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Effect of Feeding Graded Levels of Urea on Growing New Zealand White Rabbit Performance

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Abstract: Forty New Zealand white rabbits of about 1340 g live weight were used to evaluate the influence of diet supplemented with urea at different levels on growth performance, blood cellular and biochemical parameters. The rabbits were assigned into four dietary treatments control, 3 urea-supplemented groups (0.5%, 1% and 1.5% urea) for two months, at the Agriculture and Veterinary Research Station, King Faisal University. Rabbits supplied with ration containing 1.5% urea had significant increase in body weight after 4 weeks than those fed 1% urea (1995 \pm 60 vs. 1829 \pm 53 g) and 6 weeks than the 0.5% group (3175 \pm 125 vs. 2235±50 g). Additionally, no significant trend was detected for the hemogram parameters, except for the RBC count of 0.5% urea group (6.48±0.21 x106) (P<0.05) and monocyte% for the 1% group (8.56±0.82x103) and 1.5% group $(4.99\pm1.38\times103)$ (P<0.05). there were significant differences (P<0.05) among treatment groups 1.5% (5.75±0.57 g/dl) and 1% (4.13±0.57 g/dl) for total protein, albumin. Serum levels of AST were significantly decreased in all experimental groups as well as control group ($25.96\pm2088 \mu/l$) except the 1.5% urea fed rabbits which showed 4 times fold the other groups ($94.45\pm36.67 \mu/l$). The blood urea increased with increase in level of urea level in diet. Serum calcium was significantly the least at 0.5% urea level (10.13±0.05 mg/dl) while the control group had the highest (16.33±0.32 mg/dl). In Conclusion, based on the results obtained, it appears that the inclusion of up to 1.5% urea in to the diets of growing rabbits has no adverse effect on growth performance, hematological parameters and serum biochemical indices.

Key words: Rabbit • Urea • Growth • Blood Cellular And Biochemical Parameters

INTRODUCTION

Rabbits are widely raised in developing countries, because of its low investment cost, high fecundity, short generation interval and ability to utilize non human feed (forages - monogastric herbivores) [1]. Nutritionally, rabbit meat is more desired than other livestock species because of its higher protein (20 -21%), low fat (10 - 11%), low calories (1749 Kcal/Kg), low cholesterol content (169 mg/100g on dry matter basis) [2, 3].

Rabbits are capable of utilizing urea as a non-protein nitrogenous source because of caecal fermentation due to the presence of urease activity [4, 5], similar to that of ruminants [6], transfer of blood urea to caecum [7] and cecotrophy (ingestion of cecal contents) [8]. Many researchers indicated that working with weanling rabbits

did not show significant effect on growth performance due to feeding urea supplements of low protein diets [9, 10].

Moreover, feeding on diet with urea supplement had been resulted in increased plasma glucose and urea nitrogen meanwhile total serum protein did not change significantly [11]. Also, the knowledge of rabbit hematological and serum biochemical reference values due to feeding urea supplements, cannot be over emphasized [3, 12]. Since there is some discrepancy in the effect of supplementing urea in rabbit diet and insufficient data concerning its effect on blood cellular elements, this research was postulated to assess the growth performance, blood cellular and biochemical parameters of growing New Zealand White rabbits supplemented with different levels of urea.

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MATERIALS AND METHODS

This research project (financially supported by Deanship of Scientific Research) was conducted to assess the growth performance, carcass and blood parameters of growing New Zealand White rabbit supplemented with different urea levels at the Agriculture and Veterinary Training and Research Station of King Faisal University, Kingdom of Saudi Arabia.

Rabbits and Procedures: Forty apparently healthy White New Zealand growing rabbits with average body weight (1340 g), obtained from lab animal center at king Faisal University Research Station, were allotted into 4 equal groups (10 rabbits each). The rabbits were treated with Ivomec to be free from internal and external parasites. Sulphadimidine powder also offered to rabbits as a safeguard against coccidiosis. Metal-wire bottomed cage allowing easy feces collection were used for keeping the experimental rabbits. Feed and water were provided ad libitum. All cages were placed in a semi - controlled environmental temperature in a suitable building. The building was well ventilated and electrically lightened (14:10 hours light to dark photoperiod) throughout the experiment. The diets were provided regularly at 9.0 o'clock a.m. daily.

Diet Preparation: Ingredients and chemical composition of the used experimental diets are presented in Table 1.

Ingredients of diets were finely ground by using hammer mill screen size 3.0 mm, then weighing of different ingredients at required amount for each experimental group and thoroughly mixed with the liquid portion (oil). The mixed feed ingredients blended with a good quality steam and the required molasses quantity were added at this stage of feed processing. The conditioned feed was passed through 3.5 mm holes then sun dried.

Experimental Design: Four diets were formulated to study the effect of different urea levels added to diets. The diets were formulated according NRC [13] to meet the requirements of the growing rabbits. The control (No. 1) had no added urea (Table 1). Diets 2 - 4 contained 0.5, 1.0 and 1.5% of urea respectively. Diets were supplemented with vitamin-mineral premix and molasses (as binder).

Data Collection: The rabbits were weighed individually biweekly and the live body weight (kg) change was taken as the measure of growth. Body weight gain (expressed in grams) was calculated. Average daily gain (ADG) was calculated as the difference between two successive weights divided by the time period (days).

Blood Samples: Two types of blood samples were obtained from each rabbit, being fastened overnight, before slaughtering through ear vein puncture.

 The first blood samples were obtained in vaccutainer tubes with EDTA as anticoagulant and were used for carrying out hemogram or complete blood count (CBC) by using the electronic cell counter (UDIHEM-UDI). These parameters included:

Total erythrocytic count (RBCs), Hemoglobin concentration (Hb), Packed cell volume (PCV- HCT), Total leucocytic count (WBCs), Differential leucocytic count (monocytes, lymphocytes, granulocytes) on a stained blood film using Giemsa stain [14].

• The second blood samples were obtained in plain vaccutainer tubes and used for obtaining serum for biochemical analysis of the selected parameters. These blood samples were allowed to clot in room temperature for 1-2 hours then centrifuged at 3000 rpm for 30 minutes. Only clear and non-hemolysed serum were obtained and kept frozen until used for biochemical analysis of the selected parameters [14], except for glucose level in serum that were determined as soon as we get serum samples.

The biochemical parameters of the blood sera samples included:

Calcium, Phosphorus, Magnesium, Total proteins, Albumin, Cholesterol, Triglycerides, Glucose, Blood urea nitrogen, creatinine, uric acid and Liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The concentrations of the selected biochemical parameters were measured calorimetrically with auto analyzer (Ellipse-UDI) machine, using commercially available test kits.

Statistical Analyses: Data were analyzed by the General Linear Model (GLM) procedure [15]. The Least Square Mean (LSM) \pm standard errors were calculated and tested for significance using the "t" test [16].

Growth Curve: Under the Gompertz function, the animal's relative growth rate is considered as a simple function of its current size [40]. The weights of each rabbit were assumed to follow the Gompertz model:

Gompertz Model: $W_t = A(EXP(-EXP(-B(-Ct))))$

where Wt is the weight at time t, A is the asymptotic live weight for Gompertz and Logistic models and indicates initial live weight (A0) for the Quadratic and Cubic models. Initial live weight is converted to asymptotic live weight (A) where A0 is the initial live weight; B and C are the model parameters which characterize the shape of the curve and were estimated from a nonlinear regression analysis using the NLIN procedure [15].

RESULTS

Growth Performance: The growth performance pattern of New Zealand White rabbit weights as a function of age in weeks; using standard Gompertz Model parameters is indicated in Figure (1).

Although there were no significant differences in rabbit weights due to feeding ration with different levels of urea after 2 weeks of experiment initiation, marked weight variation (P<0.05) was detected after 4 and 6 weeks of starting urea feeding.

Rabbits supplied with ration containing 1.5% urea had 166 g weight gain increase after 4 weeks than those fed 1% urea (1995±60 vs. 1829±53 g). The same trend was also continued at 6 weeks for the same group (1.5%) as its weight was significantly the heaviest 3175 ± 125 g while the 0.5% group was the least (2235±50 g).

On the other hand, the average daily gain (ADG) did not support the previous findings. Control group showed a significant increase in ADG at 2 - 4 weeks period (20.01 ± 1.72 g) compared with those of 0.5% and 1% urea groups (13.62 ± 1.36 and 13.98 and 13.98 ± 1.57 g). Similarly, the 0% urea fed group had almost doubled ADG at 6-8 weeks compared to the 1 and 1.5% groups (30.5 ± 5.54 vs. 16.12 ± 0.19 and 17.69 ± 3.25).

The previous finding was reversed for the ADG at 4 - 6 weeks period. The 1.5% rabbit urea group had the highest ADG (78.34 \pm 4.34) followed by the 1% group (68.67 \pm 00), while the 0.5% group was the least (26.5 \pm 3.17g) (P<0.05). In addition, the ADG of the control group was the maximum (26.75 \pm 2.5) and the 1% was the least (17.64 \pm 0.85) during the whole experimental period (0-8 weeks) (P<0.05).

Hematological (Blood Cellular Elements) Characters: The results of the hematological indices due to the effect of urea inoculation in rabbit feed at different levels are listed in Table 2. No significant trend was detected for the hemogram parameters, except for the RBC count of 0.5% urea group $(6.48\pm0.21 \times 10^6 \text{ and control group} (5.96\pm0.16\times10^6) (P<0.05)$ and monocyte% for the 1% group $(8.56\pm0.82\times10^3)$ and 1.5% group $(4.99\pm1.38\times10^3) (P<0.05)$.

The values for the RBC count ranged from $9.65\pm0.16 \times 10^6$ for control group to $6.48\pm0.21\times10^6$ for the 0.5% urea group, while the WBC values ranged from $7.25\pm0.8\times10^3$ for the control to $9.41\pm0.62\times10^3$ for the 1% urea group.

Moreover, monocyte% was $5.5\pm1\%$ for the control group to $8,56\pm0.82\%$ for the 1% urea fed group; neutrophil% was $43.25\pm3.33\%$ for the 1% urea group to $50.23\pm6.7\%$ for the control group; Lymphocyte% was $48.05\pm7.06\%$ for control and $58.46\pm4.91\%$ for the 1.5% urea group; The Packed Cell Volume (PCV) ranged from $37.97\pm1.21\%$ for the control group to $40.22\pm1.52\%$ for the 0.5% urea group; The hemoglobin content (Hb) was 11.06 ± 0.28 g/dl for control group and 11.63 ± 0.27 g/dl for the 0.5% urea fed group.

Serum Biochemical Analysis: The results of the serum biochemistry are presented in Table 3. Although there were significant differences (P<0.05) among treatment groups 1.5% (5.75 ± 0.57 g/dl) and 1% (4.13 ± 0.57 g/dl) for total protein, albumin showed no significant effect (P>0.05) among all treatment groups and control one. The values for albumin ranged from 3.08 ± 0.33 to 3.84 ± 0.14 g/dl.

Serum levels of AST were significantly decreased in all experimental groups as well as control group $(25.96\pm2088 \ \mu/l)$ except the 1.5% urea fed rabbits which showed 4 times fold the other groups $(94.45\pm36.67 \ \mu/l)$. whereas, serum levels of ALT did not show any significant variation by addition of urea, $(22.44\pm4.51 \ \mu/l)$ for 1% and 28.67±5.48 μ/l for 1.5%) compared to the control group (29.1±6.61 μ/l). Meanwhile, unexplainable the level of ALT for the 0.5% urea fed group was 55.72±30.37 μ/l , a finding that would be due to unknown reason.

The blood urea values ranged from (2.54 to 5.06 mmol/L). The lowest and highest values were in control and 1.5% (P<0.05). The blood urea level was increased with the increase in the level of urea in diet. The creatinine value ranged from 22.54 to 104.8 mmol/L, with the highest value in the control group and the lowest in the highest urea level (1.5%).

The blood glucose values ranged from 5.37 ± 1.84 (1% urea group) to 17.06 ±6.07 mmol/L (1.5% urea group) (P<0.05), while the control rabbit had 10.34 ±3.85 mmol/L. Serum total blood cholesterol values ranged from 2.12 ±0.43 mmol/L (control group) to 10.93 ±4.03 mmol/L (1.5% urea fed group).

Table 1. Ingredient composition	Table 1. Ingredient composition (76) of the asea diets feet to different factor groups						
Ingredients	1	2	3	4			
Berseem hay (BH)	35.65	35.65	35.65	35.65			
Wheat bran	17.5	17.5	17.5	17.5			
Barley	16.25	17.75	19.25	20.75			
Yellow corn	9	10	11	12			
Soybean meal (44%)	16	13.0	10.0	7.0			
Urea	0	0.5	1.0	1.5			
Palm oil	1.0	1.0	1.0	1.0			
Molasses ¹	3.0	3.0	3.0	3.0			
Limestone ²	0.25	0.25	0.25	0.25			
Dicalcium phosphate (DCP)3	0.25	0.25	0.25	0.25			
Sodium Bicarbonate	0.30	0.30	0.30	0.30			
Premix ⁴	0.25	0.25	0.25	0.25			
Salt	0.50	0.50	0.50	0.50			
DL-Methionine	0.05	0.05	0.05	0.05			
Moisture %	10.1	11.2	10.4	10.9			
Crude protein %	17.1	17.3	17.4	17.2			
Ether extract %	2.3	3.7	6.8	3.9			
Crude fiber %	13.4	13.1	13.2	13.3			
Total ash %	9.1	8.9	9.1	8.8			
NFE % ⁵	47.8	45.8	43.1	45.9			
Calcium %	1.09	1.06	1.04	1.07			
Phosphorus %	0.43	0.45	0.44	0.42			
DE (Kcal/kg) ⁶	2552	2633	2710	2633			

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Table 1: Ingredient composition (%) o	of the used diets fed to different rabbit groups
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1- Molasses: characterized by the following composition; Brix (81%), Sucrose (32.5%), reducing sugar (19%), Total sugar as invert (53.18%), Ash (16.8%), Protein (4%) and Density (1.4%). 2-Limestone: contain 34% calcium.

3- Di-calcium phosphate: contain 20% P and 25% calcium. 4- Premix: Muvco premix (Mineral and vitamin premix) each 2.5 Kg contain the following: vitamin A 12000000 IU, vitamin D₃ 200000 IU, vitamin E 10g, vitamin K₃ 2.5g, vitamin B₁ 1 g, vitamin B₂5 g, vitamin B₆ 1.5g, vitamin B ₁₂10g, pantothenic acid 10g, niacin 30g, folic acid 1g, choline chloride 500g, biotin 50 mg, iron 30mg, manganese 40 mg, zinc 45 mg, copper 3 g, cobalt 100mg, iodine 300 mg, selenium 100mg.

5- NFE calculated by difference = 100 - (moisture % + CP% + EE% + CF% + Ash%). 2- calculated according to NRC (1977). 6- DE of DSBT was calculated according to NRC (1977).

Table 2: The effect of different levels of urea on Hematological (Blood cellular elements) characters of rabbits (Means ± SE)

Blood Parameter	Urea Conc.	No	Means \pm SE	Blood Parameter	Means \pm SE
WBC X10 ³	0	10	7.25±0.80	RBC X10 ⁶	5.96±0.16ª
	0.5	10	7.57±1.01		6.48±0.21 ^b
	1.0	10	9.41±0.62		6.33±0.16
	1.5	10	7.08±1.17		6.04±0.12
Monocyte %	0	10	5.50±1.00	Neutrophil %	50.23±6.70
	0.5	10	6.73±1.37		44.32±4.77
	1.0	10	8.56±0.82ª		43.25±3.33
	1.5	10	4.99±1.38 ^b		47.95±3.30
HCT(PCV)	0	10	37.97±1.21	Lymphocyte %	48.05±7.06
	0.5	10	40.22±1.52		55.16±6.03
	1.0	10	38.27±1.14		48.25±3.63
	1.5	10	38.20±0.78		58.46±4.91
Hemoglobin g/dl	0	10	11.06±0.28		
	0.5	10	11.63±0.27		
	1.0	10	11.11±0.27		
	1.5	10	11.25±0.26		

^{a-c} different letters between treatments are significant (P<0.05)

HCT (PCV) % = Packed cell volume

TRAIT	Urea Conc.	No	Means \pm SE	TRAIT	$Means \pm SE$
Albumin g/dl	0	10	3.67±0.27	Total Protein g/dl	4.37±0.37ª
	0.5	10	3.68±0.35		4.50±0.52ª
	1.0	10	3.84±0.14		4.13±0.57 ^{ab}
	1.5	10	3.08±0.33		5.75±0.57 ^{ac}
ASTµ/l	0	10	25.96±2.88ª	ALT μ/l	29.10±6.61
	0.5	10	19.78±2.46 ^a		55.72±30.37
	1.0	10	21.16±1.88 ^a		22.44±4.51
	1.5	10	94.45±36.67 ^b		28.67±5.48
Blood UREA Nitrogen mmol/L	0	10	2.54±0.21ª	CREATININ µmol/L	104.8±44.2ª
	0.5	10	3.78±1.06 ^{ac}		27.23±15.4 ^{ac}
	1.0	10	2.87±0.314ª		41.99±19.3 ^{ac}
	1.5	10	5.06±1.01 ^{bc}		22.54±11.4 ^{bc}
Cholesterol mmol/L	0	10	2.12±0.43ª	GLUCOSE mmol/L	10.34±3.85ª
	0.5	10	2.23±0.63ª		11.57±3.11ª
	1.0	10	2.19±0.36ª		$5.37{\pm}1.84^{ab}$
	1.5	10	10.93±4.03 ^b		17.06±6.07 ^{ac}
Calcium mg/dl	0	10	16.33±0.32ª	Phosphorus mg/dl	4.56±0.86
	0.5	10	10.13±0.05 ^b		$2.80{\pm}0.07$
	1.0	10	15.60±0.34ª		3.93±0.46
	1.5	10	15.57±0.34ª		4.27±0.70

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Table 3: The effect of different levels of urea on Serum biochemical analysis of rabbits (Means± SE)

AST=Aspartate aminotransferase ALT=Alanine aminotransferase

^{a-c} different letters between treatments are significant (P<0.05)



Fig. 1: New Zealand White rabbit weights as a function of age in weeks: using standard Gompertz Model parameters

Serum calcium was significantly the least at 0.5% urea level (10.13 ± 0.05 mg/dl) while the control group had the highest (16.33 ± 0.32 mg/dl). The serum phosphorus values were 2.8 - 4.27 for the treated groups and 4.56 mg/dl for the control one (P>0.05).

DISCUSSION

Although there was a marked (P<0.05) increase in body weight mean values of rabbits fed diet supplemented with urea particularly at 1.5% level at 4 (1995 g) and 6 weeks (3175 g) of experimentation, the final body weight after 8 weeks did not show any significant variation due to urea supplement at different levels compared to control ones.

Moreover, the ADG did not support the body weight increase during the first half of experiment, but ADG at 4-6 week period was maximized for the 1.5% level (78.34g) followed by the 1% level (68.67 g) (P<0.05). The non favorable effect on growth performance due urea supplementation agree with the findings of Lebas and colin [9]. However, the findings of Yono *et al.* [17] and [18] support the significant differences in body weight and ADG between urea fed group and control group.

Monocytes% showed significant increase in rabbits fed 1% urea (8.56%), but no variation was detected (P>0.05) between control (5.5%) and other urea fed groups (4.99-6.73%). These values were close to the range reported by Al-Shami *et al.* [19] due to feeding urea as non protein nitrogenous substance to lambs. Both neutrophils (43.25 - 50.23%) and lymphocytes (48.05 - 55.16%) did not show any variation (P>0.05) due to urea supplementation, a finding agree with the ranges (38.83 - 57-04%) recorded by Al-Shami *et al.* [19].

There were no significant differences (P>0.05) among treatments for packed cell volume (PCV) (37.97 to 40.22%) was close to the range of 31 to 38% reported by Shah *et al.* [20] and Njidda and Isidahomen [21]. This suggests detoxification of urea processing was good enough as demonstrated in the normal PCV range of values observed for rabbits on diets containing urea. PCV is a blood toxicity reduction index and its abnormal level point to the presence of a toxic factor which has a drastic effect on blood formation [22].

The hemoglobin (Hb) also, did not increase with the increase in the levels of urea supplements with a normal value (11.06 g/d1) for the control and 11.11-11.63 g/dl for urea treatments. These values were within the range of 10.67 to 12.60g/dL recorded by Njidda *et al.* [23] and Njidda and Isidahomen [21]. These results showed that the experimental diets contained good quality proteins that met the rabbit nutritional requirements. It was reported by Adejumo [24] that hematological parameters especially PCV and Hb were positively correlated with the nutritional status of the animal.

The values of the WBC counts did not increase with the increased level of urea supplement in the rabbit diet (P<0.05) with 1% level having the highest value of 9.41 x 10³mm³ and control having a lower value of 7.25 x 10³/mm³. WBC counts were close to the range of 5 to 13 x 10³/mm³ reported by Njidda and Isidahomen and Hillyer [21, 25]. These findings shows that the rabbits remained healthy because the number of WBC were within the normal range being an indication of non allergic conditions, free parasitism or presence of foreign body in circulating system [26]. The RBC counts showed significant difference (P<0.05) between 0.5% urea level (6.48x10⁶) and control group (5.96x10⁶). The values were within the range 3.8 to 7.9 x 10⁶/mm³ reported by Anon [27].

There were significant differences (P<0.05) between 1.5% and 1% groups (5.75 vs. 4.13 g/dl) for total protein but albumin showed no significant effect (P>0.05) among treatment groups and control (3.08 - 3.84 g/dl). These values were close to the normal values of 4.1 to 4.2 g/dl reported by Njidda and Isidahomen [21] for albumin and 5.0 to 7.5g/dL for total proteins reported by Njidda and Isidahomen and Onifade and Tewe [21, 36]. The normal values for albumin and total protein obtained in this study indicate nutritional adequacy of the dietary proteins for rabbits as being indicated by last authors.

Serum levels of AST were significantly (P<0.05) decreased in all experimental groups (19.78 - 21.16 μ /l) and

control group (25.96 μ /l) in comparison with 1.5% urea level (94.45 μ /l), whereas, serum levels of ALT increased only by addition of 0.5% urea only (p<0.05), with a range value of 22.44 - 29.1 for other groups and control one. Serum levels of ALT and AST are usually used to diagnose hepatotoxicity [37]. The recorded values of the two enzymes suggest that no damage to the liver had occurred by addition of urea although they were higher than those reported by Abdel-Rahman *et al.* [38] who reported a significant decrease in AST due to feeding 1% urea (13.76 μ /l) compared to the control group (16.6 μ /l).

Blood urea nitrogen was 2.54 mmol/L in control and increased in rabbits fed urea (2.87-5.06 mmol/L), suggesting the effectiveness of processing methods and increase in activities of urea enzymes [39]. The blood urea values were lower than that reported by Anon [27], Ovuru et al. [28] 5.4 mmol/l [29], Amao et al. [30] (5.4-8.61mmol/l), but similar to that reported by Njidda et al. [23] and Njidda and Isidahomen [21] (2.50 to 5.80 mmol/L). The creatinine value ranged from 22.54 to 104.8 µmol/L, with the highest value in control and the lowest in 1.5% urea. No specific trend was detected due to urea feeding and the low values are unexplainable, however the control level are within the wide range has been previously recorded in rabbits (from 0.752 to 1.522 mg/dl) [28-32]. The change in creatinine level could be due to stress or generating insufficient dietary energy to maintain a normal physiological condition [19, 33].

The blood glucose value for control group was 10.34 mmol/L. There was a significant decrease in 1% urea group compared to 1.5% (5.37 vs. 17.06 mmol/L). The higher values observed in 1.5% may not pose any problem, as Flurharty and Leorch [34] reported that high energy did not cause any detrimental effects on the health of the animals, but rather it increase the growth rate in the tropics. The range of blood sugar level obtained for rabbits in this study are close recorded by Njidda and Isidahomen [21] (6.9 - 10.9 mmol/L).

Serum total blood cholesterol values ranged from 2.12 to 2.23 mmol/L for control and urea treated groups, except for 1.5% urea fed rabbit group (10.93 mmol/L). These values are less than the range of 3.8 to 8.00 mmol/L reported by Njidda *et al.* [23] and Njidda and Isidahomen [21]. The recored values of calcium (10.13-16.33 mg/dl) and phosphorus (2.8-4.56 mg/dl) are close to those found in rabbit by Gbore and Akele [35] and Al-Shami *et al.* [19] while feeding urea to Suakin lambs (Ca 10.8-10.95 mg/dl and ph 3.66-5.24 mg/dl).

CONCLUSIONS

Based on the results obtained, it appears that the inclusion of up to 1.5% urea in to the diets of growing rabbits has no adverse effect on growth performance, hematological parameters and serum biochemical indices.

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