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Association Between Serum Biochemistry of Leghorn Chickens and Changes in Renal Tissues Induced by High Calcium and High Urea Diets

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Abstract: An experiment consisted of three groups of hens that were given basal diet only and/or supplemented with either high calcium or high urea was designed to assess the correlation between serum constituents and the severity of damage to the kidney's tissues. At weeks 6, 12, 18, 24 and 30 of age, six birds from each group were sacrificed, histology of kidney tissues was examined microscopically and the abnormal changes in tissue were recorded to each bird and scored. Collected blood from 6 birds of each group at weeks 6, 12, 18, 24 and 30 of age was used to estimate serum levels of uric acid, phosphorous, magnesium, calcium, urea and creatinine. The outcome of the study revealed that severity score of kidney tissues was positively correlated with serum levels of uric acid ($r = 0.992$, $p<0.01$) and calcium ($r = 0.914$, $p<0.01$) in the group that was fed high calcium diet. It was also correlated with uric acid ($r = 0.994$, $p<0.01$) and calcium ($r = 0.881$, $p<0.05$) in group that was fed high urea diet. However, levels of serum urea, creatinine, phosphorous or manganese were not correlated ($p>0.05$) with severity score.

Key words: Correlation, calcium, uric acid, serum biochemical analysis, kidney

INTRODUCTION

High calcium diet during rearing periods and increase nitrogen intake in the form of urea; lead to degenerative changes in various tissues, nephritis and the induction of uroliths in chickens[1,2].

Differences of biochemical analysis in chicken's sera with nephritis induced by diets that are high in calcium or containing urea have been documented[1]. However, the association between the serum biochemistry and nephropathic changes has not been investigated. This study was carried out to assess the correlation between serum constituents and the severity of damage to the kidney's tissues as a result of feeding high calcium and high urea diets to chicken.

MATERIALS AND METHODS

Birds: A total of 162, day old White leghorn chicks were obtained from local hatchery (Al-Ahsa, Saudi Arabia) and placed in floor pen houses; at the Agricultural and Veterinary Training and Research Station affiliated to King Faisal University, Al-Ahsa, Saudi Arabia. Strict sanitation practices were employed to the house before and during the course of the study.

The chicks were allocated at random into three groups. Fifty four chickens were assigned at random to each treatment in three replicates and kept in three cages (eighteen chickens per cage) until sixteen weeks of age. After which chickens were transferred to layer-open-sided house. During the laying period, each experimental group were divided into nine cages, each cage contained two chickens. Temperature and lighting cycles of the house were maintained as described by North[3] and vaccination program applied based on layer raisers' recommendations at the area of the study.

Experimental diets: Birds in group 1 were fed the basal diet only[4]. Birds in groups 2, and 3 were fed the basal diet that was supplemented with 3.5% calcium and 2.5% urea, respectively. The chicks were kept on the starter diet containing 21% crude protein and 2900 Kcal kg^{-1} Metabolizable Energy (ME) till 4 weeks of age when they were switched to the commercial grower diet for 14 weeks. Pre-laying and laying diets were provided between 15-18 and 19-40 weeks of age, respectively. Food and water was given *ad libitum* throughout the experimental period.

Blood analysis: Blood was drawn from the brachial vein from two chickens in each replicate of each group at 6, 12, 18, 24 and 30 weeks of age and sera were separated and stored at -20°C. Sera were used to estimate serum levels (mg dL^{-1}) of uric acid, phosphorous, magnesium, calcium, urea and creatinine according to methods described

elsewhere[5-10], respectively. All kits required for the estimation processes were obtained from Biömerieux (France).

Bird sampling and lesions' scoring: At weeks 6, 12, 18, 24 and 30 of age, six birds from each group were sacrificed and examined for lesions. Tissue samples of kidney subjected for histology investigation were fixed in 10% buffered neutral formalin, paraffin-embedded, sectioned at 5 μm and stained with Haematoxylin and Eosin (H and E).

Kidney lesion scoring: The sections were examined microscopically. Abnormal changes in kidney tissue were recorded to each sacrificed bird and scored according to the following scheme:

Observed score	Weighted severity	Description
-	0	No lesions
1+	1	Nephrosis
2+	2	Nephritis
3+	3	Gout
4+	4	Glumerulo-nephritis
5+	5	Glumerulo-sclerosis with tubular necrosis

Severity score was calculated according to the equation below:

$$\text{Severity score} = \eta \times \beta/\gamma$$

Where as:

η = Number of sacrificed birds in a group with pathological changes in kidney tissues.
β = Lesion score of each bird.
γ = Total number of sacrificed birds in the group.

Statistical analysis: Analysis of variance using General Linear Model (GLM) procedure in the PC-SAS®[11] was used to estimate the variations among the means serum constituents. Comparison of means in different groups was made by Duncan's multiple-range test[12]. $p<0.05$ was accepted as statistically significant. Correlation coefficient was employed to examine the association between means of serum constituents in different groups and the correspondence severity score.

RESULTS AND DISCUSSION

Total mortality rate in groups 1, 2 and 3 was 1.85, 51.85, and 14.82%, respectively throughout the experiment Fig. 1. Death amongst experimental chickens was most likely as a result of kidney atrophy, visceral gout and obstruction of ureters. Pathological investigations revealed that 90% of chickens autopsied in group 2 had

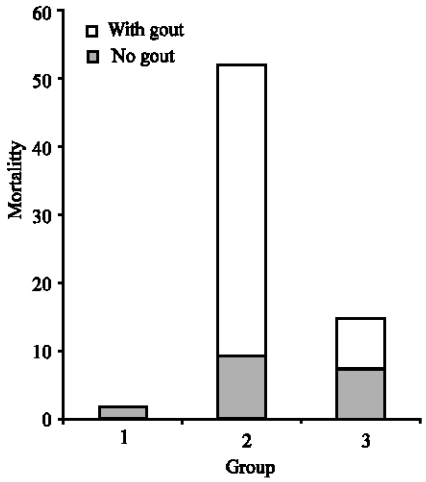

Fig. 1: Effect of high calcium and high urea diet on mortality rate among leghorn chickens

atrophied kidney and the majority had diffused deposition of visceral urates, ureters were distended and partially obstructed with calculi. On the other hand, 80% of birds in group 3 had kidney damage but were less severe whereas, controls remained free of pathological changes Table 1.

It has been documented that high dietary calcium: phosphorous ratios inhibit the secretion of parathyroid hormone and such inhibition directly leads to increase urinary calcium excretion (Hypercalciuria) and decreased urinary phosphorous execration (Hypophosphaturia)[13,14]. Furthermore, Lent and Wideman[15] revealed that excess calcium significantly reduced glomerular filtration rate, affected renal plasma flow and phosphorous excretion rates as well as significantly increased calcium excretion and urine pH. It is believed that mortality accompanied by visceral urate deposition occurs when the amount of unobstructed renal tissues fall below the functional mass necessary to excrete uric acid and other waste products[16,17]. These may explain the significant difference in mortality rate between the groups in this study.

Serum constituents of uric acid, creatinine and calcium were significantly ($p<0.05$) higher in groups 2 and 3 in comparison with contro Table 2 and serum uric acid as well as calcium levels in group 2 were increased with age of the birds and decreased at 30 weeks of age. This is probably due to the fact that level of calcium in the diet was sufficient to satisfy the requirement of egg production at that stage of age[13,18]. Correlation coefficient test indicated that the severity score of kidney tissues was positively correlated with serum levels of uric acid ($r = 0.992$, $p<0.01$) and calcium ($r = 0.914$, $p<0.01$) in group 2 as well as with uric acid ($r = 0.994$, $p<0.01$) and calcium

Table 1: Histopathological changes of kidney tissues of different groups and their scores

	Group					
	1		2		3	
Period (Weeks)	Examined No.	Lesion score	Examined No.	Lesion score	Examined No.	Lesion score
6	6	-	3	-	1	-
			2	2+	3	1+
			1	4+	2	2+
Severity score		0		1.33		1.17
12	6	-	6	3+	1	-
					2	1+
					3	2+
Severity score		0		3.00		1.33
18	6	-	1	3+	3	-
			5	4+	3	4+
Severity score		0		3.83		2.00
24	6	-	1	3+	4	2+
			1	4+	2	3+
			4	5+		
Severity score		0		4.50		2.33
30	6	-	6	4+	1	-
					2	1+
					3	3+
Severity score		0		4.00		1.83

Table 2: Correlation coefficient (r) between mean serum levels (mg dL^{-1}) of uric acid (Ua), urea (Ur), creatinine (Cr), calcium (Ca^{2+}), phosphorous (P^{2+}), magnesium (Mg^{2+}) and Severity Score (SS) of kidney's tissues

Groups and treatments								
		1	2	3		1	2	3
		Basal diet (BD)	BD+3.5% Ca^{2+}	BD +2.5% Urea		Basal diet	(BD)BD+3.5%	Ca^{2+}BD+2.5%Urea
Age (Week)		Mean±SD				Mean± SD		
6		2.41±0.44^{b}	4.23±0.35^{a}	3.15±0.72^{ab}		5.75±1.10^{a}	6.02±0.88^{a}	5.68 ± 0.91^{a}
12		2.50±0.23^{c}	6.96±0.95^{a}	4.50±0.80^{b}		5.35±0.93^{c}	13.22±2.15^{a}	6.39±0.14^{b}
18	Ua	5.00±0.79^{c}	9.55±1.50^{a}	7.11±0.78^{b}	Ca^{2+}	8.39±2.03^{b}	12.01±2.68^{a}	10.37±3.18^{ab}
24		4.40±0.25^{c}	10.62±1.13^{a}	8.54±0.81^{b}		17.9±2.15^{b}	20.62±2.13^{a}	18.39±6.21^{b}
30		4.25±0.48^{b}	9.71±1.35^{a}	6.23±1.28^{ab}		16.3±1.08^{a}	16.81±1.05^{a}	16.05±1.18^{a}
r	(with SS)	-	0.992**	0.994**		-	0.914*	0.881*
P		-	0.001	0.001		-	0.030	0.049
6		6.58±0.48^{b}	7.33±0.35^{b}	17.65±0.17^{a}		6.48±0.60^{a}	4.82±0.45^{b}	7.12±1.11^{a}
12		4.19±0.23^{b}	4.02±0.15^{b}	15.31±0.11^{a}		5.95±5.95^{a}	3.12±0.65^{c}	4.99±0.34^{b}
18	Ur	3.98±0.69^{b}	4.51±0.58^{b}	49.95±2.68^{a}	P^{2+}	6.09±0.53^{a}	3.01±0.88^{b}	6.17±1.28^{a}
24		8.47±0.95^{b}	8.62±2.13^{b}	29.57±3.81^{a}		9.13±1.15^{a}	8.62±1.18^{a}	8.49±2.21^{a}
30		8.59±1.98^{b}	8.51±1.95^{b}	19.92±3.38^{a}		6.39±1.09^{b}	7.61±0.95^{ab}	8.35±3.29^{a}
r	(with SS)	-	0.182	0.624		-	0.466	0.543
P		-	0.769	0.261		-	0.429	0.344
6		0.47±0.12^{b}	0.83±0.02^{a}	0.72±0.11^{a}		2.48±0.18^{a}	2.52±0.08^{a}	2.42±0.21^{a}
12		0.31±0.03^{b}	0.92±0.15^{a}	0.79±0.14^{b}		2.35±0.13^{a}	2.29±1.04^{a}	2.59±0.09^{a}
18	Cr	0.29±0.03^{b}	0.51±0.08^{a}	0.37±0.18^{b}	Mg^{2+}	2.79±0.29^{a}	2.86±0.48^{a}	3.47±0.48^{a}
24		0.53±0.15^{c}	9.62±1.13^{a}	6.19±1.21^{b}		6.53±1.17^{a}	6.52±0.93^{a}	6.39±1.40^{a}
30		0.39±0.08^{c}	8.71±0.85^{a}	3.45±0.88^{b}		6.39±1.58^{a}	5.81±1.09^{a}	6.75±1.27^{a}
r	(with SS)	-	0.669	0.714		-	0.715	0.752
P		-	0.217	0.175		-	0.175	0.143

Mean with different superscripts within the raw are significantly different ($p<0.05$). * Significantly correlated with severity score ($p<0.05$). ** Significantly correlated with severity score ($p<0.01$)

($r = 0.881$, $p<0.05$) in group 3 Table 2. However, levels of serum urea, creatinine, phosphorous or manganese were not correlated ($p>0.05$) with severity score.

Level of serum urea were significantly ($p<0.05$) higher in group 3 compared to other groups throughout Table 2. This finding is in accordance with those of Chandra *et al.*[19] and Mallinson *et al.*[20]. Serum phosphorus level significantly ($p<0.01$) increased in group 2 up to 24 weeks of age. These results are in agreement with Page *et al.*[21]. This is probably was due to the high phosphorus intake during the rearing period[22] or could be due to the role of phosphorus in egg shell formation[19,23].

No significant differences were observed in serum magnesium level between layers fed the different

experimental diet throughout table 2. These results are in agreement with[19].

Serum creatinine level was significantly increased ($p<0.05$) in birds of groups 2 and 3 compared to group 1 at weeks 24 and 30 of the experiment. These results coincided with the findings of Karasaw *et al.*[24] who attributed such changes to kidney dysfunction as a consequence to renal tubular damage and presence of micro-calculi in the tubules.

Serum magnesium significantly correlated with phosphorous ($r = 0.970$, $p<0.01$) in group 2 and with calcium ($r = 0.951$, $p<0.01$) in group 3. It seems that results in the current study support what was hypothesized by Garland[21] who stated that numerous interactions or interrelationships between the major mineral elements such as calcium, phosphorus and magnesium and who also indicated that severity of the lesions depends on the degree of the deficiency and the amount of other mineral present since excess of one will increase the severity of the deficiency of the other.

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