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ORIGINAL RESEARCH ARTICLE

Influence of dietary neem (*Azadirachta indica* A. Juss) kernel on serum biochemical components of growing male pigs

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ABSTRACT

The increasing cost of maize as a source of dietary energy for swine necessitates investigation into unconventional alternative energy sources. Neem kernel (NK) has a higher dietary energy hence; the influence of dietary NK on serum biochemical components and selected organs of growing male pigs was investigated. 40 crossbred (Large White x Landrace) male weanling pigs were allotted into experimental treatment in a completely randomised design to evaluate the influence of NK inclusion in swine diets at 0, 50, 100 and 150 g/kg on serum chemistry profile. Data obtained were analysed using descriptive statistics and ANOVA. Results indicated significant (P<0.05) effect of dietary NK on serum proteins, cholesterol, creatinine, urea nitrogen, potassium and calcium. The general trend is observed comparable mean values for pigs on 0 and 50 g/kg NK diets, while elevation or depression in mean values were indicated at 100 and 150 g/kg NK diets for most parameters. Significant (P<0.05) effects were observed for cholesterol concentration (204.6mg/dl) and aspartate transaminase activity (27.2iu/l) in pigs on 150 g/kg NK compared to that of 134.4 mg/dl and 11.46 iu/l respectively from those on control. Results indicated that neem kernel can be included up to 50 g/kg in boars' diets without deleterious effects on their serum biochemical constituents.

Keywords: Pigs, neem kernel, serum biochemistry

INTRODUCTION

Reports on the effects of neem on the serum biochemical components and internal organs in livestock are presently scanty. Verma *et al*. (1995) observed from their investigation of the effect of feeding water washed neem seed kernel cake to growing goats on their blood and plasma constituents, that glucose, urea nitrogen and total protein were significantly lower in diets containing neem. However, creatinine and cholesterol were similar among dietary groups. They argued that the lowered blood glucose could be attributed to the anti-hyperglycaemic effect of neem. Neem products are known for their anti-hyperglycaemic effect (Mitra, 1963). Anti-hyperglycaemic effect was observed by Tyagi (1989) with water washed neem seed kernel in buffalo calves and Garg (1989) with de-oiled neem seed kernel cake in calves. Agrawal *et al*. (1987) and Sastry and Agrawal (1992) also observed similarly depressed blood urea nitrogen. The plasma transaminases and alkaline phosphatase did not, however, differ significantly between water washed neem seed kernel cake and control fed goats. The depressed plasma protein concentration corroborated with the findings of Gangopadyay (1981) who observed similarly depressed plasma protein levels in Murrah milch buffaloes on replacement of concentrate mixture with 15 and 20 parts of neem seed cake. Anandan *et al.* (1996) reported from their study of the growth rate and nutrient efficiency of growing goats fed urea ammoniated neem seed kernel meal as protein supplement, that the concentration of blood biochemical constituents: glucose and urea nitrogen and the activities of the transaminases and alkaline phosphatase did not differ significantly between treatments. They observed a non-occurrence of the anti-hyperglycaemic effect of neem in their study of goats fed with ammoniated neem seed kernel meal. Annongu *et al.* (2003), from their work on the effect of the detoxification of neem kernel meal with Lyle under carbon dioxide environment on biochemical indices in swine showed that serum total protein, albumin, globulin, urea nitrogen, alanine transaminase and alkaline phosphatase were adversely affected from the group of pigs fed untreated neem kernel meal relative to the groups receiving treated neem kernel meal and the standard diets. However, growing female pigs fed diets containing sun-cured neem leaf meal gave similar mean values for all biochemical parameters studied except for albumin, alanine transaminase and aspartate transaminase (Sokunbi *et al*., 2002). The influence of the inclusion of neem kernel on serum proteins, chemical components, enzyme activities, and some selected organs of growing pigs was thus investigated.

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-calorific 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), such that each diet had five pigs 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 Ivomectin® (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 every 7 days thereafter until the end.

Collection of blood samples

Blood samples were collected from the anterior vena cava of all the experimental pigs at the end of the feeding trial that lasted for 12 weeks into a set of sterilized dry glass tubes containing no traces of anticoagulant for serum separation within 45 minutes of blood collection. Serum samples were immediately analysed for glucose and the rest stored at -20ºC for future analyses, of chemical components; sodium (Na^+), potassium (K^+), chloride (Cl^-), bicarbonate (HCO_3^-), calcium (Ca^+), phosphorus, urea, creatinine, cholesterol, serum proteins; total serum protein, albumin and globulin, and serum enzyme activities; alanine transaminase (ALT), aspartate transaminase (AST) and alkaline Phosphatase (ALP).

Serum biochemistry

Na^+ and K^+ were determined using flame photometry, and Cl^-, HCO_3^-, Ca^+ and P were determined according to AOAC (1990). Glucose was determined by *o*-Toluidine method using acetic acid (Cooper and McDaniel, 1970). Urea was determined by urease method and creatinine by Folin-Wu filtrate methods as described by Toro and Ackermann (1975). Total serum protein was determined using the Buiret method as described by Reinhold (1953) while albumin was determined using the BCG (Bromocresol green) method as described by Peters, Biamonte and Doumas (1982). Alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities were determined using spectrophotometric methods as described by Rej and Hoder (1983), Hoder and Rej (1983) and McComb, Bowers and Rosen (1983), respectively.

Statistical analysis

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

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 Vitamin A (10,000,000.00 I.U.); Vitamin D_3 (2,000,000.00 I.U.); Vitamin E (20,000.00 mg); Vitamin K_3 (2,000.00 mg); Vitamin B_1 (3,000.00 mg); Vitamin B_2 (5,000.00 mg); Niacin (45,000.00 mg); Calcium Pantothenate (10,000.00 mg); Vitamin B_6 (4,000.00 mg); Vitamin B_{12} (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: Serum proteins, chemical components and enzyme activities of boars fed diets containing neem kernel

Parameters	Dietary neem kernel (g/kg)				Group SEM
	0	50	100	150	
Serum Proteins (g/dl)					
Total Protein	8.96[a]	8.86[ab]	8.60[b]	8.40[b]	0.08
Albumin	4.31[a]	4.28[ab]	4.16[b]	4.00[b]	0.04
Globulin	4.65[a]	4.58[a]	4.44[ab]	4.41[b]	0.04
Albumin/Globulin	0.93	0.94	0.94	0.91	0.05
Chemical Components (mmol/l)					
Cholesterol	135.40[a]	170.14[b]	174.78[b]	204.56[c]	5.82
Creatinine	1.43[a]	1.84[b]	1.99[b]	2.30[c]	0.08
Glucose	77.60[a]	65.00[b]	62.60[b]	60.00 [b]	1.05
Urea nitrogen	14.00[a]	13.60[a]	16.60[ab]	18.80[b]	0.58
Sodium	147.40	145.80	145.60	144.60	0.82
Potassium	5.04[a]	6.08[b]	6.40[b]	6.48[b]	0.15
Chloride	106.00	105.00	105.40	105.00	0.23
Phosphorous	6.08[a]	6.04[ab]	5.90[bc]	5.82[c]	0.03
Calcium	10.38[a]	10.48[a]	10.80[b]	11.00[b]	0.07
Enzyme Activities (iu/l)					
Alanine transaminase	15.82[a]	15.69[a]	20.83[b]	23.12[b]	0.80
Aspartate transaminase	11.46[a]	13.21[a]	14.39[a]	27.14[b]	1.47
Alkaline phosphatase	106.30[a]	109.32[a]	107.12[a]	85.94[b]	2.22

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

RESULTS

Serum proteins, chemical components and enzyme activities in growing male pigs fed diets containing neem kernel

Summarized in Table 2 are the mean values of serum proteins, chemical components and enzyme activities of growing male pigs fed diets containing neem kernel. Results indicated significant ($P < 0.05$) effect of NK inclusion in swine diets on total serum protein, albumin and globulin. The general trend from these observations is the decrease in serum protein concentrations as the concentration of NK increased in the experimental diets. Differences in mean values for most of the chemical components investigated in this study were significant ($P < 0.05$) except for sodium and chloride. Results show significant elevation ($P < 0.05$) of cholesterol, creatinine, urea nitrogen, potassium and calcium concentrations as the level of NK increased in the experimental diets, while significant reduction ($P < 0.05$) in mean values was observed for phosphorous. A slight depression ($P < 0.05$) in glucose concentration was indicated in pigs on 50 g/kg NK diet while further increase in NK inclusion in swine diets from 100 g/kg NK diet led to significant ($P < 0.05$) reduction in glucose concentration.

Mean values of the three enzymes investigated were significantly ($P < 0.05$) different. The observed trend was an increase in activity for alanine transaminase and aspartate transaminase with increase in NK concentration in swine diets, and a marked ($P < 0.05$) depression in alkaline phosphatase activity at 150 g/kg NK diet.

DISCUSSION

Serum proteins

The significant depression in total protein, albumin and globulin observed from growing male pigs fed NK diets was not unusual, since it corroborates the findings of several researchers on utilization of neem products by livestock. Gangopadhyay (1981) and Sokunbi *et al.* (2002) observed depressions in plasma and serum protein concentrations in Murah Milch buffaloes on replacement of concentrate mixture with 15 and 20 parts of neem seed cake and female growing pigs on replacement of wheat bran with 5, 7.5 and 10 parts of neem leaf meal respectively. Verma *et al.* (1995) also reported similar observations in growing goats on replacement of concentrate mixture with 15 and 25 parts of water washed neem seed kernel cake. Annongu *et al.* (2003) in their comparative study of raw and treated neem kernel meal observed significant depression in total serum protein and albumin values from pigs on raw neem kernel meal when compared to those on treated neem kernel meal and standard diets. Reduced total protein or albumin manifests an alteration in normal systemic protein metabolism, attributed in part to possible interference in protein utilization. Previous reports of Rajagopal and Nath (1981) and Sadre *et al.* (1984) had indicated that the chemical compounds in neem products might be the major factors affecting protein utilization in feed offered animals. Raw neem kernel contains trypsin inhibitors and its

trypsin inhibiting activity is 15 units/mg protein (Rajagopal and Nath, 1981). The depression in serum protein in this study cannot be said to be severe when one looks at the albumin/globulin values, especially for pre-pubertal boars where differences in values were non-significant.

Chemical components

The significant increase in cholesterol concentration observed from the experimental pigs could be as a consequence of impaired lipid metabolism by pigs on NK. The significant increase indicated for young boars on neem diets is difficult to explain with the present scope of investigation. Creatinine normally decreases in certain pathological conditions like muscular dystrophy and anaemia (Livingson and MacFate, 1969), and increases in conditions associated with tissue catabolism (Oser, 1965) probably when animals are in stress or on insufficient dietary energy for maintaining normal physiological processes.

A significant reduction in blood glucose level was observed from the experimental pigs due to the incorporation of NK in their diets. Neem products are known for their serum glucose reducing effect (Mitra, 1963). This effect was observed by Tyagi (1989) with water washed neem seed kernel (WWNSK) in buffalo calves, Garg (1989) with de-oiled neem seed kernel cake in calves, and Verma *et al*. (1995) with WWNSK in goats. However, Anandan *et al*. (1996) reported a non-occurrence of serum glucose reducing effect of neem in their study of goats fed ammoniated neem seed kernel meal. However, they suggested that, this might be due to the fact that the serum glucose reducing factors present in neem seeds were probably inactivated during urea ammoniation. The increase in urea nitrogen correlates strongly with the inclusion level of NK in swine diets. Verma *et al*. (1995) reported significant differences in urea nitrogen concentration of goats on water washed neem seed kernel leading to reduced concentrations as the level of WWNSK increased in the diet. In this study, the reverse is the case. Inclusion of NK led to increased concentration of urea nitrogen at 100 g/kg WNK diet. Annongu *et al.* (2003) also reported an increase in concentration of urea nitrogen on diets containing raw neem kernel meal relative to the treated meal or standard diet. Sathyamoorthy *et al*. (1981) gave similar observation from rats offered raw green gram and green gram trypsin inhibitors based diets. They reported increases in metabolic wastes and argued that the increased activities of amino acids degrading enzymes, arginase and ornithin-trans-carbamoylase are responsible for elevated levels of blood metabolites. Elevated level of blood urea for instance, is an index of high ammonia production, which is extremely toxic, especially in monogastrics. Increased blood metabolic wastes observed could be explained as the consequence of high level of phytotoxins in untreated neem products, which reduced protein utilization while increasing the catabolism of amino acids that are subsequently degraded into these metabolites (Annongu *et al*., 2003).

Potassium and calcium concentrations were significantly increased in response to NK's incorporation into swine diets while phosphorous concentrations were significantly reduced. Sokunbi and Egbunike (2000) reported similarities among diets for most chemical components of rabbits on replacement of wheat bran with 5, 7.5 and 10 parts of neem leaf meal except for potassium and phosphorous. However, it is important to note that serum potassium concentration is not an accurate indicator of the true status of potassium balance. This can only be confirmed through the analysis of intracellular potassium concentrations in muscle biopsy. In muscle, the proportion of potassium to nitrogen is 3 mmol to each gram. Storage of nitrogen as muscle protein therefore demands additional potassium. It has been suggested that a loss of 5 kg of muscle protein requires 600 mEq of potassium together with the protein nitrogen necessary for its replacement. It is thus safe to assume that the higher concentration of potassium observed in pigs on NK is a direct response to high concentration of urea nitrogen in the extracellular fluids. Ca^{2+} is absorbed by an active transport process occurring mainly in the upper small intestine. The process is regulated by 1, 25 – dihydroxycholecalciferol, a metabolite of vitamin D that is produced in the kidney in response to low plasma Ca^{2+} concentrations. Thus Ca^{2+} absorption is adjusted to body needs. Absorption is facilitated by vitamin D, lactose and protein. The more alkaline the intestinal contents, the less soluble the calcium salts. However, there was no evidence of metabolic disorder as a result of impaired urea synthesis such as ammonia intoxication. Clinical symptoms such as vomiting, irritability, lethargy and mental retardation were not evident.

Enzyme activity

Linear increases were observed for the transaminases while the reverse was observed for alkaline phosphatase. Sastry and Agrawal (1992) reported similarities in blood enzyme profile in pigs fed water washed neem kernel and those fed control diet. Anandan *et al*. (1996) and Verma *et al.* (1995) reported similar findings from goats fed different forms of processed whole neem kernel. However Sokunbi *et al*.

(2002) gave a different picture of blood enzyme profile of pigs fed neem leaf meal. Mean values observed were significantly different for alanine transaminase and aspartate transaminase with a general trend of reduction in values as neem leaf meal (NLM) inclusion increased. A similar trend was observed from rabbits fed NLM (Sokunbi and Egbunike, 2000). Annongu *et al*. (2003) reported significant reduction in alanine transaminase and alkaline phosphatase and total acid phosphatase while this study revealed opposite responses by experimental pigs. Alanine transaminase is essential in the metabolism and energy processes of cells. The increase in alanine transaminase activity observed in this study might be indicative of deviations in cellular metabolism and energy in the body of the experimental animals. Alkaline phosphatase is a membrane-associated enzyme present in most animal tissues. The organs with high alkaline phosphatase activity are those involved in active transport mechanism like the liver, kidney, heart and intestine (Kaplan, 1972). The presence of alkaline phosphatase in amounts determined by the body's physiological needs is essential for proper functioning of organs. However, very low or extremely high alkaline phosphatase activity can precipitate a threat to the proper functioning of body cells, which depend on phosphate esters for vital processes (Brain and Kay, 1927). The low alkaline phosphatase activity observed from pigs in this study might be due to the deleterious effects of phytotoxins in NK acting in concert with other anti-nutrients to produce adverse effects on the activity of enzymes in the body (Annongu *et al*., 2003).

CONCLUSION

It appears from this investigation that neem kernel can be included up to 100 g/kg in boars' diets without deleterious effects on their serum biochemical constituents.

CONFLICT OF INTEREST

There is no conflict of interest in the conduct and publication of this research work

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