Effect of housing system and dietary biotin supplementation on 2- Egg quality traits and some blood constituents

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ABSTRACT

The present study was aimed to investigate the effect of housing system and dietary *biotin* supplementation on productive and metabolic performance of Benha line chickens. A total number of 224 chickens 20 weeks old and mean of body weight (1742 ± 20.1), were equally divided into two groups in a factorial arrangement design (2x4x3). Pullets of the first group were housed in cages of laying battery (with artificial insemination) two birds were kept per cage of 48×40cm (960cm^2/bird). Pullets of the second group were kept on deep litter laying houses (with natural mating) in a density of 900cm^2/ bird. Chickens of each group were then subdivided into four sub groups each of 25 females and 3 males according to dietary biotin supplementation. Pullets of the 1st sub group were fed on basal layer diet and considered as control group, the 2nd, 3rd and 4th sub groups were fed on basal diet supplemented with biotin at a level of 100, 150 and 200µg/kg diet, respectively. The results obtained showed that, pullets housed in cages improved significantly relative weights of egg shell and albumin, indexes of egg shape, albumin and yolk, hatchability percentage and chick weight at hatch, it also decreased early, mid and late embryonic mortality, plasma total proteins, aspartate aminotransferase, creatinine and plasma uric acid. However pullets housed on deep litter improved yolk index, Haugh unit, fertility percentage, plasma levels of albumin, globulin and alanine aminotransferase. Biotin supplementation to layer diets at a level of 150 µg / kg diet increased relative weight of egg shell and albumin, yolk index, fertility and hatchability percentage and decreased early, mid and late embryonic mortality. Chick weight at hatch, egg shape index, relative weight of albumin and plasma level of albumin significantly increased by adding 100 µg biotin to layers diets. It could be concluded that biotin supplementation at a level of 150 and 100 µg/ kg in layer diet and reared in battery cages and on deep litter system, respectively seemed to be adequate to achieve the favorable results.

Key words: laying hens, housing system, biotin, Egg quality, blood.

Introduction

Laying-hen housing may have different schemes in modern production agriculture, including traditional cages, enriched cages, cage-free floor-raised house or aviary, or free range system. Each housing scheme has come into practice for various reasons as the scale of production has increased from the family farm to commercial-scale operations. Each system offers benefits to the producer, the bird, the consumer, or a combination. Alternative housing systems for laying hens must be designed to balance the health and the welfare of the birds with consumer preferences, the needs of the industry, and the impact on environment. Housing systems for laying hens have considerable effects on performance and production traits such as egg weight, feed efficiency and daily feed consumption **(Suto *et al.*, 1997)**. Egg quality is important for consumer appeal, and the economic success of a producer depends on the total number of eggs sold. Egg quality has a genetic basis and its parameters vary between strains of hens **(Silversides *et al.*, 2007)** and also influenced by the housing system under which the hens are kept (**Vits *et al.*, 2005**). Blood biochemical analysis is very important among the research because it supports and interpret the results of the research (**Ozbey and Esen. 2007 a**). The study of the variation in blood picture and constituents in the fowls sets an important foundation to the study of growth and egg production. Moreover, it helps in explaining the reaction of developed strains of poultry to their environments.

Biotin (also known as vitamin H), a water-soluble vitamin belonging to the B-complex group (Ploux, 2000), is necessary for normal embryonic development and hatchability (**Whitehead *et al.*, 1985**). Its physiologically active form is linked to enzymes of great metabolic importance like biotin carboxylase and biotin decarboxylase and seems to be a key-enzyme in important processes like gluconeogenesis ,fatty acids and protein synthesis, controlling scleroprotein production. Because of its functions, this vitamin contributes to such important processes as growth, skin regeneration, bone development and reproduction, increasing feed conversion in animals (**McMahon, 2002**). Therefore, the objective of this study was to evaluate the effect of housing system and dietary levels of biotin supplementation on egg quality traits and some blood constituents of Benha line chickens as a native breed of chickens in Egypt.

Materials and Methods

Experimental birds and housing:

A total number of 224(200 hens and 24 cocks), Benha line chickens 20 weeks old and overall mean

of body weight (1742 ± 20.1) were equally divided into two groups. Pullets of the first group were housed in cages of laying battery (with artificial insemination) two birds per cage of 48×40×40 cm (960cm^2/bird). Pullets of the second group were kept on deep litter laying houses (with natural mating) in a density of 900cm^2/ bird. Chickens of each group were then subdivided into four sub groups each of 25 females and 3 males according to dietary biotin supplementation. Pullets of the 1st sub group were fed on basal layer diet and considered as control group, the 2nd, 3rd and 4th sub groups were fed on basal diet supplemented with biotin at a level of 100, 150 and 200µg/kg diet, respectively. The basal layer diet (Table, 1) was formulated according to (NRC, 1994) with 15.68 % crude protein and 2726 kcal/ kg. The experimental diets and fresh water were supplied ad libitum.

Table 1. Composition* and calculated nutritional value of starting, growing and basal layer diet.

Ingredients %	Ration		
	Starting 0-8 wks age	**Growing 9-19 wks age**	**Laying 20 wks after**
Yellow corn	65.00	63.00	66.00
Soybean meal 44%	30.45	15.50	21.30
Wheat bran	0.65	17.78	2.94
Di-calcium phosphate	1.80	1.25	1.50
Limestone	1.40	1.80	7.60
Salt	0.30	0.30	0.30
Vit & Min. premix**	0.30	0.30	0.30
DI-Methionine	0.10	0.07	0.06
Total	100.00	100.00	100.00
Calculated analysis:			
Curd protein %	19.278	15.196	15.676
M.E. (k cal/kg)	2868.665	2689.866	2726.418
Crude fiber %	3.723	4.472	3.329
Crude fat %	2.723	3.051	2.766
Calcium %	1.016	1.030	3.285
Avail. Phosphorus %	0.485	0.397	0.412
Lysin %	1.051	0.709	0.799
Methionine %	0.428	0.326	0.334
Cystin %	0.180	0.180	0.180
Meth. + Cys. %	0.737	0.571	0.588

*As recommendation as recommended by NRC, (1994), which used during the period from sexual maturity to the end of the experimental period.
**Composition of premix in 3 kg is: Vit. A 10,000,000 IU, Vit. D3 2,000,000; Vit. E 10,000 mg, Vit. K3 1,000 mg, Vit. B1 1,000 mg, Vit. B2 4,000 mg, Vit. B6 1,500 mg, Vit. B12 10 mg; Niacin 20,000 mg; Pantothenic acid 10,000 mg, Folic acid 1,000 mg, Biotin 50 mg, Choline chloride 500, 000 mg, Cu 3,000 mg, Iodine 300 mg, Fe 30,000 mg; Mn 40,000 mg, Zn 45,000 mg, Selenium 100mg.

Parameters estimation and data collection:-

During two successive days out of each week, 20 eggs of each treatment were collected and individually subjected to the following measurements and estimation. Egg components (egg shell, albumen and yolk) in terms of the percentage of egg were determined, egg shape index (ESI) was calculated according to the following equation reported by **Stadelman (1977),** [ESI = (egg width/egg length) x 100]. Albumen index was calculated according to **Romanoff and Romanoff, (1949**) as follows: AI= [albumen height / (albumen diameter)/2) ×100].Yolk index was calculated according to **Wesley and Stadelman (1959).** Haugh unit was calculated according to**, Haugh (1937)** as follows: **HU =100 log (H+ 7.37-1.7 EW$^{0.37}$),** Where: H =Albumen height (mm). EW = Egg weight (g). Data were then presented at sexual maturity, at the peak of egg production and at the end of the experimental period (lasted 180 days of egg production).

During egg production periods, eggs were collected and incubated monthly (6 batches). At hatching time, infertile eggs were examined to determined fertility percentage. Fertility percentage was calculated using the following equation: Fertility (%) = Number of fertile eggs / Number of setting eggs x100, hatchability percentage was calculated according to the following equation: Hatchability (%) = Number of hatched chicks / Number of fertile eggs x100**,** chick weight at hatch was calculated and Percentages of early, mid and late embryonic mortality were also calculated.

Blood samples were taken for chemical determination at sexual maturity, at the peak of egg production and at the end of the experimental period. Blood samples were withdrawn from four females randomly selected from each experimental treatment into the tube contain *EDTA*, Plasma was separated by using centrifugation for 20 minutes of 2500 rpm. Then all samples were transferred and stored in the deep freezer at approximately -20 C^o until the time of

the biochemical analysis. Plasma total protein, albumin and globulin, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), creatinine and plasma uric acid were calorimetrically estimated using commercial kits purchased from (Spectrum Bio- Diagnostic Company, Hannover-Germany).

Statistical analysis

Analysis of variance was carried out using SAS procedure guide **(SAS, 2004).** According to the following linear model 1:

$\mathbf{X_{ijk} = \mu + Hi + B_j + P_k + e_{ijk}}$

$\mathbf{X_{ijk}}$ = the k Th observation, **μ** = the overall mean. $\mathbf{H_i}$ = the effect of the housing system. (I, 1-2), $\mathbf{B_j}$ = the effect of the biotin supplementation. (J, 1-4) $\mathbf{P_k}$ = the effect of the Experimental period. (K, 1-3) and $\mathbf{e_{ijk}}$ = the experimental error.

Significant differences among groups means were tested using Duncan multiple range test **(Duncan, 1955).**

Results and Discussion

Egg quality

The results obtained for relative weights of egg component in terms of egg shell, albumin and yolk at age of sexual maturity, at the peak of egg production and at the end of the experimental period are shown in Table (2). During the different periods of the experiment, it could be noticed that relative weights of yolk was significantly ($p<0.01$) increased in egg produced from birds reared on deep litter (30.12%) than those produced from birds reared in cages (29.95%). While relative weight of egg shell and albumin were not significantly affected by housing system, it was clearly observed that egg produced from birds reared in cages showed the higher relative weights of egg shell and albumen (12.86% and 59.37%,respectively) than those produced from birds reared on deep litter (12.80% and 59.34%,respectively).The non-significant difference in egg shell percent due to housing system may be attributed to those hens received a balances diet which may be sufficient to cover their requirements, thus the genetic effect become dominant for controlling this trait rather than housing system (**El-Gendi, 1985).**

Table 2. Least –square means and standard error (LSM ± S.E) for relative weights of egg shell, albumen and yolk as affected by the studied factors

Factors	Trait	Relative weights of egg component (%)		
	Treatment	Shell	Albumen	Yolk
Housing system	Cages	12.86 ±0.27	59.37±0.49	29.95±0.28^{b}
	Deep litter	12.80 ±0.27	59.34±0.49	30.12±0.28^{a}
Biotin levels (µg/kg)	Control	12.70 ±0.38	58.94±0.70	30.05±0.40^{ab}
	100	12.93±0.38	59.64±0.70	29.80±0.40^{b}
	150	12.96 ±0.38	59.34±0.70	29.83±0.40^{b}
	200	12.72 ±0.38	59.39±0.70	30.47±0.40^{a}
Experimental period	Sexual maturity	13.43±0.43^{a}	58.49±0.60^{a}	28.32±0.34^{c}
	Peak	12.69±0.43^{ab}	60.07±0.60^{a}	30.23±0.34^{b}
	End	12. 37±0.43^{b}	59.50±0.60^{b}	31.55±0.34^{a}

(a, b, c, d) means within each factor with different superscripts are significantly different.

The results obtained agreed with those reported by **Singh *et al.*, (2009)** who found that eggs produced in cages had significantly lower yolk weights than those laid on floor. However**, El-Anwer *et al.*, (2009) and Hassan (2001)** who, stated that there were no significant differences in egg shell percent and albumin percent under either housing system battery cages and/or floor deep litter.

Pullets fed diet supplemented with biotin at a level of 150 µg / kg diet showed the higher value of relative weight of egg shell (12.96 %), while pullets fed diet supplemented with biotin at a level of 100 µg / kg diet showed the higher value of relative weight of albumin (59.64 %). However increasing biotin level to 200 µg / kg diet significantly increased relative weight of yolk (30.47 %). **Bennett (1992), Narushin, (1997), Moyle et al., (2008) and Butcher and Miles (2011)** stated that there is a little information regarding biotin supplementation on egg quality characters.

Shell weight percentage increased significantly at sexual maturity then decreased toward the end of the experimental period. However, the relative weights of albumen and yolk significantly increased at the peak of egg production and at the end of the experimental period, respectively. A number of studies had shown that egg shell quality decreases as birds grow older (**Nys, 1985 and Roberts and Ball, 2004)**.

The results obtained in Table (3) showed that eggs produced from pullets reared in cages had the higher egg shape index (80.01 mm), albumen index (15.79 mm) and yolk index (53.13mm) than those laid by pullets reared on deep litter (75.22 **,** 14.88 and 51.89 mm, respectively). The results obtained agree with those reported by **El-Anwer *et al.*, (2009) and Singh *et al., (*2009)** they found that indexes of egg shape, albumen and yolk from cages eggs were significantly ($P \leq 0.05$) higher in pullets reared in cage than those laid on floor.

Pullets fed diet supplemented with biotin at a level of 100 µg biotin / kg diet produced the highest average of egg shape index (79.21 mm), while those fed diet supplemented 150µg biotin / kg diet showed the highest averages of egg albumen index (15.56mm) and yolk index (53.27 mm). Albumen and yolk index increased gradually reaching its maximum values at the end of the experimental period.

Data listed in Table (3) revealed that pullets reared on deep litter laid eggs with higher Haugh unit value (87.04) than those laid by pullets reared in cages (86.81). These results disagree with those reported by **Ozbey and Esen (2007 b)** who observed a significant difference in eggs Haugh unit values in favor of cage system. Haugh unit value increased with increasing biotin level supplementation, eggs laid from pullets fed diet supplemented with biotin at a level of 200 and 150 µg/ kg diet showed the higher averages of haugh unit value (87.11and 87.08, respectively). These results agree with those reported by **Abdel-Mageed and Shabaan (2012)** who found that biotin addition to layers diets gave the best improvement in Haugh unit.

Haugh units increased gradually with increasing of birds age reaching its maximum values at the end of experimental period.

Table 3. Least –square means and standard error (LSM ± S.E) for egg shape, albumen, yolk indexes as affected by the studied factors

Factors	Trait / Treatment	Index (mm) of			Haugh unit
		Egg shape	Albumen	Yolk	
Housing system	**Cages**	80.01±1.71	15.79±0.46	53.13±1.34	86.81±0.48
	Deep litter	75.22±1.71	14.88±0.46	51.89±1.34	87.04±0.48
Biotin levels (µg/kg)	**Control**	75.86±2.43	15.13±0.65	50.60±1.90	86.56±0.69
	100	79.21±2.43	15.40 ±0.65	53.00±1.90	86.95±0.69
	150	77.15±2.43	15.56±0.65	53.27±1.90	87.08±0.69
	200	78.25±2.43	15.25±0.65	53.18±1.90	87.11±0.69
Experimental period	**Sexual maturity**	75.54±2.10	14.68±0.56	50.29±1.65	85.23±0.59^{b}
	Peak	79.54±2.10	15.61±0.56	51.54±1.65	87.63±0.59^{a}
	End	77.78±2.10	15.73±0.56	53.11±1.65	87.91±0.59^{a}

(a, b) means within each factor with different superscripts are significantly different.

Fertility and hatchability:-

Data presented in Table (4) revealed that, no significant variations was found in fertility due to the effect of housing system, it was clearly observed that eggs produced from birds reared on floor deep litter (natural mating) showed a higher fertility percentage (82.70%) than those produced from birds reared in cages (artificially inseminated) it was mounted (81.94%), this result may be attributed to the type of mating rather than the effect of housing system **(Balcazar, 2014)**. Eggs produced from birds reared in cages had significantly higher average of hatchability percentage (88.10%) than those produced from birds reared on floor (80.64%), this result may be attributed to the differences in the internal and external characteristics of incubated eggs collected from pullets reared under these two different housing systems (**El-Gendi, 1985).**

Fertility and hatchability percentages of eggs produced from birds of the experimental groups increased significantly by increasing biotin level supplementation reaching its maximum values (86.87 and 89.29%, respectively) for birds fed diets supplemented with biotin at a level of 150 µg/ kg diet, then it decreased significantly at the higher level of biotin (200 µg/ kg diet) reaching to (84.51 and 82.34%, respectively). These results agree with those reported by **Chen *et al.*, (1994)**, **Nofal and Salem (2000) McMahon, (2002) and ROBEL (2002)**.

Table 4. Least –square means and standard error for fertility and hatchability as affected by the studied factor

Factors	Trait / Treatment	Fertility (%)	Hatchability (%)
Housing system	**Cages**	81.94±1.63	88.10±1.55^{a}
	Deep litter	82.70±1.63	80.64±1.55^{b}
Biotin levels (µg/kg)	**Control**	74.50±2.30	81.20±2.19^{b}
	100	83.35±2.30	86.61±2.19^{ab}
	150	86.87±2.30	89.29±2.19^{a}
	200	84.51±2.30	82.34±2.19^{b}
Experimental period	**Sexual maturity**	79.04±1.99	78.70±1.86^{b}
	Peak	84.31±1.99	91.61±1.86^{a}
	End	83.60±1.99	84.19±1.86^{b}

(a, b) means within each factor with different superscripts are significantly different.

Embryonic mortality:-

Data obtained in Table (5) revealed that incubated egg from pullets reared in cages significantly decreased ($p<0.05$) percentage of early (3.19%), mid (1.76%) and late (2.67%) embryonic mortality, than those reared on floor (6.90%, 4.03% and 3.72%, respectively). Pullets fed diet supplemented with biotin at a level of 150 µg/ kg diet showed significantly ($P<0.05$) the lowest percentages of early (2.93%), mid (2.20%) and late (2.79%) embryonic mortality compared with different levels of biotin applied and control group. The results obtained agree with those reported by **Abdel-Mageed and Shabaan (2012)** who found that increasing biotin level from 162.5 to 325.5 µg/ kg gave a significant decrease in the percentages of early and late embryonic mortality.

Chick weight at hatch:

Data presented in Table, (6) revealed that significant ($P<0.05$) variations due to the effect of housing system on chick weight at hatch , it was clearly observed that incubated eggs produced from pullets reared in cages had a higher chick weight at hatch (36.17 g) than those reared on deep litter (33.32 g). This result is quite true and logic since the increasing in egg weight may be attributed to the increasing chick weight at hatch (**Van de Ven et al., 2009)**. Incubated eggs produced from pullets fed diet supplemented with biotin at a level of 100 µg/ kg had significantly ($p<0.05$) the higher chick weight at hatch (36.03 g), followed by those fed diet supplemented with 150 µg/ kg (35.33 g). The increase chick weight at hatch observed in this study may be due to the increase in egg weight. In this respect, **Abiola (1999)** found a close correlation between egg weight and hatching weight in domestic birds. **Abdel-Mageed and Shabaan (2012)** found that supplemented biotin at a level of 100 µg/ kg to layer diet gave significant increase in chick weight at hatch. Chick weight at hatch increased significantly by advancing age reaching its maximum value at the end of the experimental period.

Table 5. Least –square means and standard error (LSM ± S.E) for early, mid and late embryonic mortality as affected by the studied factors

Factors	Trait / Treatment	Embryonic mortality (%)		
		Early	Mid	Late
Housing system	Cages	3.19±0.22^{b}	1.76±0.14^{b}	2.67±0.15^{b}
	Deep litter	6.90±0.22^{a}	4.03±0.14^{a}	3.72±0.15^{a}
Biotin levels (µg/kg)	Control	6.58±0.31^{a}	3.83±0.21^{a}	3.64±.22^{a}
	100	5.31±0.31^{b}	2.52±0.21^{bc}	3.25±.22^{ab}
	150	2.93±0.31^{c}	2.20±0.21^{c}	2.79±.22^{b}
	200	5.37±0.31^{b}	3.02±0.21^{b}	3.12±.22^{ab}
Experimental period	Sexual maturity	8.35±0.27^{a}	5.12±0.18^{a}	6.60±0.19^{a}
	Peak	2.15±0.27^{c}	0.57±0.18^{c}	1.01±0.19^{c}
	End	4.64±0.27^{b}	2.98±0.18^{b}	1.98±0.19^{b}

(a, b, c) means within each factor with different superscripts are significantly different.

Table 6. Least –square means and standard error (LSM ± S.E) for chick weight at hatch as affected by the studied factors

Factors	Trait / Treatment	Chick Weight at hatch (g)
Housing system	Cages	36.17±0.32^{a}
	Deep litter	33.32±0.32^{b}
Biotin levels (µg/kg)	Control	33.16±0.46^{c}
	100	36.03±0.46^{a}
	150	35.33±0.46^{ab}
	200	34.46±0.46^{b}
Experimental period	Sexual maturity	33.25±0.40^{b}
	Peak	35.00±0.40^{a}
	End	36.01±0.40^{a}

(a, b) means within each factor with different superscripts are significantly different.

Blood constituents:-

The obtained data presented in Table (7) showed that pullets kept on deep litter laying house had significantly the higher level of plasma total proteins (6.05 g /dL) than caged ones (4.52 g/dl), while, pullets kept in cages had significantly the higher levels of plasma albumen and globulin (3.15 and 3.19 g /dL, respectively). These results agree with those reported by **El- Anwer *et al.*, (2009)** who

found that there was a significant increase in plasma total proteins at 36 wks in floor hens.

Plasma protein fractions significantly affected by dietary biotin level, pullets fed diet supplemented with biotin at a level of 100 and 150 µg/kg showed significantly the higher plasma albumin concentration (3.43 and 3.00 g/ dL). However control group significantly increased in plasma total protein and globulin compared with different biotin level supplementations these results disagree with those reported by **Al-Salih *et al.*, (2012)** who found that the treated rabbits with a dose of 200 µg biotin / kg evoked a significant increase in the concentrations of each total protein, albumin, and globulin.

Table 7. Least –square means and standard error (LSM ± S.E) for plasma total proteins, albumin and globulin as affected by the studied factors

Factors	Trait / Treatment	Total proteins (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Housing system	**Cages**	4.52±0.14^{b}	3.15±0.09^{a}	3.19±0.15^{a}
	Deep litter	6.05±0.14^{a}	2.86±0.09^{b}	1.36±0.15^{b}
Biotin levels (µg/kg)	**Control**	5.55±0.19^{a}	2.95±0.12^{b}	2.60±0.22^{a}
	100	5.47±0.19^{ab}	3.43 ±0.12^{a}	2.03 ±0.22^{a}
	150	5.26±0.19^{ab}	3.00±0.12^{b}	2.25±0.22^{a}
	200	4.88±0.19^{b}	2.64±0.12^{b}	2.23±0.22^{a}
Experimental period	**Sexual maturity**	5.41±0.17	3.03±0.11	2.37±0.19
	Peak	5.09±0.17	2.85±0.11	2.24±0.19
	End	5.37±0.17	3.14±0.11	2.22±0.19

(a, b) means within each factor with different superscripts are significantly different.

Data presented in Table (8) showed that the higher levels of plasma AST and ALT transaminases (133.01 and 48.16 U/L) were found in pullets housed in deep floor litter and battery cages, respectively.

Pullets fed diet supplemented with biotin at a level of 200 µg/ kg had significantly the highest averaged of plasma AST (159.79) and ALT transaminases (67.30 U/L). These results agree with those reported by **Grier *et al.*, (1990)** and **Mutluay et al., (2008)** they found that there were significantly affected on serum AST and ALT Levels ($p < 0.05$) due to biotin supplementation to layer diet. Plasma AST significantly increased with advanced age reaching its maximum value (148.21 U/L) at the end of the experimental period.

Data presented in Table (9) revealed highly significant ($P<0.05$) variation in plasma uric acid and creatinine due to housing system applied, the higher averages of plasma creatinine (1.22 mg/dL) and uric acid (7.60 mg/dL) were found in pullets kept on deep litter laying houses.

Regarding the effect of biotin level, it is clearly observed that pullets fed diet supplemented with biotin at a level of 150 µg/ kg showed significantly the highest averages of plasma creatinine and uric acid level (1.26 and 8.16 mg/ dL, respectively). However, the lower plasma creatinine and uric acid levels (1.11 and 5.87 mg/ dL, respectively) showed in pullets fed diet supplementation with biotin at a level of 200 µg /kg. These results agree with those reported by **Al-Salih et al., (2012)** they found that treated rabbits with a dose of 200 µg biotin/kg evoked a significant decrease in uric acid concentration in their blood serum.

Plasma creatinine and uric acid showed the lowest averages 0.88 and 6.26 mg/ dL at the end of the experimental period and at the peak of egg production, respectively. (Table 9)

It could be concluded that biotin supplementation at a level of 150 and 100 µg/ kg in layer diet and reared in both battery cages and on deep litter system, respectively seemed to be adequate to achieve the favorable results.

Table 8. Least –square means and standard error (LSM ± S.E) for plasma Aspartate aminotransferase (AST) and Alanine Aminotransferase (ALT) as affected by the studied factors

Factors	Trait / Treatment	AST (U/L)	ALT (U/L)
Housing system	**Cages**	120.21±8.97^{a}	48.16±7.17^{a}
	Deep litter	133.01±8.97^{a}	45.95±7.17^{a}
Biotin levels (µg/kg)	**Control**	115.00±12.68^{b}	44.42±10.15^{ab}
	100	74.73±12.68^{c}	28.02±10.15^{b}
	150	156.92±12.68^{a}	48.47±10.15^{ab}
	200	159.79±12.68^{a}	67.30±10.15^{a}
Experimental period	**Sexual maturity**	111.90±10.98^{b}	47.77±8.79^{a}
	Peak	119.72±10.98^{ab}	41.57±8.79^{a}
	End	148.21±10.98^{a}	51.82±8.79^{a}

(a, b) means within each factor with different superscripts are significantly different.

Table 9. Least –square means and standard error (LSM ± S.E) for plasma creatinine and uric acid as affected by the studied factors

Factors	Trait / Treatment	Creatinine (mg/dL)	Uric Acid (mg/dL)
Housing system	**Battery**	1.13±0.13	6.38±0.44^{b}
	Floor	1.22±0.13	7.60±0.44^{a}
Biotin levels (µg/kg)	**Control**	1.13±0.19^{a}	6.98±0.63^{ab}
	100	1.21±0.19^{a}	6.94 ±0.63^{ab}
	150	1.26±0.19^{a}	8.16±0.63^{a}
	200	1.11±0.19^{a}	5.87±0.63^{b}
Experimental period	**Sexual maturity**	1.41±0.16^{a}	7.57±0.54^{a}
	Peak	1.23±0.16^{ab}	6.26±0.54^{a}
	End	0.88±0.16^{b}	7.14±0.54^{a}

(a, b) means within each factor with different superscripts are significantly different.

Reference

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