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Effect of feeding kulthi (*Dolichos biflorus* L.) extract on induced avian urolithiasis and kidney damage in cockerel

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Abstract
The present study was planned to prove the hypothesis that kulthi or its extract cures urolithiasis (as mentioned in Unani and Ayurvedic system of medicine).
For the studies, 100 day old male WLH chicks reared upto 60 days on a standard chick mash diet. The chicks were duly vaccinated: (Ranikhet F1 strain-7th day; Infectious bursal disease – 14th day; Ranikhet F1 strain (repeat) – 28th day; Ranikhet (R_2B strain) – 42nd day). The debeaking was done during the 7th week of age. After that 50 cockerels were selected and divided into five dietary treatment groups, randomly. Each group had 10 cockerels and they were housed individually. The five groups were: I- (Control) NCNP - Normal calcium (1%), normal phosphorus (0.6%); II- HCLP- High calcium (3.25%), low phosphorus (0.4%); III- HCLP+1% $NaHCO_3$; IV – HCLP + 1% $NaHCO_3$+ Kulthi extract; V – HCLP + 1% $NaHCO_3$ + Kulthi extract in double amount. The diets were fed from 9 weeks of age for 5 months. Body weight and feed intake were recorded during the experimental period. At monthly interval, blood serum calcium, phosphorus and magnesium estimated. A metabolic trial was conducted at the end of experiment and cockerels were sacrified for X-ray examination of kidneys, gross and histopathology.
The result of study indicated that urolithiasis and kidney damage was induced by HCLP diet (Group II). The gain in body weight and feed intake were not affected by the diet containing kulthi extract but the same could not prevent urolithiasis and kidney damage. The use of double the amount of kulthi extract affected liver adversely indicating liver damaging effect.

Keywords: kulthi, urolithiasis, kidney, HCLP (High calcium low phosphorus)

Introduction
Horse gram (*Dolichos biflorus* L.) locally known as kulthi is extensively grown in penninsular India. Kulthi is an important legume especially in tribal areas. In the Unani and Ayurvedic 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 extract 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 + 1% $NaHCO_3$ + kulthi extract - HCLP(B) KEx and V- HCLP + 1% $NaHCO_3$ + Double the quantity of kulthi extract - HCLP(B) KEx^2. Each bird was housed individually in "grower's cage". The cages were provided with individually feeder and waterer. Diets were fed from 9 to 32 weeks of age. During the experimental period, body weight, feed intake, feed efficiency and levels of serum Ca, P & Mg were determined. The results of study indicated that HCLP diet adversely affected growth, feed intake and feed efficiency but reduced significantly with kulthi extract. 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.

Materials & 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 (ap0.5%) – 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% $NaHCO_3$ + kulthi extract - HCLP(B)KEx and V- HCLP + 1% $NaHCO_3$ + Double the quantity of kulthi extract - HCLP(B)KEx^2 (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) [1].

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) [1].

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) KEx and HCLP (B) KEx^2 with which it was at par. Feed intake differed significantly from HCLP (P<0.01) diets except HCLP (B) KEx^2 with which it was at par. Feed efficiency was reduced in HCLP diets except the HCLP diet supplemented with Kulthi extract HCLP (B) KEx and HCLP (B) KEx^2. All the HCLP diets adversely affected cumulative gain in weight. Incorporation of kulthi extract prevented the adverse effect of HCLP diets probably due to higher levels of methionine, cystine and lysine in these diets.

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 were at par with the NCNP diet. As far as phosphorus was concerned, with HCLP diet per cent 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; Glahn *et al*., 1988b using 3% calcium and 0.6% ap. It has been suggested by Wideman *et al*., 1987 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.

Table 1: Composition of different diets

	Diets				
	NCNP I	**HCLP II**	**HCLP (B) III**	**HCLP(B) KEx IV**	**HCLP (B) KEx^2 V**
Ground Maize	64.0	66.0	65.5	65.5	65.5
Wheat Bran	12.2	2.6	2.1	2.1	2.1
Groundnut cake (decorticated)	16.0	16.0	16.0	16.0	16.0
Fish meal	6.0	8.5	8.5	8.5	8.5
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	5.8	5.8
Sodium bicarbonate	-	-	1.0	1.0	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	2715	2715
Calcium (%)	1.03	3.20	3.20	3.20	3.20
Phosphorus (%)	0.83	0.64	0.64	0.64	0.64
Available phosphorus (%)	0.50	0.32	0.32	0.32	0.32
Methionine (%)	0.29	0.32	0.32	0.32	0.32
Cystine (%)	0.22	0.21	0.21	0.21	0.21
Lysine (%)	0.66	0.71	0.71	0.71	0.71

Mineral- Contained trace animals –

Mixture* $ZnSo_4$, 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.

a) Meriplex (Merind Pvt. Ltd.) 5g/100 kg feed.

b) 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.

Aurofac*** - Pfizer Pvt. Ltd.

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.

	Diets				
	NCNP	HCLP	HCLP (B)	HCLP (B) KEx	HCLP (B) KEx^2
	I	II	III	IV	V
Gain in body weight (g)	1180.6^a ±35.44	986.3^b ±41.40	1033.5^b ±29.35	1055.5^{ab} ±30.68	1100.7^a ±23.42
Feed intake (g)	6730.3^a±84.54	5635.1^b±234.43	6046.2^b±89.23	5935.4^b±185.64	6068.8^{ab}±141.53
Feed efficiency (Feed intake/ gain in body weight)	5.726^{ab}±0.11	5.721^{ab} ±0.10	5.866^a ±0.11	5.617^b ±0.07	5.514^b ±0.09

Figures bearing different superscripts differ significantly (< 0.01)

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

	Diets				
	I	II	III	V	VI
Calcium	8.228 ± 0.13	8.486 ± 0.14	8.444 ± 0.14	8.419 ± 0.13	8.316 ± 0.19
Phosphorus	3.861 ± 0.11	3.694 ± 0.11	3.571 ± 0.11	3.604 ± 1.13	3.537 ± 0.09
Magnesium	2.298 ± 0.12	2.355 ± 0.13	2.324 ± 0.15	2.180 ± 0.13	2.164 ± 0.16

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 (B)KEx	1702.00±108.31	1596.00±106.20	106.00±5.96	6.22^b±0.41
HCLP (B) KEx^2	1525.00±132.48	1410.00±140.22	115.00±12.60	7.54^b±1.43

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 (B) KEx	204.00±6.10	148.00±6.80	56.00^b±5.70	27.40±1.64
HCLP (B) KEx^2	185.00±14.80	135.00±11.20	50.00^b±3.60	27.18±0.68

Figures bearing different superscripts differ significantly (< 0.01)

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