Effect of Stocking Density, Dietary Vitamin D3 and Probiotic Supplementation on Carcass Traits and Blood Parameters of Broiler Chickens

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Abstract

The present study was conducted to evaluate the effect of stocking density, dietary supplementation of probiotic and Vitamin D3 on carcass yield and blood biochemistry of broiler chickens. A total number of 360 unsexed broiler chicks (Evian), one-day old, nearly similar in live body weight were used in this study. Chicks were randomly divided into equal 12 treatments in a 2X3X2 factorial arrangement experiment. Birds were allocated in two main experimental groups (each of 180 birds), the first main group kept under normal stocking density (LSD) with 10 birds /m^2 and the second main group kept under high stocking density (HSD) with16 birds/m^2. Each main group was divided into three groups (each of 60 birds) fed on diets supplemented with probiotic at a levels of 0, 1 and 2 g/kg diet, respectively. Each group divided into two sub-group (each of 30 birds) and supplemented with fed vit.-D3 at a level of 250 and 500 IU/kg diet, respectively. Results obtained showed that raised broiler chickens under LSD had significantly improved absolute and relative carcass, giblets and total edible weight Moreover, it has significantly increased plasma total protein, albumin (A), globulin (G), cholesterol, triglyceride and it has decreased plasma transaminase enzymes of AST and ALT, compared with those rose under HSD. Broiler chicks fed on diet supplemented with probiotic at level of 2 g/ kg diet showed higher plasma total protein, albumin and A/G ratio, cholesterol and triglycerides than those fed on the other levels of probiotic supplementations. While, it has recorded lowest plasma AST and ALT levels, compared with the other experimental groups. Feeding broiler chickens on high level of Vit.-D3 (500 IU/ Kg diet) has significantly increased plasma protein fractions and decreased plasma AST and ALT levels, compared with those fed diet supplemented with low level of Vit.-D3 (250 IU/Kg) that caused significant decreased plasma cholesterol and triglycerides. Finally, it could be recommended to apply LSD (10 birds/ m^2), high level of Vit.-D3 (500 IU/ Kg) and probiotic at a level of 2 g/ kg diet to improve carcass yield and blood biochemistry of broiler chickens.

Key words: broilers, stocking density, probiotic, Vit.-D3, carcass and blood parameters

Introduction

The performance of broilers is governed by different factors like environment, nutrition, management, and genetic makeup. Optimal environment and nutrition must be provided for better performance of broilers. High stocking density (**HSD**) has been reported for its beneficial economical impact if it remains within optimum range **(Dozier *et al.*, 2006).** HSD reduces the fixed cost of production and produces more kilograms of chicken per area up to a certain extent with increasing profitability **(Puron *et al*., 1995).** A higher profitability per kilogram chicken can be obtained by increasing the stocking density if the performance of birds remains constant **(Feddes e*t al*., 2002).** However, it seems to be impractical due to the fact that the health and performance of broilers is compromised if the placement density goes beyond 34 to 40 kg/ m^2 that assuming to be 2.5 kg final body weight (BW) on an average at the end of growing period **(Estevez, 2007).** High stocking density (HSD) reduces the performance of broilers due to a number of factors. It accounts for increased ammonia and reduced access to feed and water in addition to poor air quality because of insufficient air exchange at the level of bird. Consequently, a high environmental temperature is observed in the microclimate of the bird resulting in a reduced dissipation of body heat to the environment **(Feddes *et al*., 2002),** thus, the ability of the farmers to keep the balance between HSD and the confortable environmental management conditions in the farm, it may be the main key to increase the production performance of broiler chicken under HSD condition. High stoking density poses a stress on the broilers and it is believed that stressful conditions such as heat stress and crowding disrupt the microbial ecology of the bird's intestine, thereby causing dysbiosis**(Guardia *et al*., 2011)**.One of the possible approaches employed to maintain the performance, intestinal health, humeral immunity, and to avoid physiological stress in broiler exposed to heat stress is the use of dietary probiotics **(Sohail *et al*., 2012)** which enhance the performance without being digested**.** Direct feed microbial, known as probiotics, can improve the performance **(Huang *et al*., 2004)**, nutrient digestibility **(Li *et al.* 2008),** immunity **(Teo and Tan, 2007),** gut microflora ecology **(Yu *et al*., 2008),** and inhibition of pathogens **(Mountzouris *et al*., 2009)** in birds. In poultry feeding, cholecalciferol (VIT-D3) is necessary for the intestinal absorption, blood transport and efficient metabolism of calcium and phosphorus. In addition, the VIT-D3 has an important role for in the development of skin and blood cells, as well it is involved in controlling cellular activities of host immune system **(Lymboussaki *et al.*, 2009).**Vitamin D is an essential metabolite for bone formation and maintenance in all vertebrate animals primarily because cholecalciferol (D3) controls calcium (Ca) and phosphorus (P) homeostasis within the vertebrate body **(Hurwitz *et al.* 1984).**The D3, the first metabolite of vitamin D is transferred from the skin and gastrointestinal tract via serum to the liver where it undergoes the first hydroxylation to 25 hydroxy cholecaciferol [25(OH)D3]. For 25(OH)D3, the second metabolite of vitamin D is the storage form of vitamin D. Thus25(OH)D3 remains in serum until it is further hydroxylated in the kidney to become the active hormonal metabolite of vitamin D known as 1,25-dihydroxy cholecalciferol [1,25-(OH)2D3] (**Fraser and Kodicek (1970).** Until recently, tibial dyschondroplasia was the main leg problem affecting broilers **(Almeida Paz et al., 2008),** specially under the HSD during the growing period of broiler chickens. Vitamin D is added to broiler diets to provide Ca balance, and to improve Ca intestinal absorption **(Waldenstedt, 2006).** The aim of this study was to evaluate the effect of stoking density, dietary supplementation of probiotic and Vitamin D3 on carcass yield and some blood biochemical parameters of broiler chickens.

Material and Methods:

Experimental design:

A total number of 360 unsexed broiler chicks (Evian), one-day old, nearly a similar live body weight were used in this study. Chicks were randomly divided into equal 12 treatments in a 2X3X2 factorial arrangement experiment. Birds were allocated in two main experimental groups (each of 180 birds), the first main group kept under normal stocking density (SD) with 10 birds/m^2 and the second main group kept under high stocking density (HSD) with16 birds/m^2. Each main group was divided into three groups (each of 60 birds) fed on diets supplemented with probiotic at levels of 0, 1 and 2 g/kg diet, respectively. Each group divided into two sub-groups (each of 30 birds) and supplemented with fed Vit.-D3at levels of 250 and 500 IU/kg diet, respectively. Chicks were kept under similar, standard hygienic and environmental conditions. Wood shaving was used at 10 cm depth as a litter and the wetting litter was continually changed by a fresh one. Floor brooders with gas heaters were used for brooding chicks. Brooding temperature was maintained at 35°C during the first 5 days of chick's age, and then decreased by 2°C weekly until the end of the 4th week. The lighting program was 24 h light during the first 5 days of age, then it decreased from 6 to 42 days of age (the end of the experiment) to 23 hours light and 1 hour dark was applied. Feed and water were offered ad-libitum. Chicks were fed starter and grower diets. The basal diet was formulated according to the recommended requirements of **NRC (1994)** as showed in table (1):

Table 1. The composition and calculated analyses of experimental starter and grower diets:

Ingredients %	Starter (0-4) wks	Grower (4-6) wks
Yellow corn (8.5%)	61.00	66.00
Soybean meal (44% CP)	35.50	26.00
Corn gluten meal (60% CP)	0.00	1.00
Vegetable oil	0.00	3.00
Di. calcium phosphate	1.70	1.70
Calcium carbonate	1.10	1.20
Sodium chloride	0.30	0.30
Vit. and M.n. permix*	0.30	030
DL. Methionine 99%	0.10	0.16
L. Lysine hydrochloride 78%	0.00	0.04
Total calculated analysis	100	100
ME.(Kcal / Kg)	3135	2839
Crude protein	20.86	17.81
Crude fat	2.67	5.80
Crude fiber	2.73	2.49
Calcium	0.93	0.95
Available P	0.43	0.42
Methionine	0.46	0.47
Methionine + Cystine	0.83	0.79
Lysine	1.21	0.99

Calculated analysis %**

*Each 3.0 Kg of the Vit. and Min . premix contains :

Vit.A, 12000000 IU; Vit.E, 10 g; Vit.K3, 2.0 g; Vit.B1; 1.0 g; Vit.B2, 5 g; Vit. B6;1.5 g; Vit. B12, 10 mg; choline choloride, 250 g; Biotn, 50 mg; folic acid,1 g; nicotinic acid, 30 g; Ca Pantothenate, 10 g; Zn, 50 g; Cu, 10 g; Fe, 30 g; Co,100 mg; Se, 100 mg; I, 1 g; Mn, 60 g and antioxidant, 10 g and complete to 3.0 kg by calcium carbonate.

Parameters estimation and data collection:

Slaughtering and carcass characteristics:

Carcass characteristic for random sample of 3 birds from each treatment were performed at the end of the experimental period (6 weeks). Birds' chosen were deprived from feed for 16 hours before slaughtering after which they were individually weighed to the nearest g and killed by cutting the throat and the jugular veins with a sharp knife near the first neck vertebra. Birds were individually reweighed after complete bleeding. Shank and head were separated, the birds were then eviscerated and intestine, gizzard, lungs, spleen, liver, heart and all internal organs were removed. The carcass and giblets (empty gizzard, liver and heart) were separately weighed. The proportional weights to live body weight of giblets, carcass and total edible parts were calculated as follows:

$$\textbf{Giblets weight (\%)} = \frac{\textbf{GW}}{\textbf{LW}} \times \textbf{100}$$

$$\textbf{Edible parts (\%)} = \frac{\textbf{EW} + \textbf{GW}}{\textbf{LW}} \times \textbf{100}$$

Whereas: LW = live body weight
EW = eviscerated weight
GW = giblets weight

Blood samples for chemical analysis were individually obtained from 5 birds randomly chosen from each treatment at 4 and 6 weeks of birds' age from the wing vein. Heparinized blood samples were centrifuged at 2500 rpm for 15-min. Plasma samples were then stored in the deep freezer at approximately -20±l°C until the time of chemical analysis. Plasma total protein, albumin, globulin , A/G ratio, triglycerides, cholesterol , creatinine, uric acid, calcium, inorganic phosphorus, asparatate aminotransferase (AST) and glutamic oxaloacetic transaminase(ALT) were calorimetrically determined using commercial kits.

Statistical analysis

Analysis of variance was calculated using SAS procedure guide **(SAS 2004)** using the following linear model:

$$X_{ijhk} = \mu + S_i + P_j + V_h + SP_{ij} + SV_{ih} + PV_{jh} + SPV_{ijh} + E_{ijhk}$$

Whereas:

X_{ijhk} =the kth observation

μ = overall mean

S_i = the effect of ithstocking density

P_j= the effect of jth level of probiotic

V_h = the effect of hth level of vitamin D3

SP_{ij}= the effect of the interaction between the ith stocking density and the jth probiotic.

SV_{ih}= the effect of the interaction between the ith stocking density and the hth vitamin D3.

PV_{jh}= the effect of the interaction between the jthprobiotic and the hth vitamin D3.

SPV_{ijh}= the effect of the interaction between the ith stocking density, the jth levels of probiotic and the hth levels of vitamin D3

E_{ijhk} = the experimental error

Significant differences among group were tested using Duncan multiple range test
(Duncan. 1955).

Results and Discussion

Carcass traits:

Results obtained in table (2) showed highly significant variations were found in absolute weights of carcass, giblets and total edible parts due to the effect of stocking density (SD) of birds. While, no significant variations were found in absolute weights of carcass, giblets and total edible parts due to either probiotic (P) or Vitamin-D3 (V.D3) supplementation levels. However, significant variations were found in all carcass traits, except proportional weights of carcass and total edible parts of broiler chickens due to effect of the interaction between SD X V.D3 X P. Broiler chickens kept under LSD (10 bird/m^2) recorded the highest absolute weights of carcass, giblets and total edible parts (1806.1, 100.3 and 1906.4 g respectively), compared with those kept under HSD (16 bird/ m^2) which recorded the lowest absolute weights of carcass, giblets and total edible parts (1475,3, 83.6 and 1558.9g respectively). These results agreed with those concluded by **Adeyemo *et al.* (2016)** who found that broiler chickens raised in high stocking density up to stocking density 14 birds /m^2 showed significant improve of carcass characteristics. However, it is disagree with those reported by **Rudzani *et. al*., (2017) and Goo *et al*., (2019)** they found no significant (P>0.05) effect of stocking density on carcass, eviscerated carcass, breast, and abdominal fat yields of broiler chickens.

Although there were no significant effects of Probiotic and Vit.-D3on absolute and relative weights of carcass, giblets and total edible parts, it is clearly observed that chicks fed on diet supplemented with probiotic at a level of 1 g/kg diet showed a higher absolute weights of carcass, giblets and total edible parts (1682.5, 93.3 and 1775.8g, respectively) than those fed on probiotic at a level of 2 g/kg diet and those fed diet without probiotic. Moreover, chicks that fed on diet supplemented with V.D3 at a level of 500 IU/Kg diet showed higher averages of absolute weights of carcass, giblets and total edible parts than those fed diet supplemented with V.D3 at a level of 250 IU/Kg diet. The interaction between LSD X P2 X V2 showed significantly the higher averages of absolute and relative weights of carcass and total edible parts. While the interaction between LSD X P0 X V2 showed significantly the higher averages of absolute and relative weights of giblets when compared with different interactions applied.

Table 2. Least –square means and pooled standard error for carcass characteristics of different experimental groups as affected by studied factors

Treatments	level	Carcass traits					
		Carcass weight		Giblets weight		Total edible weight	
		g	%	g	%	g	%
Stoking density(SD) bird/m²	10	1806.11[a]	74.39	100.27[a]	4.13	1906.38[a]	74.53
	16	1475.27[b]	73.63	83.61[b]	4.16	1558.88[b]	75.82
MSE		**18.40**	**0.36**	**1.51**	**0.07**	**18.30**	**0.38**
Probiotic (P) g /kg	0	1570.0	73.24	91.08	4.30	1662.08	77.54
	1	1682.50	74.32	93.33	4.10	1775.83	78.50
	2	1669.58	74.44	92.41	4.05	1760.0	78.48
MSE		**22.22**	**0.44**	**1.85**	**0.09**	**22.41**	**0.46**
Vitamin D3 (V.D3) IU/kg	250	1626.11	74.22	91.38	4.17	1717.50	78.41
	500	1655.27	73.80	92.50	4.12	1747.77	77.94
MSE		**18.14**	**0.36**	**1.51**	**0.07**	**18.30**	**0.38**
Interaction							
LSDX P_0 X V_1	10 x 0 x 250	1705.00[bc]	73.56	96.66[ab]	4.16[ab]	1801.66[b]	77.73
LSDX P_0 X V_2	10 x 0 x 500	1663.33b	73.40	101.66[a]	4.50[a]	1765.0[ab]	77.90
LSDX P_1 X V_1	10 x 1 x 250	1801.66[ab]	75.16	98.33[a]	4.10[ab]	1900.0[a]	79,26
LSDX P_1 X V_2	10 x 1 x 500	1891.66[a]	74.20	96.03[ab]	4.26[ab]	2000.0[a]	78.46
LSDX P_2 X V_1	10 x 2 x 250	1868.33[ab]	74.76	100.33[a]	4.01[ab]	1968.33[a]	78.76
LSDX P_2 X V_2	10 x 2 x 500	1906.66[a]	75.26	96.66[bc]	3.80[b]	2003.33[a]	79.06
HSDX P_0 X V_1	16 x 0 x 250	1418.33[d]	73.73	85.11[bc]	4.43[ab]	1503.33[c]	78.16
HSDX P_0 X V_2	16 x 0 x 500	1493.33[d]	72.26	85.01[bc]	4.10[ab]	1578.33[c]	76.36
HSDX P_1 X V_1	16 x 1 x 250	1503.33[d]	73.93	85.01[bc]	4.13[ab]	1588.33[c]	78.13
HSDX P_1 X V_2	16 x 1 x 500	1533.33[d]	74.20	81.66[c]	3.93[ab]	1615.0[c]	78.17
HSDX P_2 X V_1	16 x 2 x 250	1460.33[d]	74.1	83.33[c]	4.23[ab]	1543.33[c]	78.40
HSDX P_2 X V_2	16 x 2 x 500	1443.33[d]	73.50	81.66[c]	4.16[ab]	1525.0[c]	77.70
MSE		**43.83**	**0.90**	**3.69**	**0.19**	**44.29**	**0.95**

Blood constituents

Plasma protein fractions:

The obtained results in table (3) showed that the broiler chicks kept under LSD recorded significantly the higher averages of plasma total protein, albumin and globulin at 4th and 6th week of age, than those kept under HSD. These results are agree with those reported by **Tong *et al*., (2012)** who found significant differences in protein fractions (P>0.05) of plasma broiler chicken due to the effect of stocking density. Concerning to the effect of probiotic on plasma protein fractions, it is clearly observed that chicks fed on diet supplemented with probiotic at a level of 2 g/kg diet showed significantly the higher averages of plasma total protein, albumin, globulin and A/G ratio at 4 and 6 wks of age, followed by those fed diet witha level of 1 g/kg diet or non- supplemented one, respectively. These results agreed with those reported by **Mohammedi *et al*., (2016)** who showed that the supplementation of probiotic in broiler diets had positive effect on plasma albumin value of broiler chickens compared with control. However, it was disagree with those reported by **Sherif (2009b)** who found that plasma blood parameters of broiler chickens weren't affected by adding probiotic (Avian plus) in their diets. Chicks fed diet supplemented with V.D3 at level of 500 IU/Kg diet showed significantly the higher averages of plasma total protein, albumin and globulin at 4 and 6 wks of bird's age than those fed diet supplemented with V.D3 at a level of 250 IU/Kg diet. The interactions between LSD X P1 X V2 showed the highest averages of plasma total protein and globulin mounted (3.16 and 1.86 g/dI), respectively at the 6th week of age. However, the interaction between HSD X P1X V2 showed the highest average of plasma albumin and A/G ratio at the 6th week of bird's age when compared with different interaction effects.

Table 3. Least –square means and pooled standard error for plasma protein fractions (total protein, albumin and globulin and A/G ratio) of different experimental groups as affected by studied factors

Treatment	level	Plasma total protein(g/dI) at		Plasma albumin (g/dI) at		Plasma globulin (g/dI) at		Plasma A/G ratio(g/dl) at	
		4 wks	6wks	4wks	6wks	4wks	6wks	4wks	6wks
Stoking density(SD) bird/m^2	10	3.0	2.74^{a}	1.20^{a}	1.46^{a}	1.53^{a}	1.74^{a}	0.78	0.72
	16	2.98	2.57^{b}	1.13^{b}	1.38^{b}	1.44^{b}	1.60^{b}	0.78	0.86
MSE		**0.011**	**0.013**	**0.007**	**0.011**	**0.009**	**0.013**	**0.005**	**0.011**
Probiotic (P) g /kg	0	2.56^{c}	2.89^{c}	1.25^{b}	1.12^{c}	1.44^{b}	1.64^{b}	0.77	0.76
	1	2.67^{b}	$3{,}08^{b}$	1.37^{a}	1.17^{b}	1.49^{a}	1.71^{a}	0.78	0.80
	2	2.74^{a}	3.11^{a}	1.53^{a}	1.21^{a}	1.53^{a}	1.73^{a}	0.79	0.81
MSE		**0.016**	**0.013**	**0.013**	**0.009**	**0.01**	**0.016**	**0.006**	**0.013**
Vitamin D3(V.D$_3$) IU/kg	250	2.63^{b}	2.94^{b}	1.15^{b}	1.30^{b}	1.47^{b}	1.64^{b}	0.78	0.79
	500	2.68^{a}	3.04^{a}	1.18^{a}	1.34^{a}	1.50^{a}	1.70^{a}	0.78	0.79
MSE		**0.013**	**0.011**	**0.007**	**0.05**	**0.01**	**0.013**	**0.013**	**0.011**
Interaction									
LSDX P_0 X V_1	10 x 0 x 250	2.62^{dc}	2.81^{e}	1.13^{dc}	1.16^{d}	1.48^{bc}	1.65^{cd}	0.76	0.71
LSDX P_0 X V_2	10 x 0 x 500	2.73^{ab}	2.98^{dc}	1.20^{ab}	1.26^{c}	1.51^{ab}	1.71^{bc}	0.79	0.74
LSDX P_1 X V_1	10 x 1 x 250	2.74^{ab}	3.03^{bc}	1.20^{ab}	1.28^{c}	1.54^{ab}	1.75^{bc}	0.77	0.73
LSDX P_1 X V_2	10 x 1 x 500	2.76^{a}	3.16^{a}	1.22^{ab}	1.30^{c}	1.54^{ab}	1.86^{a}	0.79	0.69
LSDX P_2 X V_1	10 x 2 x 250	2.78^{a}	3.00^{dc}	1.22^{a}	1.30^{c}	1.56^{a}	1.70^{cd}	0.78	0.76
LSDX P_2 X V_2	10 x 2 x 500	2.81^{a}	3.05^{bc}	1.25^{a}	1.26^{c}	1.57^{a}	1.78^{ab}	0.80	0.70
HSDX P_0 X V_1	16 x 0 x 250	2.46^{e}	2.83^{e}	1.08^{de}	1.27^{c}	1.38^{d}	1.56^{f}	0.78	0.82
HSDX P_0 X V_2	16 x 0 x 500	2.45^{e}	2.93^{d}	1.06^{e}	1.30^{c}	1.38^{d}	1.63^{de}	0.77	0.79
HSDX P_1 X V_1	16 x 1 x 250	2.56^{d}	3.01^{dc}	1.13^{dc}	1.40^{b}	1.43^{cd}	1.61^{de}	0.79	0.86
HSDX P_1 X V_2	16 x 1 x 500	2.61^{dc}	3.11^{ab}	1.13^{dc}	1.50^{a}	1.48^{bc}	1.61^{de}	0.76	0.92
HSDX P_2 X V_1	16 x 2 x 250	2.63^{dc}	2.98^{dc}	1.16^{bc}	1.40^{b}	1.46^{bc}	1.58^{f}	0.79	0.89
HSDX P_2 X V_2	16 x 2 x 500	2.73^{ab}	3.03^{bc}	1.20^{ab}	1.43^{ab}	1.52	1.60^{ef}	0.79	0.89
MSE		**0.03**	**0.02**	**0.01**	**0.02**	**0.02**	**0.03**	**0.01**	**0.02**

Plasma cholesterol and triglycerides:

Results obtained in table (4) revealed that, broiler chicks that kept under LSD recorded the higher averages of plasma cholesterol and triglycerides at 4 and 6 wks of bird's age, than those kept under HSD. This result disagree with those reported by **Thaxton *et al*. (2006)**who found that stocking density didn't result in a recognizable trend in plasma cholesterol of broiler chickens. Concerning to the effect of probiotic on plasma cholesterol, it is clearly observed that chicks fed on diet supplemented with probiotic at a level of 2 g/kg diet showed a higher average of plasma cholesterol at the 6th week (157.5 g/dI) of age, followed by those fed diet supplemented with 1g(156.4 g/dI), compared with those fed diet without probiotic supplementation (155.0 g/dI).Opposite results were observed in plasma triglycerides which decreased with increasing dietary levels of probiotic supplementation. These results disagree with those stated by **Ignatova *et al*. (2009)** they found that probiotic supplementation leads to a significant decrease in blood plasma cholesterol and/or triglycerides of broiler chickens. Chicks fed diet supplemented with V.D3 at a level of 250 IU/Kg diet showed the lowest averages of plasma cholesterol (154.3 g/dI) and triglycerides (148.6 g/dI) at the 6 wks of age when compared with those fed diet supplemented with V.D3 at a level of 500 IU/Kg (158.3 and 148.9 g/dI) for plasma cholesterol and triglycerides, respectively. The interaction between HSD X P0 XV1 showed the lowest level of plasma cholesterol at the 6thwk of bird's age. However, the interaction effect between LSD X P1 X V1 showed the lowest averages of plasma triglycerides at the 6 wk of bird's age, compared with different interaction effects**.**

Table 4. Least – square means and pooled standard error for plasma cholesterol and triglycerides of different experimental groups as affected by studied factors

Treatment	level	Plasma cholesterol (g/dI)at		Plasma triglycerides (g/dI)at	
		4wks	6wks	4wks	6wks
Stoking density (SD) bird/m²	10	151.61ᵃ	157.72ᵃ	113.7	149.16
	16	144.44ᵇ	154.88ᵇ	113.2	148.33
MSE		**0.38**	**0.49**	1.12	4.45
Probiotic (P) g /kg	0	146.91ᵇ	155.00ᵇ	113.66	149.50
	1	148.33ᵃ	156.41ᵃᵇ	113.25	147.91
	2	148.80ᵃ	157.50ᵃ	113.58	148.83
MSE		**0.46**	**0.60**	1.37	4.45
Vitamin D3 (V.D₃)IU/kg	250	146.05ᵇ	154.27ᵇ	113.94	148.55
	500	150.00ᵃ	158.33ᵃ	113.05	148.94
MSE		**0.38**	**0.49**	1.12	4.45
Interaction					
LSDX P₀ X V₁	10 x 0 x 250	148.00ᵈᵉ	156.33ᶜᵈ	113.16	148.36
LSDX P₀ X V₂	10 x 0 x 500	152.33ᵇᶜ	157.00ᶜᵈ	113.24	148.33
LSDX P₁ X V₁	10 x 1 x 250	149.66ᵈᶜ	155.00ᶜᵈ	113.12	147.45
LSDX P₁ X V₂	10 x 1 x 500	154.66ᵃᵇ	159.33ᵃᵇ	113.62	149.53
LSDX P₂ X V₁	10 x 2 x 250	149.66ᵈᶜ	156.00ᶜᵈ	113.35	147.90
LSDX P₂ X V₂	10 x 2 x 500	155.33ᵃ	162.66ᵃ	113.58	148.95
HSDX P₀ X V₁	16 x 0 x 250	142.66ʰ	150.00ᵉ	113.37	148.45
HSDX P₀ X V₂	16 x 0 x 500	144.66ʰᵍ	156.66ᶜᵈ	113.22	148.64
HSDX P₁ X V₁	16 x 1 x 250	142.66ʰ	153.33ᵈᵉ	113.25	148.54
HSDX P₁ X V₂	16 x 1 x 500	146.33ᶠᵉ	158.00ᵇᶜ	113.12	149.55
HSDX P₂ X V₁	16 x 2 x 250	143.66ʰᵍ	155.00ᶜᵈ	113.35	147.90
HSDX P₂ X V₂	16 x 2 x 500	146.66ᶠᵉ	156.33ᶜᵈ	113.58	148.95
MSE		**0.93**	**1.21**	12.37	4.48

Mean having similar letters in each column are not significantly different.

Plasma aspartate aminotransferase (AST) and plasma alanine aminotransferase (ALT):

Results obtained in table (5) revealed that the broiler chicks kept under LSD recorded significantly the lower averages of plasma AST and ALT than those kept under HSD at 4 and 6 wks of bird's age. This result agreed with those reported by **Byung *et al* (2018)** who stated that plasma blood aspartate aminotransferase and alanine aminotransferase were significant higher (P< 0.05) in the high stocking density groups than those raised in low and medium stocking density one. Chicks fed diet supplemented with probiotic at a level of 2 g/kg diet showed significantly the lowest averages of plasma AST and ALT (19.11 and 16.11 U/L, respectively), followed by those fed diet supplemented with 1/g (19.15 and 16.27, respectively U/L), compared with those fed on diet with non supplemented probiotic (21.29 and 18.54U/L, respectively) at the 6 wk of bird's age. Concerning to the effect of V. D3 on liver function, it is observed that chicks fed diet supplemented with V.D3 at a level of 500 IU/Kg diet decreased significantly averages of plasma AST and ALT, than those fed on diet supplemented with V.D3 at a level of 250 IU/Kg diet at the 4thand 6thwks of bird's age. The interaction effect between LSD X P2 X V1 significantly decreased average of plasma AST. While, the interaction effect between LSD X P1 X V2 significantly decreased average of plasma ALT at 4 and 6 weeks of bird's age, compared with different interaction effects.

Table 5. Least –square means and pooled standard error for plasma AST and ALT of different experimental groups as affected by studied factors

Treatments	level	Plasma (AST) (U/L)at		Plasma (ALT) (U/L)at	
		4wKs	6WKs	4wKs	6WKs
Stoking density(SD)	10	15.16^{b}	19.55^{b}	11.27^{b}	16.70^{b}
bird/m^2	16	15.55^{a}	20.08^{a}	11.71^{a}	17.23^{a}
MSE		**0.07**	**0.07**	**0.06**	**0.10**
Probiotic (P) g /kg	0	15.94^{a}	21.29^{a}	12.08 ^{a}	18.54^{a}
	1	15.15^{b}	19.15^{b}	11.27^{b}	16.27^{b}
	2	14.98^{b}	19.11^{b}	11.12^{b}	16.10^{b}
MSE		**0.07**	**0.09**	**0.10**	**0.12**
Vitamin D3(V.D$_3$)IU/kg	250	15.56^{a}	20.22^{a}	11.68^{a}	17.31^{a}
	500	15.16^{b}	19.41^{b}	11.30^{b}	16.62^{b}
MSE		**0.07**	**0.07**	**0.06**	**0.10**
Interaction					
LSDX P$_0$ X V$_1$	10 x 0 x 250	16.16^{a}	21.66^{b}	12.36^{a}	19.03^{ab}
LSDX P$_0$ X V$_2$	10 x 0 x 500	15.33^{dc}	20.00^{c}	11.43^{c}	17.16^{c}
LSDX P$_1$ X V$_1$	10 x 1 x 250	15.16^{ce}	19.36^{de}	11.31^{cd}	16.40^{cd}
LSDX P$_1$ X V$_2$	10 x 1 x 500	14.90^{ce}	19.33^{de}	10.80^{e}	15.50^{e}
LSDX P$_2$ X V$_1$	10 x 2 x 250	14.80^{de}	18.79^{ef}	10.90^{de}	15.93^{de}
LSDX P$_2$ X V$_2$	10 x 2 x 500	14.63^{e}	19.00^{e}	10.83^{e}	16.20^{de}
HSDX P$_0$ X V$_1$	16 x 0 x 250	16.33^{a}	22.33^{a}	12.50^{a}	19.50^{a}
HSDX P$_0$ X V$_2$	16 x 0 x 500	15.93^{ab}	21.16^{b}	12.03^{b}	18.46^{b}
HSDX P$_1$ X V$_1$	16 x 1 x 250	15.43^{bc}	19.70^{dc}	11.53^{c}	16.66^{cd}
HSDX P$_1$ X V$_2$	16 x 1 x 500	15.13^{ce}	18.80^{ef}	11.43^{c}	15.83^{de}
HSDX P$_2$ X V$_1$	16 x 2 x 250	15.64^{e}	19.33^{de}	11.46^{c}	16.36^{cd}
HSDX P$_2$ X V$_2$	16 x 2 x 500	15.05^{ce}	19.20^{de}	11.30^{cd}	16.60^{cd}
MSE		**0.17**	**0.19**	**0.14**	**0.25**

Mean having similar letters in each column are not significantly different.

Plasma uric acid and creatinine

Data presented in table (7) revealed that, no significant variations were found in plasma uric acid and creatinine of broiler chickens due to the effect of stoking density (SD), probiotic (P), Vit.D3 (V.D3) supplementation and the interaction between them at all time of estimations. Although there were no significant effects of studied factors on kidney function, broiler chicks kept under HSD showed the lowest average of plasma uric acid (4.75 and 4.50 mg/dI at 4 and 6 weeks of age, respectively). However chicks kept under LSD decreased plasma creatinine (0.22 mg/dI) at 6 weeks of age). Concerning to the effect of Probiotic on plasma uric acid and creatinine, it is clearly observed that chicks fed diet supplemented with probiotic at a level of 1 g/kg diet showed lowest plasma uric acid at the 6 week of age (4.68 mg/dI), However chicks fed diet supplemented with 2g/kg showed the lowest plasma creatinine (0.14mg/dl) at the 6 week of age. These result disagree with those reported by **Li et al. (2008)** who demonstrated that, there was a decrease in blood uric acid by 22.39% due to probiotic supplementation (*Bacillus subtilis*) of quail birds. Chicks fed diet supplemented with V.D3 at a level of 500 IU/Kg diet showed the lowest averages of plasma uric acid (4.73 and 4.64 mg/dI at 4 and 6 weeks of age, respectively) while, chicks fed diet supplemented with V.D3 at a level of 250 IU/kg showed the lowest averages of plasma creatinine (0.22 and 0.19IU/kg at 4 and 6 weeks of age, respectively). The interaction effects between LSD X P2 XV1 and between LSD X P1X V2 decreased plasma creatinine and uric acid, respectively at the 6 weeks of age when compared with different interaction applied.

Table 7. Least – square means and pooled standard error for plasma uric acid and creatinine of different experimental groups as affected by studied factors

Treatments	level	Plasma creatinine (mg/dL) at		Plasma uric acid(mg/dl) at	
		4WKS	6WKS	4WKS	6WKS
Stoking density (SD) bird/m²	10	0.25	0.22	4.77	4.73
	16	0.17	0.39	4.75	4.50
MSE		**0.017**	**0.018**	**0.53**	**0.53**
Probiotic (P) g /kg	0	0.10	0.14	4.79	4.70
	1	0.09	0.15	4.85	4.68
	2	0.10	0.14	4.63	4.71
MSE		**0.021**	**0.022**	**0.65**	**0.44**
Vitamin D3 (V.D3) IU/kg	250	0.22	0.19	4.78	4.67
	500	0.24	0.30	4.73	4.64
MSE		**0.018**	**0.017**	**0.53**	**0.36**
Interaction					
LSDX P_0 X V_1	10 x 0 x 250	0.25	0.37	4.86	4.89
LSDX P_0 X V_2	10 x 0 x 500	0.24	0.24	4.78	4.68
LSDX P_1 X V_1	10 x 1 x 250	0.28	0.27	4.89	4.71
LSDX P_1 X V_2	10 x 1 x 500	0.20	0.26	4.78	4.45
LSDX P_2 X V_1	10 x 2 x 250	0.22	0.15	4.67	4.76
LSDX P_2 X V_2	10 x 2 x 500	0.34	0.42	4.40	4.78
HSDX P_0 X V_1	16 x 0 x 250	0.20	0.22	4.87	4.53
HSDX P_0 X V_2	16 x 0 x 500	0.27	0.16	4.79	4.63
HSDX P_1 X V_1	16 x 1 x 250	0.46	0.18	4.86	4.57
HSDX P_1 X V_2	16 x 1 x 500	0.17	0.27	4.83	4.61
HSDX P_2 X V_1	16 x 2 x 250	0.32	0.29	4.40	4.51
HSDX P_2 X V_2	16 x 2 x 500	0.25	0.26	4.63	4.67
MSE		**0.043**	**0.046**	**1.53**	**0.92**

Mean having similar letters in each column are not significantly different.

Plasma calcium and inorganic phosphorus:

Results obtained in table (8) showed highly significant variations in plasma calcium and inorganic phosphorus at 4 and 6 weeks of age due to the effect of Vit.D3 supplemented level and the interaction effects between all factors studied showed highly significant variations on plasma calcium and inorganic phosphorus at 4 and 6 weeks of bird's age. Broiler chicks fed on diet supplemented with V.D3 at a level of 500 IU/Kg diet showed significantly the higher averages of plasma calcium and inorganic phosphorus at 4 and 6 weeks of birds age than those fed on diet supplemented with V.D3 at a level of 250 IU/Kg. Chicks fed diet supplemented with probiotic at a level of 1 g/kg diet showed the highest averages of plasma calcium and inorganic phosphorus (10.57 and 6.57mg/dI, respectively) at the 6 week of age. The interaction between HSD X P0 X V2 and between LSD X P1 X V2 showed significantly the highest average of plasma calcium (13.96 mg/dl) and inorganic phosphorus (7.90 mg/dl) at the end of the experimental period compared with different interaction applied. These results are agree with those reported by **Whitehead (2004)** who sated that plasma blood ionized calcium, adjusted to pH 7.4, showed increases ($P < 0.05$) with increasing vitamin D3 supplementation for all combinations of Ca and P except, the diet containing 13 g Ca/5 g P. However, these results disagreed with those found by **Felix Shih-Hsiang Hsiao1 (2018)** who reported that, no significant effect on serum calcium and serum phosphorus of broiler chickens dietary supplementation of vitamin D3.

Table 8. Least –square means and pooled standard error for plasma calcium and inorganic phosphorus of different experimental groups as affected by studied factors

Treatments	level	Plasma calcium (mg/dI) at		Plasma inorganic phosphorus (mg/dL) at	
		4wKS	6WKS	4wKS	6WKS
Stoking density (SD) bird/m^2	10	12.12	10.57	8.20	6.54
	16	12.14	10.41	7.95	6.45
MSE		**0.22**	**0.16**	**0.15**	**0.10**
Probiotic (P) g /kg	0	12.19	10.36	7.98	6.54
	1	12.12	10.57	7.99	6.57
	2	12.08	10.54	8.25	6.37
MSE		**0.26**	**0.19**	**0.19**	**0.13**
Vitamin D3 (V.D$_3$)IU/kg	250	10.81^b	9.24^b	7.35^b	5.43^b
	500	13.45^a	11.74^a	8.80^a	7.55^a
MSE		**0.22**	**0.16**	**0.15**	**0.10**
Interaction					
LSDX P_0 X V_1	10 x 0 x 250	9.43^b	10.70^b	7.60^{bc}	5.53^b
LSDX P_0 X V_2	10 x 0 x 500	11.36^a	13.26^a	8.90^a	7.50^a
LSDX P_1 X V_1	10 x 1 x 250	9.70^b	10.76^b	7.20^c	5.60^b
LSDX P_1 X V_2	10 x 1 x 500	11.70^a	13.36^a	8.96^a	7.90^a
LSDX P_2 X V_1	10 x 2 x 250	9.33^b	10.93^b	7.65^{bc}	5.10^b
LSDX P_2 X V_2	10 x 2 x 500	11.90^a	13.90^a	9.00^a	7.63^a
HSDX P_0 X V_1	16 x 0 x 250	8.60^b	10.83^b	7.13^c	5.43^b
HSDX P_0 X V_2	16 x 0 x 500	12.06^a	13.96^a	8.30^{abc}	7.70^a
HSDX P_1 X V_1	16 x 1 x 250	9.16^b	11.36^b	7.10^c	5.50^b
HSDX P_1 X V_2	16 x 1 x 500	11.76^a	13.00^b	8.70^{ab}	7.30^a
HSDX P_2 X V_1	16 x 2 x 250	9.16^b	10.30^a	7.50^{bc}	5.64^b
HSDX P_2 X V_2	16 x 2 x 500	11.76^a	13.40^b	8.96^a	7.30^a
MSE		**0.33**	**0.54**	**0.40**	**0.26**

Mean having similar letters in each column are not significantly different

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