudsen, 2001).

Haematological and biochemical indices of 3 strains of chickens: Haematological parameters are good indicators of the physiological status of animals. Addass *et al.* (2012) reported that the majority of haematological parameters for indigenous chickens increase with advancing age with male chickens generally

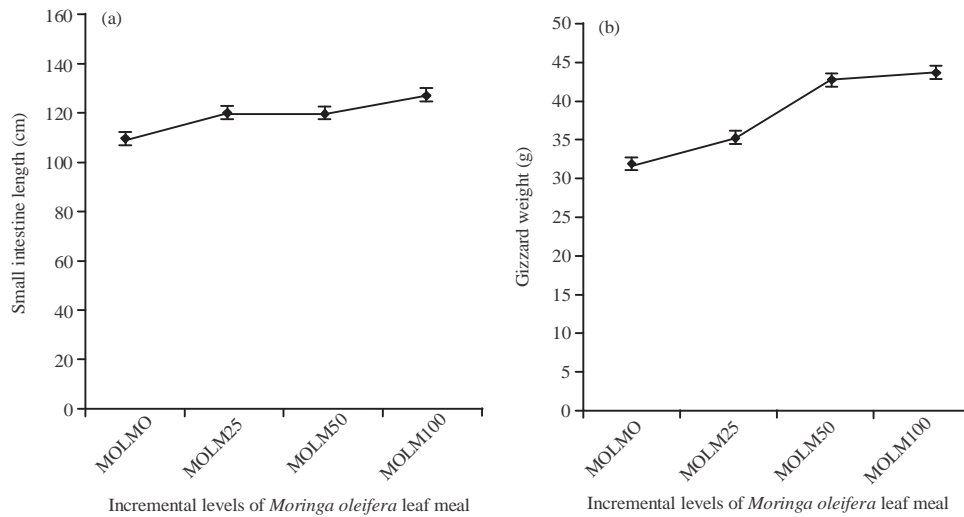


Fig. 1(a, b): Effect of incremental levels of *Moringa oleifera* leaf meal on small intestine length, (a) and gizzard weight (b) of male chickens

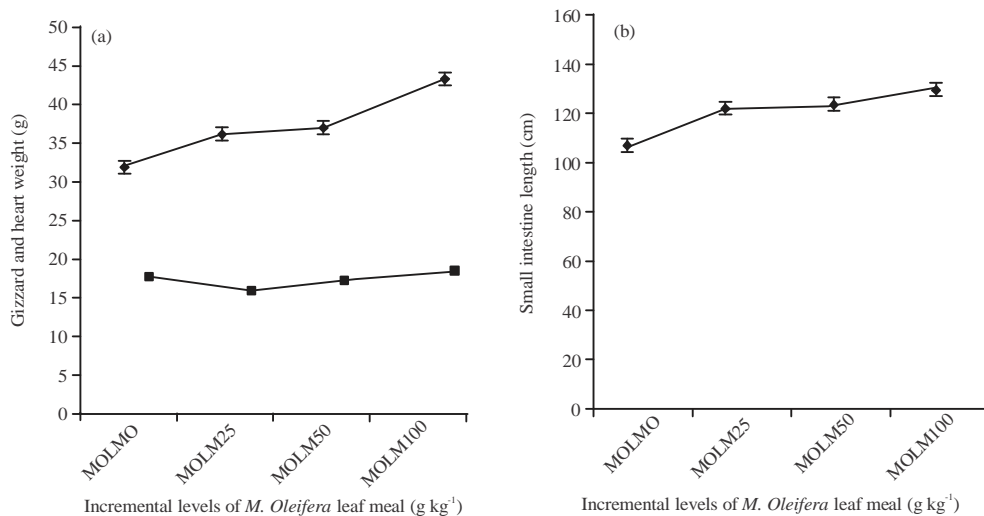


Fig. 2(a, b): Effect of incremental levels of *Moringa oleifera* leaf meal on small intestine length (a), heart and gizzard weight (b) of female chickens

exhibiting higher values than female chickens. Haematological values of blood cells of the three different chicken strains were within the normal range reported for growing chickens (Jain, 1993). The differences between male and female haematological parameters have been fully established in literature (Peters *et al.*, 2011; Addass *et al.*, 2012). In addition, Peters *et al.* (2011) reported that male chickens generally had higher mean values than the females across all genotypes. The results also reveal that haematological values vary among chicken strains. Different strains responded differently to incremental levels of MOLM. Diet MOLM25 promoted higher RBC counts than other diets in female BA chickens. In males, BA strain had lower RBC counts while OV and PK strains had higher counts. Possibly this

observation could be attributed to genetic variation of strains. Red blood cells (erythrocytes) serve as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Johnston and Morris, 1996. Jennings *et al.* (1996) opined that increased RBC values are associated with high quality dietary protein and with disease free animals. In addition, this could be attributed to *Moringa oleifera* leaf meal protein content compared to control diet (Fuglier, 2005; Owusu *et al.*, 2008). Incremental levels of MOLM resulted in elevated Hb in all chicken strains. Inclusion of *M. oleifera* leaf meal in chicken diets may have resulted in higher iron intake which promotes synthesis of haemoglobin and increases production of red blood cells. Peters *et al.* (2011) reported

variation in haematological parameters of Nigerian native chickens; normal-feathered birds had higher mean values compared to frizzled feather and native neck genotype. In both male and female chickens, highest Hct counts were observed at higher inclusion levels of *M. oleifera* leaf meal. This observation could still be related to better dietary protein quality in chickens offered MOLM. Haemoglobin, hematocrit (PCV) and Mean Corpuscular Hemoglobin Concentration (MCHC) are very responsive to protein deficiency or low protein intake (Edozien and Switzer, 1977). Therefore, MOLM provided sufficient quality dietary protein which resulted in optimum concentration of blood constituents. According to Isaac, etc., Haematocrit (Hct) is involved in the transport of oxygen and absorbed nutrients. In female chickens, the total WBC count was highest in OV and PK strain fed with higher levels of MOLM. Animals with low white blood cell count are at high risk of disease infection while those with high counts are capable of generating antibodies and have high degree of resistance to diseases (Soetan *et al.*, 2013). The highest inclusion level of Moringa leaf meal (MOLM100) elevated WBC count, possibly due to the presence of antioxidants which can improve the immune response of chickens and thus reduce mortality (Siddhuraju and Becker, 2003). In both strains incremental levels of MOLM caused a curvilinear response in total protein, albumin and globulin. Albumin functions as an osmotic pressure regulator and transport protein in birds while globulin transport nutrients to the muscles. Increased levels of MOLM in the diet significantly elevated the serum total protein which is consistent with the findings of Teye *et al.* (2013). Total serum protein has been reported as an indication of the protein retained in the animal body (Akinola and Abiola, 1991; Esonu *et al.*, 2001) while total blood protein and creatinine contents have been shown to depend on the quantity and quality of dietary protein ingested (Eggum, 1970; Iyayi and Twwe, 1998; Awosanya *et al.*, 1999; Esonu *et al.*, 2001). It is evident from the present findings that total serum protein differed among the strains. This is similar to the report of Ladokun *et al.* (2008) who reported a higher total serum protein in normally feathered than in naked neck chickens. Higher levels of MOLM promoted the highest creatinine concentration than the control. Serum creatinine concentrations are directly related to muscle volume and activity. Creatinine concentration was higher with incremental level of MOLM in agreement with Bishop *et al.* (2005) who established a direct relation between the amount and quality of ingested protein and creatinine serum level. However, while there were differences between the effect of the control and MOLM diets on creatinine, all values were within the normal ranges for chickens. Haematocrit values increased as the dietary MOLM ratio increased, possibly due to the additional amino acids in

Moringa oleifera leaf which may have increased the quantity and quality of dietary protein available to the birds. This is in agreement with the results of Edozien and Switzer (1977) who stated that hemoglobin, Hematocrit (Hct) and Mean Corpuscular Hemoglobin Concentration (MCHC) are very responsive to protein intake. The lowest level of blood urea was observed in female BA chickens at higher inclusion levels of MOLM. The same response was observed in all male chickens. The fact that higher levels of MOLM inclusion reduced blood urea concentration may be an indication of better absorption and efficient utilization of dietary protein compared to control diet. Possibly this observation could be stimulated by quality dietary protein in MOLM. Furthermore, digestion and absorption are essential parts of protein quality. The lowest values of ALT and AST were observed with high inclusion levels of MOLM in both male and female chicken strains. This indicates that MOLM had no toxic effect within the liver parenchyma of the birds. These results are in agreement with Olugbemi *et al.* (2010) who reported that *Moringa oleifera* leaves have no negative effect on the health of broilers. Instead they reported beneficial effects such as enhanced immune responses of the birds. Djuricic, etc., also indicated that the higher activities of these enzymes normally occur as the result of accelerated muscular tissue turnover. However, it was not significant to the current study with chickens fed MOLM. Cholesterol level in chickens was not affected by MOLM inclusion, this affirms the MOLM's potential as a hypocholesterolemic agent (Ghasi *et al.*, 2000). However, increase in AST may have resulted from handling or muscle injury during the collection of samples which may have resulted in the leakage of intracellular AST into the blood.

Internal organs: Birds respond quickly to changes in dietary fiber content as seen by changes in the intestinal length and weight of internal organs as well as the rate of passage through the different segments of the GIT (Mateos *et al.*, 2012). Male BA chickens had longer intestinal length as compared to OV and PK male chickens. This could be attributed to genetic variation between the strains. Diets MOLM25, MOLM50 and MOLM100 resulted in chickens with longer small intestine length than those offered the control diet (MOLM0) in both male and female chickens. This is in agreement with Borin *et al.* (2006) who reported that high amounts of undigested materials in the digesta increased the lengths of the intestinal sections. This is possibly due to stretching of the intestinal wall in response to increased contents of digesta in the small intestine. However, these results contradict the findings by Amerah *et al.* (2009) and Sklan *et al.* (2003) who observed a reduction in the length of small intestines with incremental levels of insoluble dietary fiber. Longer

intestines are assumed to digest feed efficiently and provide greater surface area for nutrient absorption. Chickens offered diet MOLM100 had larger gizzard weight compared to diets with lower proportions of MOLM. Musa, etc., indicated that benefits of a larger gizzard include improved gut motility and improved digestibility of nutrients through effective grinding in the gizzard (Amerah *et al.*, 2007). In addition, these authors also reported that feed with high fiber content increased the gizzard weight. Fiber particles are, in general, harder to grind than other dietary components, so they, tend to accumulate in the gizzard, thus, stimulating muscle development and functioning of this organ.

CONCLUSION

M. oleifera indicated hepatoprotective influence and proved to have favourable effects on some haematological, blood biochemical parameters and stimulated development and function of the gizzard and small intestine of male and female chicken strains. All chicken strains exhibited beneficial responses with higher inclusion level of MOLM. It is, therefore, concluded that MOLM can be used as a feed supplement for the investigated chicken strains without a risk of toxicity, compromised immunity or sub-optimal nutritional status.

ACKNOWLEDGEMENTS

This project was supported by Faculty Research Funds (North West University) and National Research Funds (NRF). Thanks to the Department of Animal Science (North West university) staff for the support. The support received from Ms Lungisile Tshitshiin analysing blood samples is highly acknowledged.

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