Caecal Fermentation, Blood Biochemical Profile and Histopathological Changes in Broiler Rabbits Fed Graded Levels of Neem (*Azadirachta indica*) Seed Kernel Cake

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ABSTRACT : The expediency of promoting rabbit production on underutilized by-product feedstuffs prompted the current investigation of caecal fermentation pattern, blood biochemical profile and histopathological changes of vital organs in 48 broiler rabbits fed diets containing 0, 5, 10 and 20% level of neem seed kernal cake (NSKC) for 6 weeks and subsequently sacrificed. The NSKC incorporation in the diet did not exert any adverse effect on caecal fermentation although the weight of caecum and its contents was significantly ($p<0.01$; $p<0.05$) lower in rabbits fed the diet containing 20% NSKC. Except for blood glucose concentration, none of the blood biochemical constituents (serum urea nitrogen, creatinine, total protein, haemoglobin) and the activities of different enzymes (alanine aminotransferase, asparatate aminotransferase, alkaline phosphatase) varied significantly due to NSKC feeding. A histopathological examination of the vital organs (liver, kidney, heart, spleen, lungs, intestine and stomach) revealed a variable degree of villus atrophy in the intestine and degenerative changes in the liver and tubular epithelium of kidney in some rabbits when NSKC was fed at levels above 10%. (*Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 1 : 30-34*)

Key Words : Broiler Rabbits, Neem Seed Kernel Cake, Caecal Fermentation, Blood Traits, Histopathology

INTRODUCTION

Rabbit production is gaining momentum as an economical meat producer in tropical developing countries where there is an abundance of underutilized by-product feedstuffs. Nevertheless, improved feed formulations and strategies for enhancing the productive potential of broiler rabbits are not fully exploited, especially in tropical countries.

Such nutritional strategies will ensure greater productivity of rabbits fed non-conventional by-product feedstuffs and assist in matching rabbit production with domestically available feed ingredients. One such approach of potential benefit is dietary supplementation with raw neem (*Azadirachta indica*) seed kernel cake (NSKC), a by product of the neem oil industry. Supporting evidence for nutritional feasibility and benefits of dietary incorporation of raw NSKC up to 20% has been obtained from our studies with broiler rabbits (Vasanthakumar et al., 1999a, b). This study was carried out to investigate caecal fermantation pattern, blood biochemical profile and histomorphology of vital organs in rabbits fed diets containing different levels of raw NSKC as a substitute for a conventional protein supplement.

MATERIALS AND METHODS

Animals, feeds and feeding

Forty eight, 6 week old Soviet Chinchilla (SC, 24) and White Giant (WG, 24) rabbits were randomly allotted to four dietary treatments each having 6 SC and 6 WG rabbits of both sexes in equal number. They were kept in hygienic and uniform management conditions by housing them individually in clean metallic cages, fitted with feeders and waterers, located in a well ventilated cement floored shed. The rabbits were dewormed with Mebex (Mebendazole IP 40 mg/g, M/S. Cipla Ltd., Bangalore, India) fortnightly at the dose rate of 15 mg/kg body weight.

The rabbits were fed *ad libitum* from 6 to 12 weeks of age iso-nitrogenous and iso-caloric composite diets [25 parts ground maize (*Zea mays*) hay, 75 parts concentrate] in mash form as per NRC (1977) requirements for growing rabbits. The ingredient composition of concentrate and chemical composition of diets are shown in table 1. The experimental diets contained graded levels (0, 5, 10, 20% in the diet) of raw NSKC (%DM; organic matter, 84.3; crude protein, 33.7; ether extract, 8.6; crude fibre, 13.6; nitrogen free extract, 28.4; ash, 15.7) in place of 0, 25, 50 and 100% of de-oiled groundnut cake (DGNC). Weighed quantities of feed were offered daily so as to allow *ad libitum* intake. Feed residues were weighed 24 h post-feeding to ascertain daily feed intake. Feeds offered and the residues were analysed for DM at weekly intervals to assess average DM consumption during the experimental period.

Representative samples of composite feed and raw NSKC were subjected to analysis of proximate principles (AOAC, 1980), fibre fractions (Goering and Van Soest, 1970) and gross energy using a Gallenkemp ballistic bomb calorimeter. The rabbits

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Table 1. Ingredient and chemical composition of diets

Ingredient/constituent	% Dietary level of NSKC			
	0	5	10	20
Ingredients, % air dry feed				
Maize hay (ground)	25	25	25	23
Yellow maize	35	35	23	35
Deoiled groundnut cake	18	13.5	9	0
Neem seed kernel cake	0	5	10	20
Meat meal	8	8.5	8.5	9.5
Wheat bran	5.5	4	3	0
Molasses	5	5	5	5
Tallow	1.0	1.5	2.0	3.0
Mineral mixture	2.0	2.0	2.0	2.0
Sea salt	0.5	0.5	0.5	0.5
Chemical composition, % dry matter				
Organic matter	89.3	89.2	88.8	87.5
Crude protein	16.5	16.2	16.5	16.4
Ether extract	5.3	5.7	6.7	8.7
Crude fibre	10.7	10.3	10.7	12.0
Nitrogen free extract	56.8	57.1	54.9	50.5
Ash	10.7	10.8	11.1	12.5
Neutral detergent fibre	43.7	43.6	44.1	43.2
Acid detergent fibre	16.6	15.6	16.1	17.1
Gross energy (kcal/g DM)	4.5	4.4	4.3	4.5

* Added 30 g vitabled (M/s Glaxo, Bombay) containing 50,000 IU and 5,000 IU of vitamin A and D_3 per g respectively, 50 g Tetracycline HCl (50 μg/g) (M/s Hoechst India Ltd., Bombay) and 50 g Duocoxin (Sulpha-Quinoxaline, 16.67%; Amprolium HCl, 16.67%) as Coccidiostat (M/s Dynamic Pharmaceutical Pvt. Ltd., Bombay).

were weighed individually at weekly intervals before feeding and watering. At the end of 6 weeks experimental feeding, the rabbits were slaughtered, after starving them for 12 h, by the Halal method (Ramayyan et al., 1980) for carcass characteristics (Vasanthakumar et al., 1999b), caecal fermentation pattern including weight of caecum and collection of its contents, blood and pathomorphological studies.

Caecal fermentation pattern

The caecum and its contents collected at the time of slaughter from each rabbit were weighed. The pH of uniformly mixed semi-solid caecal contents was measured by digital pH meter (pH 5651, ECIL, Hyderabad) immediately after collections and stored at -20℃ for further analysis. Caecal liquor was obtained by diluting 3 g of caecal contents with 9 ml of distilled water followed by thorough mixing and centrifugation at 3000 rpm for 10 min. Aliquots of caecal liquor were drawn for the estimation of total volatile fatty acids (Barnett and Reid, 1956) and ammonia nitrogen (Pearson and Smith, 1943).

Blood biochemical profile

Serum was separated from about 10 ml blood of each rabbit collected from the jugular vein at the time of slaughter in sterile glass tubes and stored at -20℃. Another 1 ml blood sample was collected in tubes containing ethylene diaminetetracetate at 1 mg/ml blood and sodium fluoride at 10 mg/ml blood as anticoagulants for glucose estimation (Cooper and McDaniel, 1970). The haemoglobin was estimated immediately after the collection of blood as per Sahli's acid haematin (Oser, 1971). The activity of various serum enzymes and alkaline phosphatase (King and Armstrong, 1934), alanine aminotransferase and aspartate aminotransferase (Reitman and Frankel, 1957), total protein (Hiller and Van, 1927), urea nitrogen (Rahmatullah and Boyde, 1980) and creatinine (Wootton, 1964) were estimated as per standard colorimetric methods using reagent kits supplied by M/S. Qualigens Fine Chemicals (a division of Glaxo India Ltd.) with the help of Systronic Spectrophotometer 106.

Histo-pathological studies

The vital organs (kidney, liver, spleen, heart, lungs, intestine and stomach) and carcasses of all rabbits were examined for gross pathological changes at the time of slaughter. The representative tissues of all the organs preserved in 10% formalin were microtoned after fixing in paraffin wax. The sections were stained with haematoxylin and eosin stain (Culling, 1963) and examined for histopathological changes at the Division of Veterinary Pathology, IVRI, Izatnagar.

The experimental data were subjected to analysis of variance between diets and breeds ignoring sex effect as per the methods of Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Caecal fermentation pattern

The concentration of caecal fermentation products including weight of caecum and its contents are given in table 2. The body weight changes, average daily gains and dry matter intakes of broiler rabbits between 6 and 12 weeks of age on different diets did not vary significantly. However, at the end of 6 weeks feeding, the weight of caecum and its contents were significantly ($p<0.01$; $p<0.05$) lower in rabbits on 20% NSKC diet, as compared with the highest weights found with the diets containing deoiled groundnut cake. In general, rabbits are not efficient utilizers of fibre because of rapid passage of digesta (Nordiobaldissera, 1980). This may als be the reason for comparatively lower values of caecal contents on

Table 2. Caecal fermentation pattern in rabbits

Attribute	% Dietary level of NSKC				
	0	5	10	20	SEM
Initial body weight (g)	937.3	306.4	904.5	901.0	67.7
Pre-slaughter body weight (g)	1357.3	1380.0	1386.4	1226.4	87.8
Average daily gain (g)	10.0	11.3	11.5	8.1	8.2
Dry matter intake (g/kgW$^{0.75}$)	42.3	49.7	50.4	49.9	3.4
Caecal weight (g)	94.6^{a}	84.2^{a}	88.5^{ab}	81.5^{b}	4.1**
Caecal content (g)	58.0^{a}	53.7^{ab}	48.5^{bc}	44.0^{c}	3.6*
pH	6.23	6.21	6.30	6.26	0.03
Total volatile fatty acids (m Eq/g)	0.036	0.037	0.035	0.035	0.02
Ammonia nitrogen (μmol/g)	45.0	44.4	44.2	44.1	1.1

^{a,b,c} Means with different superscripts in a row differ significantly. * $p<0.05$, ** $p<0.01$.

20% NSKC diet. However, the pH and concentrations of total volatile fatty acids and ammonia nitrogen were similar in the caecal liquor of rabbits fed control and NSKC supplemented composite diets (table 2). Similar but non-significant differences were observed between breeds in body weight changes, dry matter intake and caecal fermentation pattern.

The normal pH of caecal content, averaging 6.5 (Vernay et al., 1984), is influenced by total volatile fatty acids and ammonia nitrogen levels which in turn are dictated by feed composition, time of feeding and caecotrophy (Santoma et al., 1989). The observed volatile fatty acids and ammonia nitrogen levels during the experiment were close to normal (Deshmukh, 1989; Gowda, 1994) and indicate that inclusion of NSKC in the diet up to 20% level did not exert any adverse effect on caecal fermentation pattern.

Blood biochemical profile

For indirect nutritional assessment, an examination of the haematological indices (table 3) was conducted. Feeding of graded levels of NSKC up to 20% did not influence total serum protein, haemoglobin, serum urea nitrogen, creatinine and three clinically important enzymes viz. alkaline phosphatase (ALP, EC 3.1.3.1), alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1). Irrespective of diet, blood biochemical constituents did not differ significantly between White Giant and Soviet Chinchilla rabbits, and diets × breed interactions were also non-significant. Total protein and haemoglobin concentrations showed that the health status of the animals was normal. Serum protein and haemoglobin tend to be positively correlated with protein quality and level (Eggum, 1989; Onifade and Abu, 1998), thus there appears to be no adverse influence on feed quality owing to NSKC addition to the rabbits' diet. Serum urea nitrogen and creatinine are commonly used to indicate level of renal function and possible damage to kidney architecture (Slunnil, 1974). Comparable serum urea nitrogen and creatinine levels between rabbits on control and experimental diets indicate that activity of kidneys was normal between 6-12 weeks of age. The transaminase (ALT/AST) activity, though, varies with age and productive function (Kaneko, 1989), and its activity rises faster in hepato-cellular disorders (Pensent, 1983). The present findings do not indicate any toxic effects of NSKC on liver or muscle cells. Serum alkaline phosphatase level is an important indicator of bone formation and tissue disorders (Walmsley and White, 1994; Rosenthal, 1997). Comparable values of serum alkaline phosphatase,

Table 3. Blood biochemical profile of rabbits

Attribute	% Dietary level of NSKC				
	0	5	10	20	SEM
Alkaline phosphatase (KA units ml^{-1})	4.54	4.63	4.64	4.44	0.15
Alanine aminotransferase (IU ml^{-1})	31.65	31.49	33.16	33.20	0.90
Aspartate aminotransferase (IU ml^{-1})	27.64	27.69	27.65	28.37	0.35
Serum urea nitrogen (mg dl^{-1})	13.51	13.34	13.55	13.72	0.56
Creatinine (mg dl^{-1})	0.86	1.00	0.90	0.92	0.07
Total protein (g dl^{-1})	8.25	7.96	7.83	7.34	0.20
Blood glucose (mg dl^{-1})	90.63^{a}	85.09^{ab}	85.08^{ab}	82.75^{b}	0.49
Haemoglobin (g dl^{-1})	9.97	10.09	10.11	10.04	0.29

^{a,b} Means with different superscripts in a row differ significantly ($p<0.05$).

irrespective of diet, indicated no adverse effects of dietary supplementation of raw NSKC.

Blood glucose concentration in rabbits was significantly ($p<0.05$) depressed at 20% level of NSKC incorporation (table 3). Neem and its products are known to possess anti-hyperglycemic factors (Bhargava et al., 1985; Chattopadhyay et al., 1995). Studies of Chattopadhyay et al. (1995) showed that the alcoholic extract of neem leaves exerts this effect only when β -cells of the pancreas are normal by modifying the insulin turnover. The antihyperglycemic effect of diets with higher levels of NSKC incorporation partly corroborate these findings.

Histopathology

Most of the vital organs of the rabbits such as heart, lungs with trachea, liver, spleen and kidney did not show any significant gross pathological changes after feeding NSKC at different levels. The gastro-intestinal mucosa, however, was slightly swollen and covered with an appreciable amount of mucus in 2 and 3 rabbits on diets containing 10 and 20% NSKC, respectively. Histopathological examination of livers and kidneys revealed a mild hepatic degeneration and variable degree of cortical tubular degeneration in rabbits fed with 10 and 20% NSKC. The intestine did not reveal any significant histological changes, but in a few animals a thinning of the mucosa with villus atrophy and increased goblet cell activity was noticed at some parts of the intestinal segments on 20% NSKC. Increased mucus secretary activity and mild degeneration of chief cells in the glandular portion of stomach was noticed in two animals fed with the 20% NSKC diet. The other organs, heart, lung and spleen, were normal in appearance.

Overall, it can be confirmed clinically that rabbits fed NSKC supplemented diets do not have a biochemically determinable disadvantage to their counterparts fed groundnut cake as a vegetable protein source. Clinical chemistry substantially demonstrated normal intermediary metabolism, hepatic function and bone specific mineralization. Histopathological examination of vital organs however, revealed mild discernible alterations in liver, kidney and intestine at 20% NSKC level. The rearing of rabbits beyond 12 weeks of age necessitates further long term studies using raw NSKC as a protein supplement in diets beyond the 10% level.

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