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Effect of feeding kulthi seeds (*Dolichos biflorus* L.) with high calcium low phosphorus diet on induced/reduced urolithiasis and kidney damage in cockerel

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Abstract
In the Unani and Ayurvedic system of medicine, Kulthi is prescribed to the patients suffering from urolithiasis. Kulthi is an important legume especially in tribal areas. Horse gram (*Dolichos biflorus* L.) locally known as Kulthi is extensively grown in peninsular India. It is claimed that ingestion of Kulthi cures urolithiasis. Studies were therefore, planned to induce urolithiasis experimentally in cockerel to observe the effect of feeding Kulthi on growth, feed intake, feed efficiency and levels of serum calcium, phosphorus and magnesium.

For the present study, 50 cockerels were divided into 5 groups of 10 each and fed 5 different diets *viz*. diet I- Control having Normal Calcium (1%) and Normal available Phosphorus (ap 0.5 %) - NCNP; II- High Calcium (3.25%) and Low available Phosphorus (0.3%) – HCLP (to induce urolithiasis); III- HCLP + 1% $NaHCO_3$ (Sodium Bicarbonate) - HCLP (B); IV- HCLP (B) + 1% NH_4Cl (Ammonium Chloride) – HCLP (A); V- HCLP + 1% $NaHCO_3$ + Kulthi seeds – HCLP(B) K. Diets were fed from 9 to 32 weeks of age.

During the experimental period, body weight, feed intake, feed efficiency was recorded. The levels of serum Ca, P & Mg were determined at monthly interval. X-ray and histopathological examination done at the end of the experiment. The results of study indicated that HCLP diet adversely affected growth, feed intake and feed efficiency. Incorporation of Kulthi in HCLP diets improved feed intake and gain in body weight. All the HCLP diets has no beneficial effect in preventing urolithiasis & kidney damage except HCLP diet with NH_4Cl – beneficial in preventing urolithiasis. The mineral levels were at par in all the groups. During the last week of experiment, a metabolic trial for 3 days was conducted and the retention of minerals was studied. It was at par in all the groups.

Keywords: Kulthi (Horse gram), High Calcium Low Phosphorus (HCLP), urolithiasis

Introduction
Horse gram (*Dolichos biflorus* L.) locally known as kulthi is extensively grown in peninsular India. Kulthi is important legume especially in tribal areas. In the Unani and Ayurveda system of medicine, kulthi or its extract is prescribed to the patients suffering from urolithiasis. It is claimed that ingestion of kulthi or its extract cures urolithiasis. Studies were therefore planned to induce urolithiasis experimentally in chicken to observe the effect of feeding kulthi on growth, feed intake feed efficiency and levels of serum calcium, phosphorus and magnesium.

Materials and Methods
For the present study, 50 cockerels were divided in to 5 groups of 10 each feed 5 different diets *viz* diet I-control having normal calcium (1%) and normal available phosphorus (ap 0.5%) – NCNP; II- high calcium (3.25%) and law ap (0.3%) – HCLP (to induce urolithiasis); III- HCLP + 1% $NaHCO_3$ (Sodium Bicarbonate) - HCLP (B); IV- HCLP + 1% NH_4Cl (Ammonium Chloride) – HCLP (A); V- HCLP + 1% $NaHCO_3$ + kulthi seeds – HCLP(B)K (Table-1). Diets were fed from 9 to 32 weeks of age.

During the experimental period, gain in body weight, feed intake and feed efficiency were recorded. The levels of mineral calcium, phosphorus and magnesium were determined at monthly interval. For this 5 ml blood was collected from wing vein at the start of experiment and thereafter every month.

Serum was separated and subjected to the estimation of calcium by Connerty and Briggs (1966) [2], phosphorus by Daly (1972) [3] and magnesium by Henry (1984) [5]. Calcium and phosphorus of feed and faeces were estimated as per A.O.A.C. (1980).

At the end of experiment, a metabolic trial of 3 days was conducted. From each treatment group 4 representative birds were selected. At the end of metabolic trial retention of calcium and phosphorus was worked out (AOAC, 1980).

Data were analyzed by analysis of variance technique given by Panse and Sukhatme (1967) [6].

Results and Discussion

The data on gain in body weight (g), feed intake and feed efficiency are presented in Table 2.

Perusal of Table 2 revealed that cumulative gain in body weight differed significantly ($p< 0.01$) from HCLP diet except HCLP (B) K with which it was at par. Feed intake differed significantly from HCLP ($p<0.01$) diets except HCLP (B) K with which it was at par. Feed efficiency was reduced in all the HCLP diets. All the HCLP diets adversely affected cumulative gain in weight. Incorporation of kulthi prevented the adverse effect of HCLP diets probably due to higher levels of methionine, cystine and lysine in these diets. Incorporation of kulthi in HCLP diet improved feed intake and thereby gain in body weight.

The data on serum calcium phosphorus and magnesium are presented in Table 3. Table 3 revealed that serum calcium phosphorus and magnesium were at par in all the HCLP diets.

Table 4 and 5 depict the average intake, excretion and retention (mg/c/d) of calcium and phosphorus, respectively. The calcium and phosphorus retention was at par in all HCLP diets. Percentage of calcium and phosphorus retention differed significantly ($p< 0.01$) between control and HCLP diets.

Although blood serum level of calcium of cockerels fed HCLP diets was similar to that of NCNP diet, the percent retention of calcium was significant ($p< 0.01$) but the absolute calcium retention was found to be non-significant. This might be the reason for serum calcium level which was at par with the NCNP diet. As far as phosphorus was concerned, with HCLP diet percent retention of phosphorus diet differ significantly from the NCNP diet but the differences in absolute phosphorus retention were significant ($p< 0.05$) and lower when compared with NCNP diet. Increased urinary calcium excretion and reduced inorganic phosphorus excretion was reported by Wideman *et al*., 1987 [7]; Glahn *et al*., 1988b [4] using 3% calcium and 0.6% ap. It has been suggested by Wideman *et al*., 1987 [7] that low phosphorus availability stimulates 1, 25 – dihydroxycholecalciferol formation causing increased intestinal phosphorus absorption and an unavoidable flux of calcium. As a result the parathyroid glands are inhibited triggering hypercalciuria and hypophosphaturia similar to that observed following surgical removal of parathyroid glands of birds fed normal calcium diets. Total kidney mass of HCLP and HCLP (B) group were significantly higher, when compared with the rest of the groups, and were at par amongst themselves.

Table 1: Composition of different diets

Ingredients	NCNP I	HCLP II	HCLP (B) III	HCLP (A) IV	HCLP (B) K V
Ground Maize	64.0	66.0	65.5	66.2	51.9
Wheat Bran	12.2	2.6	2.1	-	-
Groundnut cake (decorticated)	16.0	16.0	16.0	16.0	-
Kulthi seed (ground)	-	-	-	-	27.85
Fish meal	6.0	8.5	8.5	9.0	12.0
Groundnut oil	-	-	-	-	1.0
Mineral Mixture	0.4	0.4	0.4	0.4	0.4
Dicalcium phosphate	1.4	0.7	0.7	0.7	0.7
Ground lime stone	-	5.8	5.8	5.7	5.15
Sodium bicarbonate	-	-	1.0	1.0	1.0
Ammonium chloride	-	-	-	1.0	-
Kulthi Extract	-	-	-	-	-
Vitamin mixture (g)**	10	10	10	10	10
Aurofac (g)***	25	25	25	25	25
Coccidiostat (g)****	50	50	50	50	50
Calculated					
Crude Protein (%)	16.3	16.1	16.0	16.0	16.0
ME Kcal/Kg	2727	2736	2715	2720	2734
Calcium (%)	1.03	3.20	3.20	3.21	3.21
Phosphorus (%)	0.83	0.64	0.64	0.63	0.64
Available phosphorus (%)	0.50	0.32	0.32	0.31	0.32
Methionine (%)	0.29	0.32	0.32	0.33	0.63
Cystine (%)	0.22	0.21	0.21	0.21	0.72
Lysine (%)	0.66	0.71	0.71	0.72	2.79

Mineral Mixture* - Contained trace animals –

$ZnSO_4$, 25; $MnSO_4$, 25; $FeSO_4$, 20; $CuSo_4$, 15: and NaCl, 328 (g)

Vitamin mixture**- To 100 kg mash, 10(g) of vitamin mixture was added.

1. Meriplex (Merind Pvt. Ltd.) 5g/100 kg feed.
2. Merivite (Merind Pvt. Ltd.) 5g/100 kg feed. Each (g) of Meriplex contained vitamin B, B_6, B_{12}, E, niacin and calcium pentothenate, 40, 4, 8, 40 (I.U.), 60 and 40mg respectively. B_2 and D_3 40,000 I.U., 25mg and 5000 I.U. respectively.
3. Aurofac*** - Pfizer Pvt. Ltd.
4. Coccidiostat**** - Bifuran (Smith Kline & French Ltd.)

Table 2: Cumulative gain in body weight (g), feed intake (g) and feed efficiency of cockerels on different diets

Parameters	NCNP	HCLP	HCLP (B)	HCLP (A)	HCLP (B) K
	I	II	III	IV	V
Gain in body weight (g)	1180.6$^{a\pm}$35.44	986.3$^{b\pm}$41.40	1033.5^{b}±29.35	935.7^{b}±39.55	1093.1^{a}±49.47
Feed intake (g)	6730.3^{a}±84.54	5635.1^{b}±234.43	6046.2^{b}±89.23	5405.8^{b}±209.45	6196.2^{a}±430.63
Feed efficiency (Feed intake/ gain in body weight)	5.726^{ab}±0.11	5.721^{ab}±0.10	5.866^{a}±0.11	5.782^{a}±0.07	5.745^{a}±0.41

Figures bearing different superscripts differ significantly (< 0.01)

Table 3: Serum calcium, phosphorous and magnesium of cockerels on different diets (mg/100 ml)

Parameters	I	II	III	IV	V
Calcium	8.228±0.13	8.486±0.14	8.444±0.14	8.574±0.19	8.797±0.16
Phosphorus	3.861±0.11	3.694±0.11	3.571±0.11	3.671±0.11	3.712±0.14
Magnesium	2.298±0.12	2.355±0.13	2.324±0.15	2.048±0.09	2.351±0.14

Table 4: Average calcium intake, excreted and retained (mg/c/d)

Diets	Intake	Excreted	Retained	% Retained
NCNP	486.00±39.40	400.00±42.58	86.00±7.32	17.69^{a}±2.60
HCLP	1654.00±95.94	1541.00±100.36	113.00±8.50	6.83^{b}±0.75
HCLP (B)	1612.00±144.54	1494.0±141.83	118.00±9.54	7.32 ^{b}±0.81
HCLP (A)	1461.00±31.29	1339.00±38.99	122.00±9.38	8.35 ^{b}±0.75
HCLP (B) K	1556.00±57.40	1433.00±63.53	123.00±11.10	7.90 ^{b}±1.13

Figures bearing different superscripts differ significantly (< 0.01))

Table 5: Average phosphorus intake, excreted and retained (mg/c/d)

Diets	Intake	Excreted	Retained	% Retained
NCNP	279.00±20.10	208.00±11.50	71.00^{a}±9.70	25.40±1.81
HCLP	196.00±9.50	142.00±5.90	53.00^{b}±5.10	27.00±1.75
HCLP (B)	190.00±15.50	140.00±10.00	50.00^{b}±5.60	26.30±1.00
HCLP (A)	171.00±2.60	125.00±3.60	46.00^{b}±3.80	26.79±1.06
HCLP (B)K	186.00±6.10	136.00±3.70	50.00^{b}±4.30	26.99^{b}±1.09

Figures bearing different superscripts differ significantly (< 0.01)

Table 6: Average water intake (g)/DM (g) of different diets during metabolic trial

Diets	DM Intake (g)	Water ingested			Water intake (g) / Feed DM (g)
		Drinking water (g)	Water in feed (g)	Total (g)	
NCNP	59.02±4.52	147.0	3.3	150.3	2.54
HCLP	64.82±3.61	131.2	3.6	134.6	2.07
HCLP (B)	63.27±5.71	143.7	3.0	146.7	2.31
HCLP (A)	57.66±1.22	150.8	2.6	153.4	2.66
HCLP (B)K	60.95±2.07	155.5	2.7	158.2	2.59

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