



ORIGINAL RESEARCH ARTICLE

Haematological Response of Growing Male Pigs Fed Dietary Neem (*Azadirachta indica* A. Juss) Kernel***Sokunbi O. A and Egbunike, G. N***Department of Animal Science, University of Ibadan, Nigeria***Correspondence: Email: jide.sokunbi@gmail.com; GSM: +2348055302569***ABSTRACT**

Several studies had been carried out to evaluate the suitability of utilizing neem as a feed ingredient in pig production with varied results. The negative effects of anti-nutrients in neem had been reported. The haematological response of growing male pigs fed with diets in which maize was partially replaced with neem kernel (NK) was thus investigated. Forty crossbred (Large White x Landrace) male weanling pigs were allotted into experimental treatments in a completely randomised design to evaluate the influence of NK inclusion in swine diets at 0, 50, 100 and 150 g/kg on haematological profile; packed cell volume (PCV), erythrocytes (RBC), haemoglobin (Hb), leukocytes (WBC) and corpuscular indices. Data obtained were analysed using descriptive statistics and ANOVA. Haematological profile of growing male pigs on 150 g/kg NK diet indicated significant differences ($P < 0.05$) in PCV (34.0%), RBC ($7.5 \times 10^6/\mu\text{l}$), Hb concentration (11.3g/100ml), mean corpuscular volume (45.3fl) and mean corpuscular haemoglobin concentration (33.2%) compared to control with 39.3 %, $6.3 \times 10^6/\mu\text{l}$, 12.5 g/100ml, 62.8 fl and 32.0 % respectively. Pigs on 50 and 100 g/kg NK diets gave similar values with those on control for all parameters. Results observed in this investigation indicated that neem kernel can be included up to 100 g/kg in growing male pigs' diets.

Keywords: Pigs, neem kernel, haematological profile**INTRODUCTION**

In a study conducted in southern part of Kaduna State, Nigeria on the economics of swine production, cost of feed was found to be the major constraint followed by purchase of piglets and cost of labour (Ajala, 2003). The use of non-conventional or unusual feedstuffs to bring down this cost was suggested as a possible short-term method to improve pig production in the study area. One of such promising potential feedstuffs is neem (*Azadirachta indica*). Neem kernel (NK) has a higher dietary energy hence, the performance and physiological response of swine fed with diets in which maize was partially replaced with NK should be researched into. Many uses of neem have been reported. Some of these are wood for fuel and furniture making, production of pharmaceutical products, lubricants, insecticides, organic manure, apiculture and feed for livestock. Neem products (whole seeds, cakes, oil, bark and leaves) have properties that have been reported, some of which are pharmacological, bactericidal and anti-viral. The leaves contain compounds with demonstrated antiseptic, antiviral, and anti-fungal activities. There are also hints that neem has anti-inflammatory, anti-hypertensive and anti-ulcer effects (NRC, 1992). Neem has received maximum attention from entomologists all over the world (Stark and Walter, 1995). The extracts from neem tree were found to reduce or prevent insect feeding and also to adversely

affect growth, development and reproduction (Mordue and Blackwell, 1993). The compounds responsible for the insecticidal activity of neem appear to be essentially non-toxic to mammals (Koul *et al*, 1990). Although neem appears to be safe to mammals in normal use as a pesticide, the possibility of future hazards should not be dismissed. Few toxicity tests on higher mammals such as dogs, pigs, primates, or humans have yet been published, necessitating its use on food crops or as feed ingredients for livestock to be executed with caution.

Many researchers have reported negative effects of the use of unusual ingredients on some blood parameters as a result of factors such as nutrient imbalance, improper metabolism, presence of anti-nutritional factors and toxic elements in diets offered livestock. This emerging fact makes it necessary to investigate the effect of the inclusion of ingredients with established anti-nutrients on the health status of pigs via the study of the animals' blood profile. Few studies have been carried out on the effect of neem on the haematological profile of livestock. Gangopadhyay *et al*. (1981) reported that there was no effect on the red and white blood cell counts of Murrah milch buffaloes on replacement of concentrate mixture with 15 and 20 parts of neem seed cake. Neem oil injected under the skin led to increased leukocytic cells and peritoneal macrophages showed enhanced phagocytic activity and spleen cells showed

higher lymphocyte reaction to infection (The Original Neem Company, 2002). Verma *et al.* (1995) observed similarities in haemoglobin values between goats on control diet and those on diets containing water washed neem seed kernel cake. Anandan *et al.* (1996) also reported similar observation of haemoglobin values obtained from goats on control diets and those on diets containing urea ammoniated neem seed kernel meal, both at the active phase and later phase. Sokunbi and Egbunike (2000) reported similarities in haematological profile except for white blood cells of rabbits on replacement of concentrate mixture with 5, 7.5 and 15 parts of neem leaf meal. These similarities in mean values of haematological parameters were also observed for growing female pigs fed diets containing sun-cured neem leaf meal (Sokunbi *et al.*, 2002). However, a study on the utilization of raw neem kernel meal (Annongu *et al.*, 2003) by growing pigs indicated significant depressed responses of packed cell volume, red blood cells, white blood cells and haemoglobin. The possible effects of neem kernel on the haematological profile of growing male pigs was thus investigated in the light of conflicting and yet inadequate information on this aspect of the physiology of pigs.

MATERIALS AND METHODS

Experimental animals, diets, design and management

Forty cross bred (Large White x Landrace) weanling male pigs were randomly allotted to four diets (Table 1) formulated to be iso-nitrogenous and iso-caloric with graded levels of neem kernel; 0 g/kg (diet 1), 50 g/kg

(diet 2), 100 g/kg (diet 3) and 150 g/kg (diet 4), in individual pens in a completely randomised design. All animals were penned in a dwarf-walled well-ventilated cement-floored building. The pigs were fed a commercial pigs' grower diet for two weeks to stabilize them and then placed on the experimental diets for twelve weeks. The pigs were treated against parasitic infestation (external and internal) with Ivermectin® (1 ml kg⁻⁵⁰ body weight) during the two weeks period of adjustment and feeds were provided twice daily at 8.00 hours and 16.00 hours, and animals were weighed before feeding at the commencement of the feeding trial and weekly feed intake and weight changes were monitored for individual pigs.

Collection of blood samples

To elucidate the effect of neem kernel on haematological responses by the growing pigs, blood was sampled four times (week 0, week 4, week 8 and week 12) from all the experimental pigs. Blood samples were collected from the anterior vena cava of the pigs (that were previously confined without feed for 12 hours but were allowed access to water) into sterilized glass tubes containing EDTA (ethylene diamine tetra acetic acid) for immediate analysis of haematological parameters in the laboratory.

Haematology

The packed cell volume (PCV) and haemoglobin (Hb) were determined using the micro haematocrit method and cyanmethemoglobin method respectively as described by Mitruka and Rawnsley (1977).

Table 1: Composition of experimental diets

Ingredients	Dietary neem kernel (g/kg)			
	0	5	10	15
Maize	40.00	35.00	30.00	25.00
Neem kernel	0.00	5.00	10.00	15.00
Groundnut cake	15.00	15.00	15.00	15.00
Palm kernel cake	10.00	10.00	10.00	10.00
Wheat offal	13.00	13.50	14.00	15.00
Corn bran	15.00	15.00	15.00	15.00
Fish meal (65 %)	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00
Oyster shell	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40
Premix [†]	0.50	0.50	0.50	0.50
Lysine	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30
Vegetable oil (Palm oil)	2.00	1.50	1.00	0.00

[†] Micro-Mix Growers: 2.5 kg of premix contains

Vit. A (10,000,000.00 I.U.); Vit. D₃ (2,000,000.00 I.U.); Vit. E (20,000.00 mg); Vit. K₃ (2,000.00 mg); Vit. B₁ (3,000.00 mg); Vit. B₂ (5,000.00 mg); Niacin (45,000.00 mg); Calcium Pantothenate (10,000.00 mg); Vit. B₆ (4,000.00 mg); Vit. B₁₂ (20.00 mg); Folic Acid (1,000.00 mg); Biotin (50.00 mg); Choline Chloride (300,000.00 mg); Manganese (120,000.00 mg); Iron (100,000.00 mg); Zinc (80,000.00 mg); Copper (8,500.00 mg); Iodine (1,500 mg); Cobalt (300.00 mg); Selenium (120.00 mg); Anti-Oxidant (120,000.mg)

Source: Sokunbi (2007)

Table 2: Haematological mean values of growing male pigs fed diets containing neem kernel (Week 0)

Parameters	Dietary neem kernel (g/kg)				Group SEM
	0	50	100	150	
Packed Cell Volume (%)	34.00	34.00	34.00	34.25	0.30
Haemoglobin (g/dl)	11.55	11.53	11.50	11.53	0.20
RBC ($\times 10^{12}/L$)	5.81	5.64	5.80	5.70	0.13
MCV (μ^3)	58.42	60.18	58.61	60.40	0.32
MCH ($\mu\mu g$)	19.85	20.48	19.87	20.33	0.14
MCHC (%)	33.98	33.82	33.85	33.68	0.17
WBC ($\times 10^6/L$)	10.60	10.60	10.63	10.67	0.08
Neutrophils (%)	32.00	31.75	32.25	33.00	0.64
Lymphocytes (%)	66.50	66.00	65.25	64.75	0.66

SEM = standard error of mean, WBC: White Blood Cell, MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin, PCV: Packed Cell Volume

Erythrocyte count (RBC) and Leukocyte count (WBC) were determined using the improved Neubauer haemocytometer after the appropriate dilution (Schalm *et al.*, 1975). Differential leukocyte counts were determined by scanning Giemsa's stained slides in the classic manner (Schalm *et al.*, 1975). Blood indices and corpuscular constants; mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using the appropriate formulae (Jain, 1986).

Statistical analysis

Results were subjected to statistical analysis using the analysis of variance procedure of statistical analysis software (SAS, 1999). The treatment means are presented with group standard errors of means and where significant, were compared using the Duncan multiple range test procedure of the same software.

RESULTS

Haematological response of growing male pigs to diets containing neem kernel

There were similarities for all haematological parameters of all the experimental pigs before commencement of the feeding trial as presented in Table 2. Mean values for all parameters are within reported ranges of Mitruka and Rawnsley (1977), Schalm *et al.* (1975) and Dunne and Alibasoglu (1963). Four weeks into the feeding trial, the effect of neem kernel inclusion in the pigs' diets caused significant ($P < 0.05$) differences in PCV, RBC, MCH, MCHC, neutrophils and lymphocytes while observed mean values for Hb, MCV and WBC were similar (Table 3). A marked depression was observed for PCV and RBC as mean values decreased and NK increased in the diets, while there were elevations in the mean values of the corpuscular constants, MCH and MCHC as NK

increased in the diets. While there was no effect on WBC at this period of the feeding trial, there were significant effects ($P < 0.05$) on neutrophil and lymphocyte counts as neem kernel NK in pigs' diets increased with an increased neutrophil count and a decreased lymphocyte count.

Eight weeks into the feeding trial, the pigs presented similarities in mean values for most of the haematological parameters (Table 4) except for WBC, neutrophils and lymphocytes where observed differences were significant ($P < 0.05$). There was a marked effect of neem kernel NK inclusion in pigs' diets at this period on WBC causing a depression from 100 g/kg NK diet. The effects on neutrophils and lymphocytes were as in the fourth week. Results of the haematological profile of blood collected from the experimental pigs at the end of the feeding trial (week 12) indicated significant ($P < 0.05$) effects on all parameters (Table 5). Despite the observed significant effect of neem kernel on these haematological parameters, mean values are still within or slightly outside reported ranges in literature.

Variations in time of haematological mean values of growing male pigs fed diets containing neem kernel

Packed cell volume

There was a noticeable effect of NK inclusion in pigs' diets on PCV. A depression was observed from 100 g/kg NK diet by the fourth week. This depression was reversed by the eighth week through the end of the feeding trial.

Red blood cells

There was also a noticeable depressive effect of NK inclusion in pigs' diets on RBC from 100 g/kg NK diet by the fourth week and like it was observed for PCV, this depression was reversed by the eighth week.

Table 3: Haematological mean values of growing male pigs fed diets containing neem kernel (Week 4)

Parameters	Dietary neem kernel (g/kg)				SEM
	0	50	100	150	
PCV (%)	31.50 ^a	32.25 ^a	28.50 ^b	23.25 ^c	1.00
Haemoglobin (g/dl)	11.83	12.00	11.23	11.48	0.20
RBC ($\times 10^{12}/L$)	5.14 ^a	5.38 ^{ab}	4.70 ^a	3.83 ^c	0.17
MCV (μ^3)	61.30	59.96	60.89	60.73	0.23
MCH ($\mu\mu g$)	23.03 ^a	22.3 ^a	24.04 ^a	30.05 ^b	1.00
MCHC (%)	37.58 ^a	37.20 ^a	39.47 ^a	49.45 ^b	1.44
WBC ($\times 10^6/L$)	11.50	11.65	11.80	12.01	0.23
Neutrophils (%)	29.00 ^a	38.75 ^b	34.50 ^b	36.50 ^b	1.22
Lymphocytes (%)	69.00 ^a	59.00 ^b	64.00 ^b	61.50 ^b	1.20

^{abc}: means in the same row with different superscripts differ significantly ($P < 0.05$). SEM = standard error of mean, WBC: White Blood Cell, MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin, PCV: Packed Cell Volume

However, there was an increased RBC count from pigs fed 150 g/kg NK diet by the end of the feeding trial.

Haemoglobin

A strong negative effect of NK on haemoglobin concentration of growing male pigs by the fourth week was observed. There was a depression in Hb concentration from 100 g/kg NK diet while pigs on 50 g/kg NK diet gave higher Hb concentration when compared to those on 0 g/kg NK diet. However, there was a recovery at the eighth week, but a down turn by the end of the feeding trial was observed, especially at 150 g/kg NK diet.

White blood cells

NK caused an initial elevation of white blood cells in growing male pigs by the fourth week of the feeding trial. This increase was linear as WBC count increased with increasing concentration of NK in pigs' diets. However, this elevation of WBC by growing male pigs on neem over that of those on control diet was only maintained at 50 g/kg NK diet while there was a depression from 100 g/kg NK diet for the latter phase of the feeding trial.

Neutrophils

Inclusion of NK in pigs' diet resulted in an increase in percent neutrophils as its level of inclusion increased in the diets through the feeding trial.

Lymphocytes

Inclusion of NK in pigs' diet resulted in a decrease in percent lymphocytes as its level of inclusion increased in the diets through the feeding trial.

DISCUSSION

Early feeding phase (week 0 to week 4)

Results in this trial indicated a tendency towards normochromic macrocytic anaemia by growing male pigs fed 100 and 150 g/kg NK diets by the fourth week of the feeding trial with significant reduction in PCV and RBC values but an increase in MCV values.

Table 4: Haematological mean values of growing male pigs fed diets containing neem kernel (Week 8)

It is apparent from results obtained that the introduction of male pigs to diets with varied amounts of NK through the feeding period had effects on the leukocytes with an increase in percent neutrophils and a decrease in percent lymphocytes. Though there were significant differences in the percentage neutrophils and lymphocytes at this period of the feeding trial, results did not indicate increase in absolute lymphocyte counts. It is known that stress reduces the number of lymphocytes in blood, suggesting that there might be an adrenocortical regulating mechanism that appears to affect mostly the short lived cells (Coles, 1974). However, there was a significant increase in leukocyte counts in male pigs fed 50 g/kg NK diet. This increase in leukocytic cells had earlier been observed in rats in which neem oil was injected under the skin (The Original Neem Company, 2002). There was a deviation from the observed trend of NK influence on neutrophils/lymphocytes relationship in which percent neutrophils increased at the expense of percent lymphocytes as the pig aged which is contrary to what is expected. Lymphocyte counts are expected to rise over neutrophils as the pig ages (Coles, 1974). However, this observation is not enough to suggest lymphocytosis without consideration of other important possible physiological deviations such as adrenocortical insufficiency that is often manifested by an increase in the absolute number of lymphocytes and hyperthyroidism, which is also accompanied by lymphocytosis (Wallen, 1979).

Mid feeding phase (week 4 to week 8)

A picture of normal haematological profile except for significant differences in WBC counts, percent neutrophils and lymphocytes by both male and female pigs was presented in this feeding phase. This indicated an apparent positive physiological response

Parameters	Dietary neem kernel (g/kg)				Group SEM
	0	50	100	150	
PCV (%)	36.25	36.75	36.25	36.00	0.45
Haemoglobin (g/dl)	11.90	11.93	12.00	11.88	0.11
RBC ($\times 10^{12}/L$)	5.80	6.56	5.78	5.78	0.19
MCV (μ^3)	62.54	63.19	62.73	62.24	0.39
MCH ($\mu\mu\text{g}$)	20.54	20.51	20.77	20.55	0.09
MCHC (%)	32.84	32.47	33.12	33.03	0.18
WBC ($\times 10^6/L$)	13.19 ^a	18.38 ^b	9.85 ^c	8.19 ^c	1.16
Neutrophils (%)	26.00 ^a	33.50 ^b	35.25 ^{bc}	36.75 ^c	1.14
Lymphocytes (%)	72.00 ^a	65.00 ^b	59.75 ^c	59.00 ^c	1.36

SEM = standard error of mean, and a, b, c, = means in the same row with different superscripts differ significantly ($P < 0.05$).

to the inclusion of NK in swine diets. However, the mechanism by which this is achieved cannot be explained by the scope of this investigation. It appears that NK, at low concentration seems to raise the level of antibodies and at higher concentrations elicits a depression. It has been reported that NK contains a high concentration of Glutamic acid, more than any other amino acid (40.10 mg/g) so it can be regarded as a rich source of this amino acid whose derivative L-glutamine, appears to guard against damage to the intestinal membranes at time of stress and may also be a source of fuel for cells of the immune system that secrete antibodies (International Pig Veterinary Society, 2000).

Late feeding phase (week 8 to week 12)

The haematological profile of the male pigs by the end of the feeding trial presented a change in the macrocytic nature of anaemia to microcytic. Results indicated normochromic microcytic anaemia with reductions in PCV, Hb, MCV and MCH, an increase in RBC and normal MCHC. Annongu *et al.* (2003) reported depressed responses of PCV, RBC, WBC and Hb from growing pigs fed raw neem kernel meal. This change in

anaemic form gave an insight to the mechanism by which the experimental pigs responded to the inclusion of NK in their diets by an increase in erythropoiesis from the prevalence of immature red blood cells in analysed blood samples. Neem kernel appears to affect the feedback mechanism in erythropoiesis from the abnormal nature of erythrocyte count in pigs fed 150 g/kg NK diet. However, the scope of this investigation could not explain whether the increase in RBC was as a result of stimulation by anaemia or hypoxia (Ganong, 1991), or abnormal production of erythropoietin secreted primarily by the kidney. Also, low or very slow production of erythroblast in the bone marrow can lead to production of microcytes as a result of loss of vitamin B₁₂ and folic acid and any of the intrinsic factors from the stomach mucosa (Guyton, 1991). The presence of large number of immature cells in the blood stream has been implicated to be the cause of the disease, pernicious anaemia (Ross and Wilson, 1981) that is due to a failure in normal production of red cells occasioned by lack of a factor (or factors) necessary for erythrocyte maturation.

Table 5: Haematological mean values of growing male pigs fed diets containing neem kernel (Week 12)

Parameters	Dietary neem kernel (g/kg)				SEM
	0	50	100	150	
PCV (%)	39.25 ^a	36.25 ^b	35.25 ^{bc}	34.00 ^c	0.56
Haemoglobin (g/dl)	12.53 ^a	12.20 ^a	11.93 ^a	11.30 ^b	0.14
RBC ($\times 10^{12}/L$)	6.25 ^a	6.21 ^a	6.01 ^a	7.52 ^b	0.63
MCV (μ^3)	62.78 ^a	58.40 ^b	58.65 ^b	45.25 ^c	1.72
MCH ($\mu\mu\text{g}$)	20.04 ^a	19.66 ^a	19.85 ^a	15.04 ^b	0.56
MCHC (%)	31.94 ^a	33.66 ^b	33.84 ^b	33.24 ^b	0.30
WBC ($\times 10^6/L$)	15.08 ^a	21.41 ^b	11.83 ^c	9.28 ^c	1.35
Neutrophils (%)	23.00 ^a	35.50 ^b	38.25 ^c	39.75 ^c	1.75
Lymphocytes (%)	76.00 ^a	63.00 ^b	58.75 ^c	57.25 ^c	2.00

abc: means in the same row with different superscripts differ significantly ($P < 0.05$). WBC: White Blood Cell, MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin, PCV: Packed Cell Volume

The effect of NK inclusion in swine diets on WBC by this late feeding phase was as observed in mid feeding

phase. However the observed degenerative shift to the left from male pigs fed 50 g/kg NK diet was more

pronounced in this phase, more so that the observed mean value falls slightly outside the upper limit of WBC range reported by Mitruka and Rawnsley (1977) suggesting neutrophilia. The experimental pigs did not show outward symptoms of a disease state implicating a low or high production of WBC from the bone marrow (Ganong, 1991). Furthermore, the depressed WBC count of male pigs fed 100 and 150 g/kg NK diets were also slightly outside reported ranges in literatures. However, Dunne and Alibasoglu (1963) stated that in pigs six weeks of age or older, any count lower than 10,000 would indicate a definite leukopenia. Neutrophilia has been associated with a variety of non-infectious conditions, which stimulate the stress reaction, such as chemical and metabolic intoxication (Schalm *et al.*, 1975) and the alteration in the leukocyte picture can be the consequences of a normal physiological response not necessarily a disease condition.

CONCLUSION

Generally, results of haematological studies indicated different forms of anaemic conditions from pigs fed diets containing various levels of NK and the ability of pigs to reverse these conditions initially within a few weeks of introduction to NK diets but eventual recourse to these anaemic states. This investigation indicated that pigs can tolerate up to 100g/kg neem kernel in their diets.

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