Indian Journal of Animal Sciences **93** (3): 298–302, March 2023/Article
https://doi.org/10.56093/ijans.v93i3.124411

Evaluation of herbal leaf meal as an alternative to in-feed antibiotics on haematological parameter and serum biochemical indices of growing sexed pigs

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Received: 31 May 2022; Accepted: 12 January 2023

ABSTRACT

This study was conducted at the Federal University of Agriculture, Abeokuta in September 2019 to evaluate the influence of sex and sole or combined herbal leaf meal as alternative in-feed antibiotics on serum biochemical indices. A total of thirty large white sexed pigs of eight weeks were allotted into five (5) dietary treatments for 12 weeks. Dietary treatments had no significant effect on haematological parameters and serum biochemical indices. Sex did not influence the haematological and serum biochemical profile of pigs except for monocyte, albumin, aspartate aminotransferase, and cholesterol respectively. Dietary supplementation did not influence the haematological serum biochemical profile of pigs. This indicates that the haematological indices were not influenced by sex and diet except the significant values which were within the normal range for sexed pigs which reveals that sex and diet does not compromise the immune system of the sexed pigs.

Keywords: Haematological parameter, Herbal leaf meal, Serum biochemical indices, Sex

Recently, bacteria have become resistant to a wide range of conventional medications. This has become a major source of concern around the world (Ibekwe *et al.* 2000, FAO 2016). This caused the National Agency for Food and Drug Administration and Control to impose a ban on antibiotics and growth boosters (NAFDAC 2018). Leaf meals have been used as an alternative in-feed antibiotics as a result of the ban of antibiotics in the western world (Nweze and Nwankwagu 2010). A deluge of phytochemicals found in medicinal plants has been proved to be useful in both health and disease (Romano *et al.* 2012). Some of such leaf meal used as an alternative in-feed antibiotics include *Moringa oleifera* (Moringa leaf meal) and *Azardirachta indica* (Neem leaf meal).

Sensitivity of the physiology of the animals, influence of diet and assessing animal health is reportedly discovered by haematological and serum biochemical constituents (Onasanya *et al.* 2015). The red blood cells, white blood cells, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration which are part of what makes up the hematological components are important in monitoring feed toxicity especially in feed constituents that affects the blood as well as the health status of farm animals (Oyawole and Ogunkunle 2004). El Tazi and Tibin (2014) recorded improved red blood cell indices in broiler chickens fed on moringa leaf meal diets. Moringa leaf meal (MOLM) seed extracts also exerted blood hypocholesterolemic effects in chicken mice and dogs (Ghebreselassie *et al.* 2011, Garcia *et al.* 2015). Herbal plant supplementation in the diet of pigs is beneficial but only few information exists on the harmonious impact of sex and combining one or more herbal plants in relation to haematology and serum biochemical profile of pigs.

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MATERIALS AND METHODS

Sourcing and preparation of experimental materials: Moringa oleifera and *Azardirachta indica* were sourced locally from the environment of a tropical area. The in-feed used antibiotics (oxytetracycline©) was purchased from a reputable Veterinary Pharmacy within the metropolis. The harvested leaves were air-dried for 3-5 days and constantly turned every 4 h (3 times in a day) to avoid fungal growth until leaves became crispy while retaining its green colouration. The leaves were then tightly packed in jute bags, sealed and kept at room temperature until it was used.

Experimental pigs and management: A total of thirty, eight weeks old Large White growing sexed pigs with mean weight range of 14.54 – 16.35 kg were purchased from a reputable farm. Experimental pigs were raised under an intensive housing system. The pigs were grouped: Six pigs were placed in each treatment with three replicates consisting of two pigs serving as a replicate group, body weight and sex were balanced in a 5×2 factorial experiment. The pigs in treatment 1 (negative control) were served ration that contained no synthetic in-feed antibiotic and herbal leaf meal, while those in treatment 2 (positive control) were offered feed with ration that contained synthetic infeed antibiotic. Pigs on treatments 3, 4, 5 were offered

Table 1. Gross composition (g/kg) of experimental diet

Feed ingredients	Diet 1 (Negative control)	Diet 2 (**Positive control)	Diet 3	Diet 4	Diet 5
Maize	50.00	50.00	50.00	50.00	50.00
Palm Kernel Cake	10.00	10.00	3.50	0.30	2.20
Soyabean	18.00	18.00	18.00	18.00	18.00
Groundnut Cake	10.00	10.00	6.50	9.70	7.80
Fish meal	1.00	1.00	1.00	1.00	1.00
Wheat offal	6.90	6.90	6.90	6.90	6.90
Bone Meal	3.00	3.00	3.00	3.00	3.00
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25	0.25
*Premix	0.30	0.30	0.30	0.30	0.30
Salt (NaCl)	0.25	0.25	0.25	0.25	0.25
Moringa	0.00	0.00	10.00	0.00	6.00
Neem	0.00	0.00	0.00	10.00	4.00
Total	100	100	100	100	100
Determined analysis					
Metabolizable Energy (Kcal)	2832.77	2832.77	2567.022	2825.58	2670.41
Crude Protein (%)	20.41	20.41	20.40	20.42	20.41
Ether Extract (%)	4.55	4.55	4.13	3.96	4.06
Crude Fibre (%)	4.78	4.78	5.94	4.99	5.56

**Positive Control = In-feed Antibiotics (Oxy-tetracycline). *Premix composition to be supplied per kg diet; Vit A, 12600 IU; Vit D3, 2800 IU; Vit k3, 2.8 mg; Vit B1, 1.4 mg; Vit B2, 5.6 mg; Vit B6, 1.4 mg; Vit B12, 0.014 mg; Niacin, 21 mg; Panothenic Acid, 14 mg; Folic Acid, 1.4 mg; Biotin, 0.028 mcg; Chlorine Chloride, 70 mg; Manganese, 70 mg; Zinc, 140 mg; Iron, 140 mg; Copper, 140 mg; Iodine, 1.4 mg; Selenium, 0.28 mg; Cobalt, 0.7 mg; Antioxidant, 168 mg.

diets with 10% inclusion levels of moringa, neem and composite of moringa-neem leaf meals, respectively. The feeding duration lasted for 84 days. The gross composition of the experimental diet is shown in Table 1.

Data collection: Approximately 5 ml of blood was collected from two pigs per replicate group at the 12th week of the experiment through anterior vena cava with needle and syringe into a plain bottle for haematological and serum biochemical profile analyses.

Haematological and biochemical parameters: Haematological parameters, viz. haemoglobin (Franco 1984), packed cell volume (microhaematocrit method, CLSI 2000), RBCs and WBCs (improved Neubauer haemocytometer, Dacie and Lewis 1991) were estimated as per standard methods while mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the PCV. Hb and RBC count (Baker and Silverton 2002). Serum total protein (Tietz 1995), serum albumin (Grant 1987), serum aspartate aminotransferase (Reitman and Franker 1957), alanine aminotransferase (Reitman and Franker 1957) and total blood bilirubin were estimated using Randox diagnostic kit manual. Serum globulin was calculated as:

Serum globulin (g/dl) = Total Serum Protein(g⁄dl) – Serum Albumin(g⁄dl)

Data analysis: The data generated were arranged in a complete randomized design and analyzed in two-way analysis of variance (ANOVA). Significant differences among means were separated using New Duncan Multiple Range Test as contained in SAS (2010) package (p<0 05).

RESULTS AND DISCUSSION

Sex had no significant effect (p>0.05) on haematological parameters of developing pigs besides monocytes. The higher monocyte value (0.70%) was recorded in growing boar compared to 0.10% obtained from growing gilts. The non-significant differences in the haematology parameters except the monocyte imposed by gender of the growing pigs indicate that the immune system of the growing pigs

Table 2. Effect of sex on haematological profile of growing pigs

Parameter	Boar	Gilt	SEM	p- value
Packed Cell Volume (%)	32.80	37.40	1.72	0.087
Haemoglobin (g/dl)	11.10	12.46	0.58	0.126
Red Blood Cell (10^{12}/L)	5.55	6.24	0.29	0.121
White Blood Cell (10^{9}/L)	10.56	11.29	1.36	0.712
Neutrophils (%)	30.90	35.30	1.99	0.149
Lymphocyte (%)	67.50	63.90	1.84	0.197
Eosinophils (%)	0.40	0.30	0.24	0.769
Basophils (%)	0.50	0.40	0.24	0.769
Monocytes (%)	0.70^{a}	0.10^{b}	1.41	0.013
MCV (fl)	59.12	59.94	0.48	0.249
MCH (pg)	20.00	19.98	0.46	0.764
MCHC (g/dl)	33.83	33.31	0.30	0.247

MCV, Mean Corpuscular Volume; MCH, Mean Cell Haemoglobin; MCHC, Mean Cell Haemoglobin Concentration; S.E.M, Standard Error of Mean.

Table 3. Effect of dietary inclusion of sole or combined herbal leaf meal on haematological parameters of growing pigs

Parameter	Control	Antibiotics	Neem	Moringa	Moringa+Neem	S.E.M	p- value
Packed Cell Volume (%)	33.75	30.75	35.00	38.75	37.25	2.71	0.329
Haemoglobin (g/dl)	11.35	10.50	11.75	12.90	12.40	0.91	0.433
Red Blood Cell (10^{12}/L)	5.65	5.25	5.88	6.68	6.23	0.46	0.402
White Blood Cell (10^{9}/L)	10.85	11.18	11.48	11.70	9.43	2.15	0.946
Neutrophils (%)	34.00	31.75	31.50	30.00	38.25	3.14	0.431
Lymphocyte (%)	64.25	67.50	67.75	68.75	60.25	2.91	0.293
Eosinophils (%)	0.25	0.25	0.00	0.50	0.75	0.37	0.677
Basophils (%)	0.50	0.25	0.75	0.50	0.25	0.37	0.859
Monocytes (%)	1.00	0.25	0.00	0.25	0.50	0.22	0.080
MCV (fl)	59.83	58.60	59.58	59.83	59.83	0.75	0.735
MCH (pg)	20.10	20.00	20.03	19.93	19.90	0.072	0.360
MCHC (g/dl)	33.58	34.15	33.60	33.28	33.25	0.473	0.678

^{a,b,c}Means along the same row with different superscripts are significantly different (P<0.05). 0- Control, 1- In-feed Antibiotics (Oxytetracycline), 2- Neem Leaf meal, 3- Moringa Leaf meal, 4- Neem and Moringa Leaf meal. MCV, Mean Corpuscular Volume; MCH, Mean Cell Haemoglobin; MCHC, Mean Cell Haemoglobin Concentration; S.E.M, Standard Error of Mean.

was not compromised. The monocytes (0.70%) observed in this study were below the range stated by Merck Manual (2012). Lower value observed in female than in male is similar to the report of Etim *et al.* (2014). The decrease in monocyte count in gilt can be attributed to the production of progesterone and oestrogen inducing apoptosis in monocytes (Thongngram *et al.* 2003). The monocyte boosts the immune system which helps restore the macrophages and dendritic cells in the tissues respond likewise to inflammatory mediators by differentiating into macrophages and dendritic cells as part of an immune response (Ida and Gerald 2019).

The effect of dietary levels of sole or combined herbal leaf meal had no significant effect (p>0.05) on all haematological parameters measured in Table 3. The absence of significant difference in the dietary treatment on the haematological indices of pigs in this present study correlates with NseAbasi *et al.* (2014) and Adegbenro *et al.* (2016) who reported that dietary treatments had no influence on the haematology indices of pigs which indicates that diet had no deleterious effects on haematological parameters (Stukeji *et al.* 2010).

Sex had no significant effect (p>0.05) on serum biochemical indices of growing pigs except on aspartate aminotransferase, albumin and cholesterol (Table 4). The higher albumin value (3.54–4 .75 g/dl) observed in this study is at variance with study of Abonyi *et al.* (2018) for both genders. Higher value of albumin in boar than the gilt is an expression of androgen anabolic effect (Saad *et al.* 2017). The androgen hormone such as the testosterone which is higher in male animals promotes protein synthesis (Sheffield-moore 2000) resulting in muscle cell multiplication, maintenance of life process and improved metabolic activities (Cherin 2019). Increased value of albumin improves the clotting ability of blood, thus improving the prevention of haemorrhage in the body of the pig (Robert *et al.* 2003). Aspartate aminotransferase was higher in boar than gilt in this study which aligns with the study of Prati *et al.* (2002) and could be due to hormonal differences (Jorge *et al.* 2007). Aspartate aminotransferase value (26.60–43.51 U/L) observed in this present study is within the range value as recorded by Merck Manual (2019). This implies that there was no damage to the liver as elevated serum enzyme activity an indicator of liver injury (Aro *et al.* 2012). The cholesterol value was within the normal range of 59.6-265.4 mg/dl as reported by Yeom *et al.* (2012) in miniature sexed pigs. The increase in serum cholesterol in boar can be attributed to the cellular mechanism of androgen which might have elevated cholesterol ester accumulation (Kenneth *et al.* 2011). Normal range of cholesterol indicates that the animal is not at risk of hypercholesterolemia and liver failure, and there is a normal lipid mobilization and lipogenesis in the body of the pig as reduction in serum cholesterol suggests a general decrease in lipid mobilization in the body of an animal (Adegbenro *et al.* 2016).

Table 4. Effect of sex on serum biochemical indices of growing pigs

Parameter	Boar	Gilt	S.E.M	p- value
Total Protein (g/dl)	8.12	8.07	0.37	0.9250
Albumin (g/dl)	4.75^{a}	3.54^{b}	0.25	0.0070
Globulin (g/dl)	3.34	4.52	0.40	0.0660
Glucose (mg/dl)	82.13	84.45	4.28	0.7100
Aspartate Aminotransferase (U/L)	43.51^{a}	26.60^{b}	4.13	0.0160
Alanine Aminotransferase (U/L)	24.26	30.60	2.80	0.1410
Urea (mg/dl)	10.78	9.88	0.99	0.5360
Cholesterol (mg/dl)	129.12^{a}	98.05^{b}	5.84	0.0040
Total Bilirubin (mg/dl)	0.68	0.63	0.19	0.8560
Creatinine (mg/dl)	3.07	1.85	0.31	0.2000

^{a,b,c}Means along the same row with different superscripts are significantly different (P<0.05). SEM, Standard Error of Mean.

Dietary inclusion of sole or combined herbal leaf meal had no significant influence (p>0.05) on serum biochemical

Table 5. Effect of dietary inclusion of sole or combined herbal leaf meal as alternative in-feed antibiotics on serum biochemical indices of growing pigs

Parameter	Control	Antibiotics	Neem	Moringa	Moringa+Neem	S.E.M	p-value
Total Protein (g/dl)	8.20	8.38	8.13	8.30	7.18	0.58	0.8130
Albumin (g/dl)	4.38	4.55	4.15	4.30	3.33	0.40	0.3140
Globulin (g/dl)	3.78	3.83	3.95	3.98	4.13	0.64	0.9950
Glucose (mg/dl)	74.98	83.35	83.08	97.95	77.10	6.77	0.2140
Aspartate Aminotransferase (U/L)	28.85	35.33	37.95	37.70	35.45	6.53	0.8600
Alanine Aminotransferase (U/L)	31.80	27.95	25.30	27.50	24.60	4.43	0.8000
Urea (mg/dl)	9.13	10.98	10.95	10.78	9.83	1.57	0.8880
Cholesterol (mg/dl)	119.48	117.58	122.13	119.50	89.25	9.23	0.1420
Total Bilirubin (mg/dl)	0.35	0.75	0.95	0.78	0.45	0.30	0.6210
Creatinine (mg/dl)	2.15	2.25	3.35	2.75	1.80	0.50	0.2800

SEM, Standard Error of Mean.

indices of growing pigs (Table 5). The non-significant differences in all the serum biochemicals observed in growing pigs fed test diets indicates that the renal and hepatic system of the pigs were not compromised.

The study confirms that sex did not influence the haematological and serum biochemical profile of pigs except on monocyte, albumin, aspartate aminotransferase, and cholesterol, respectively. Also, dietary supplementation did not influence the haematological serum biochemical profile of pigs.

The haematological indices were not influenced by sex and diet except on monocytes which was within the normal range value for sexed pigs, which reveals that sex does not compromise the immune system of the sexed pigs. Also sex had no influence on serum biochemical indices except on albumin, aspartate aminotransferase, and cholesterol which are all within normal range.

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