

INCREASING STOCKING DENSITY OF EGYPTIAN LAYING HENS BY USING:

3 – Increasing protein level & Betaine supplementation

By

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ABSTRACT: *This study was conducted to increasing stoking density of Egyptian laying hens from 8 to 12 birds / m². Also, to investigate the ability of increasing dietary protein level from 16 to 18% or supplemental 2 ppm betaine (trimethyl glycine) in laying hens diet to alleviate the deleterious effects of increasing stocking density to 12 birds / m². A total number of 330 pullets and 30 cocks of Dandarawy (20 wks of age) were randomly chosen and allocated into six groups. The first three groups were placed in 8 birds / m² to achieve hen densities of 1250 cm² / bird. The other three groups were placed in 12 birds / m² to achieve hen densities of 834 cm²/ bird. All the while, every stocking density category was fed the basal diet 16 % crude protein (control), basal diet with 18% crude protein, or basal diet enriched with 4 ppm betaine, respectively.*

Statistical analyses revealed that increasing stoking density reduced ($P<0.05$) body weight gain, egg production rate, total egg mass and feed consumption. However, mortality rate and erythrocyte cells were increased ($P<0.05$) while, feed conversion was improved ($P<0.05$). Conversely, increasing dietary protein from 16 to 18 % or adding 2 ppm betaine to layer diets under increasing stoking density improved ($P<0.05$) all previous parameters. Also, egg quality factors such as Haugh unit, shell thickness and yolk color were increased ($P<0.05$) by increasing dietary protein level however, betaine supplementation had no effect on interior or exterior egg quality. Increasing dietary protein had no effect on metabolic profile under taken however, betaine supplementation reflected ($P<0.05$) positive effects on total protein as well as albumin and globulin. Fertility, hatchability and hatched chick weight percentages did not affect by dietary treatments. Increasing stocking density causing raise ($P<0.05$) in heterophil cells number, reduction ($P<0.05$) in lymphocyte cells number, increase ($P<0.05$) in Heterophils / Lymphocytes ratio and also appearance of monocyte cells. Conversely, increasing dietary protein level caused increase ($P<0.05$) in

leucocytes which attribute to increase ($P < 0.05$) in lymphocytes, Heterophil cells number did not significantly differ, Heterophils / Lymphocytes ratio was increased ($P < 0.05$), and monocyte cells were disappear, indicating that the treatment can ameliorate haematological stress of increasing densities. However, betaine supplementation had partially effect on blood hematology. Briefly, increasing dietary protein level or betaine supplementation to laying hens diet can be considered as a potential protective management practice in preventing the detrimental effects of increasing stocking density of laying hens from 8 to 12 birds / m^2 and that does not lead to excessively poor bird welfare, whereupon, achieves assurance to this new stocking density.

INTRODUCTION

Poultry managers are interested to increasing the number of birds per space area, and reducing housing, and labor costs per bird. Stocking density has become a highly important economic factor due to the intensification of industrial poultry production, which resulted in decreased performance per bird housed and too small profit margins (Carey et al., 1995). Stocking density is one component of the environment that plays a critical role in determining the well being of a bird (Lee and Moss, 1995). Several studies have measured the stocking density on bird performance (Carey et al, 1995; Patterson and Siegel 1998; and Carmichael, et al 1999).

Protein level of laying hens diets has great been consideration in nutritional mind due to its great effect on the production parameters. Laying hens responses to dietary protein level under different densities has been controversy of poultry physiologists or husbandmen for many coming years. Increasing dietary protein level lead to increases egg production reported as early as by Fernandez, *et al.* (1973) and egg weight of layers was also reported by Summers (1993). Also, feed conversion was improved due to high dietary protein level (Calderon and Jensen 1990 and Angelovicova 1994). Immune responses expressed as numbers of lymphocyte cells in the thymus of chickens were increased as dietary protein level increased (Cheema *et al.*, 2003). Furthermore, several researches have compared the growth of chickens fed diets with different levels of protein during different intervals (Keshavarz and Nakajima, 1995; El-Sayed, *et al.*, 2001 and Essa *et al.*, 2003).

Additives added to diets in very small quantities with objective of obtaining some special effect. Betaine, known as glycine betaine or trimethyl glycine, is a tertiary amine formed by the oxidation of choline and implicated in methionine sparing, osmoprotective, and fat distribution

(Wang *et al.*, 2004). Betaine is a source of labile methyl groups and plays an important role in methylation reactions (Kettunen *et al.*, 2001). Betaine was effective in improving growth and feed conversion and has a methionine sparing effect in broilers and ducks (Garcia *et al.* 2000 and Wang *et al.*, 2004), and had a positive effect on immune response (Swain and Johri, 2000; Kettunen *et al.*, 2001 and Remus *et al.*, 2004). Betaine supplementation seems to be important to improve productivity and resistance to stress (Wang *et al.*, 2004). The principal physiological role of betaine is as an osmolyte and methyl donor (Craig, 2004). Betaine addition at 2 ppm may have the potential to improve the performance, feed conversion, eggshell breaking strength and liver betaine during the environmentally high temperature stress (Ryu *et al.*, 2002). However, betaine supplementation seems to be important to improve productivity and resistance to stress (Wang *et al.*, 2004). Betaine also reduces the negative effects of dehydration of high temperature or diseases (Garcia *et al.*, 2000). Very scanty studies involving dietary protein level or betaine supplementation of laying hens diets with increasing stocking density are available. For these reasons, this is the third attempt for the same authors, aims to increasing stoking density from 8 to 12 birds / m² and to evaluate the effects of increasing protein level from 16 to 18% or supplemental 2 ppm betaine in laying hens diet to alleviate the negative effects of increasing stocking density on productive performance, physiological stress and egg quality.

MATERIALS AND METHODS

The experimental work was carried out at Malloway Poultry Breeding Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Minia Governorate, Egypt.

Birds, diets and experimental design: Three hundred and sixty (330 pullets and 30 cocks), 20 wks old, Dandarawy hens having nearly similar body weight were housed in open floor pens (2m x 1.5m = 3 m² = 30000 cm²). Birds were randomly distributed into six treatment groups (Table, 1). Each of the groups 1st, 2nd and 3rd were consisted of 48 birds in two replicates (22 pullets and 2 cocks each) and placed in 8 birds /m² to achieve hen density of 1250 cm²/bird. While, each of the groups 4th, 5th and 6th were included 72 birds in two replicates (33 pullets and 3 cocks each) and placed in 12 birds / m² to achieve hen density of 834 cm²/ bird. The first and fourth groups were fed the basal diet 16% crude protein (Table, 2) and each of them considered as control. Also, second and fifth groups were fed basal diet with 18% crude protein instead of 16 %. Moreover, third and sixth groups were fed the basal diet enriched with 4 ppm betaine (97%) / kg feed.

In each treatment, the third replicate served as a reserve stock to compensate the dead birds. Similitude alive birds were substituted the weak or dead birds on day of death from a reserve stock to maintain constant densities. The birds were submitted to the same conditions of laying hens management under natural environment prevails in Upper Egypt. The male to female mating ratio were 1:11 according to Campo and Davila (2002). The experimental work lasted until 36 week of age.

Performance variables and egg quality: Individual body weights were recorded at the beginning and at the end of the study to calculate body weight gain. Feed consumption was measured weekly and feed conversion was calculated. The number of eggs and egg weight were recorded daily to calculate rate of laying and egg mass throughout the experiment period. Mortality was recorded daily. Egg quality measurements were determined at 36 week of age using 30 eggs per treatment laid on consecutive days. Egg shape index %, yolk index % and Haugh units (indicator for albumen quality) were calculated. Shell thickness was determined at three locations on the egg (air cell, equator, and sharp end) by using a dial pipe gauge. For evaluating egg fertility and hatchability, two hatches of eggs (60 eggs / treatment) were achieved when the birds were 32 and 36 weeks of age. Fertility was calculated as percentage of fertile eggs to total incubated eggs. Hatchability was calculated relative to total of fertile eggs. Weights of healthy hatched chicks were also recorded. Also, economical efficiency per square meter stocking density of egg production was calculated based on the prices of experimental diets and eggs in the Egyptian market at the experimental time.

Sample collection and laboratory analysis: At 36 wks old, 4 birds / treatment (2 birds / replicate) were slaughtered and blood samples were taken in heparinized tubes, erythrocytes and leucocytes and its differential were counted. Some of samples were centrifuged, plasma was separated and stored at -20°C for analyzes. Tri-iodothyronine T_3 (ng/dl), total protein (g/dl), albumin (g/dl), alanine transaminase ALT (U/L), aspartate transaminase AST (U/L) concentrations, and glucose (mg/dl) were determined. The manufacturer recommendations of commercial kits were used for all determinations.

Statistical analyses: Data were subjected to computerize two-way analysis of variance, and Duncan's multiple range test procedures using (SAS software, 1996). The percentage values were transferred to percentage angle using arcsine equation before subjected to statistical analysis.

RESULTS AND DISCUSSION

1 - Productive performance:

1. 1 – Body weight gain and mortality rate

As reported in Table 3, significant negative effects ($P<0.05$) on live weight gain and mortality rate were resulted by increasing stocking density due to treatments or regardless of dietary treatments (density overall mean), which has already been reported by Imaeda (2000) and Anderson et al. (2004). Conversely, live weight gain and mortality rate significantly ($P<0.05$) improved with increasing protein level to 18% or betaine supplements compared to the basal diet which contain 16% crude protein and zero betaine, due to densities or overall mean, as shown in Table (3). Parallel reports were noted by Douglas and Harms (1982); Makled et al., (2001); Zanaty et al. (2001); Essa et al. (2003) and Zanaty and Ibrahim (2005) when they increase protein level, and Augustine *et al.* (1997) and Waldenstedt *et al.* (1999) when they added betaine and reported that dietary addition improved performance of chickens.

1. 2 – Feed consumption and conversion:

Feed consumption was decreased ($P<0.05$) due to increasing stocking density comparing to normal stocking density. Feed conversion was improved ($P<0.05$) by increasing stocking density due to densities treatment or regardless of dietary treatment (Table, 3). Similar reports were noted by Sohail et al, (2001) and Tollba et al, (2006). Whilst, groups fed unsupplemented basal diet (16%) protein level consumed less ($P<0.05$) feed and impaired ($P<0.05$) feed conversion comparing to those fed (18%) or betaine supplements due to densities or overall mean (Table, 3). This result was in agreement with that reported by Makled et al. (2001), Essa et al. (2003) and Bunchasak et al. (2005) when they increased protein level. Calderon and Jensen (1990) observed an improvement in laying hens feed conversion due to high dietary protein level. However, Angelovicova (1994) found that a low-protein diet containing 14.1 % protein reduced daily feed intake average and improved feed conversion. Also, Ryu *et al.*, (2002) and Hassan *et al.*, (2005) when they reported that betaine supplementation at 0.072 and 0.144% significantly improved feed conversion rate.

1. 3 – Egg Production and egg weights:

In Table (3), increasing stocking density resulted in a significant ($P<0.05$) reduction in egg production rate or egg mass regardless of dietary treatments, which has already been reported by Sohail et al. (2001) and Anderson et al. (2004). However, increasing protein level or betaine

supplements to the diet reflected higher ($P < 0.05$) egg production rate and egg mass comparable to its control groups due to densities or overall mean. Besides, egg weight was numerically higher with increasing the level of crude protein only. Similar trends were found by Hammershoj and Kjaer (1999) and Bunchasak *et al.* (2005) who reported that high crude protein of 16 and 18 % tend to have better egg production, egg weight and egg mass than the lower level of 14 % crude protein. Also, increasing dietary protein level lead to an increase in egg production (Fernandez, *et al.*, 1973) and average egg weight of layers (Summers, 1993).

Concerning to the betaine supplements, the present results are in parallel with the finding of Castaing *et al.*, (2002) and Ryu *et al.*, (2002) reported that an improvement ($P < 0.05$) in egg production and egg mass were observed when 2,000 ppm betaine was fed to the laying hens during the environmentally high temperature stress. Also, they reported that, egg weights did not differ. They added that dietary supplemental betaine may have the potential to improve the performance of laying hens. The improving performance (body weight gain, mortality rate, feed conversion, egg Production and egg mass) due to betaine supplementation could be attributed to several reasons, as methyl donor group, its diverse physiological properties that could improve gut environment and thus enhance the ability of the chicks to withstand coccidial infection (Augustine *et al.*, 1997; Kettunen *et al.*, 2001 and Remus *et al.*, 2004), reduce intestinal membrane damage, dehydration, diarrhea and mal-digestion and/or absorption (Crompton, 1976; Kettunen *et al.*, 2001). Saunderson and Mackinlay (1990) reported that accumulation of betaine in the cell protects it from osmotic stress.

1.4 – Egg Quality factors:

All egg quality factors were unaffected significantly by increasing stocking density due to treatments or in over all mean, as showed in Table (4), support was found with report of Sohail *et al.* (2001). In general, there was a significantly ($P < 0.05$) increase only in Haugh unit, shell thickness and yolk color by increasing dietary protein level due to densities treatment or overall mean. However, Hammershoj and Kjaer (1999) stated that with increasing dietary protein, albumen quality traits and egg shell % decreased. It was observed that, no significant differences were noted in interior or exterior egg quality due to betaine supplementation. Matching results were reported by Castaing *et al.*, (2002) and Ryu *et al.*, (2002) they reported that egg weight and Haugh unit score did not differ when 2 ppm betaine was fed to the laying hens during the environmentally high temperature stress.

2– Fertility and hatchability percentage:

None of the increasing stocking density, increasing dietary protein level and betaine supplementation significantly altered the percent of fertility, hatchability, average values of relative hatched chick weight and absolute chick weight, due to densities treatment or in overall mean (Table, 5). Parallel results of density effect have been reported by Tollba et al (2006). Also, Richter and Henning (1979) reported that protein level had no significant effect on hatchability of fertile eggs.

Productive performance results mentioned above (Tables, 3, 4) show that the treatments have the potential to improve it under increasing stocking density, indicating that they can attenuate the increasing stocking density stress.

3– Blood biochemical parameters:

Supplemental betaine to layers diet caused ($P<0.05$) increase plasma concentration of total protein as well as albumin and globulin. However, T_3 , ALT or AST as liver function and glucose were not significantly affected comparing to control, due to densities or overall mean. Conversely, all previous parameters did not have any significant effects by increasing either stocking density or dietary protein (Table, 6). Similarly, Matthews and Southern (2000) and Hassan *et al.*, (2005) reported that the improvement in serum total protein, albumin and globulin due to betaine supplementation indicating further evidence about the role of methyl donor groups in protein metabolism. They added that no significant differences in the hepatic enzyme activities or liver functions as judged by liver enzymes of AST and ALT as a response to dietary betaine.

4 – Blood hematology

4.1 – Erythrocytes and Leucocytes cells:

Increasing stocking density recorded higher ($P<0.05$) values of erythrocytes and Leucocytes as well as Heterophils (H) of layers blood, with a corresponding lower ($P<0.05$) in lymphocytes cells number comparing to normal density due to treatments or regardless of dietary treatment, which has been reported by Tollba et al (2006). However, heterophil cells number did not significantly differ with either increasing dietary protein level or betaine supplementation comparing to their controls due to normal or increasing density. Increasing dietary protein level caused increasing ($P<0.05$) leucocytes cells which attributed to increase ($P<0.05$) lymphocyte cells comparing to their controls, in both densities or in overall mean (Table, 7), indicating that it's increased the immunity of hens and also can be

ameliorate haematological stress of increasing densities. Concurrent results were obtained by Cheema *et al.*, (2003) when reported that immune responses expressed as numbers of lymphocyte cells in the thymus of chickens were increased as dietary protein level increased. However, betaine supplements had no significantly affected in each of erythrocytes and leucocytes number where, the increases were numerically only. Remus *et al.*, (2004) reported that betaine had a positive effect on immune response.

4.2 – Heterophil : Lymphocyte ratio (H/L ratio)

Firstly, the heterophil to lymphocyte ratio has become widely accepted as a reliable and accurate physiological indicator of the stress response in chickens, exposure to stressors causes it to increase progressively, the reference values for it of about 0.2, 0.5 and 0.8 are characteristic of low, optimal and high degrees of stress, respectively (Gross and Siegel, 1993; Elston et al, 2000 and Shini, 2003).

The H/L ratio in the present study was significantly ($P<0.05$) increased in high density hens compared to normal density, similarly, to that of Tollba et al (2006). This result suggests that high density hens were more stressed. In response to increasing protein level, the H/L ratio in both densities was significantly ($P<0.05$) lower compared with that of their controls (Table 7). The significant decreases were due to increases in its denominator (lymphophilia), indicating that the treatment can be ameliorate haematological stress of increasing densities. Similarly, Cheema *et al.*, (2003) reported that numbers of lymphocyte cells in the thymus of chickens were increased as dietary protein level increased. The alterations in heterophils, lymphocytes cells or their ratio suggest that hens of different stoking densities should have a great effect on the stress response (Shini, 2003).

Seemingly, betaine supplements had an insignificantly decreased H/L ratio in both densities and that were due to insignificant in leucocytes which attributed to insignificant lymphocytes cells, indicating that the betaine can be ameliorate partially the haematological stress of increasing densities (Table, 7).

4.3 – Monocyte cells

Monocyte cells were associated with high stoking density (Table, 7), which has been reported previously by Tollba et al (2006). However, hens fed diet inclusive 4 ppm betaine or 18% crude protein had responded on monocyte cells, suggesting that diet treatments are effective to alleviate the

increasing stocking density stress. Shini, (2003) found an extreme stress monocytes becomes evident in avian species.

The blood hematology and biochemical results show that the supplementations involved in adjustment the H/L ratio and disappearance of monocyte cells, indicating that they can attenuate the physiological stress of increasing stocking density.

Economical efficiency:

Increasing protein level or supplementing diets with betaine resulted in increasing the relative economic efficiency % either in treatments or overall mean compared to the control groups. All the while, increasing stocking density to 12 birds / m² recorded higher relative economic efficiency % compared with that of 8 birds / m² due to overall mean (Table, 8). This improvement may be due to the high egg numbers as a result to increasing hen's density per square meter and / or adding of betaine or increasing crude protein level.

The economical efficiency should be re-evaluated when the prices of ingredients changes.

In conclusion, increasing protein level or betaine supplements provide the greatest performance in laying hens reared under increasing stocking density, and may offer a potential protective management practices to ameliorate or minimize increasing density-related depression in the performance and haematological stress of laying hens. Consequently, it is of interest to firmly recommend the new aspects (increasing stocking density to 50% with the supplements) as management practices to maximize profit, sharply increase economic return of laying hens, assurance the alternative of increasing stocking density to 12 birds /m² and that does not lead to excessively poor bird welfare.

Table (1): The experimental design was assigned as follows:

Group	birds /m ²	cm ² / bird	Crude protein	betaine
1	8	1250	16 %	0
2	8	1250	18 %	0
3	8	1250	16 %	2 ppm
4	12	834	16 %	0
5	12	834	18 %	0
6	12	834	16 %	2 ppm

Table (2): Composition and calculated analysis of the basal diet

Ingredients	% 16	% 18
Yellow corn	64.00	58.60
Soybean meal 44 %	24.78	30.55
Limestone	7.91	7.91
Di-calcium phosphate	1.61	1.61
Wheat bran	1.00	0.00
Soybean oil	0.00	0.63
Salt (NaCl)	0.30	0.30
Vit. & Min. Mixture *	0.30	0.30
DL-Methionine	0.10	0.10
Total	100.00	100.00
Calculated analysis :		
Metabolizable energy (Kcal /Kg)	2713.00	2700.77
Crude protein %	16.03	18.01
Crude fiber %	3.39	3.58
Crude fat %	2.84	3.31
Calcium %	3.34	3.41
Available phosphate %	0.42	0.43
Lysine %	0.89	1.04
Methionine %	0.39	0.41
Met + cystine %	0.66	0.71
Prices		
Price / kg of Feed (L.E)	2.19	2.27
Price of 4 ppm betaine (P.T)	0.01	0.00

* Supplied per Kg of diet: Vit. A, 10 000 IU; Vit. D₃, 2 000 IU; Vit. E, 10 mg; Vit. K₃, 1 mg; Vit. B₁, 1mg; Vit. B₂, 5 mg; Vit. B₆, 1.5 mg; Vit. B₁₂, 10 mcg; Niacin, 30mg; Pantothenic acid, 10mg; Folic acid, 1mg; Biotin, 50mcg; Choline, 260mg; Copper, 4 mg; Iron, 30mg; Manganese, 60mg; Zinc, 50mg; Iodine, 1.3mg; Selenium, 0.1mg; Cobalt, 0.1mg;

- Price of Ingredients L.E per ton: Yellow corn, 2050; Soybean meal 44 %, 2800; Limestone, 70; Di-calcium phosphate, 6000; Wheat bran, 1550; Soybean oil, 8000; Salt (NaCl), 250; Vit & Min. Mixture, 10000 and DL-Methionine, 35000 in the Egyptian market at the time of the experiment.

Table (3): Productive performance of Dandrawy laying hens fed diet fortified with betaine or increasing protein level under different stocking density (LSM±S.E)

Treatments	Control		Protein level		Betaine		Overall mean				
	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	Control	Protein level	Betaine
Body Weight gain (g)	232.56 ^c ±12.11	211.17 ^d ±14.22	268.25 ^a ±11.14	244.26 ^b ±15.26	262.19 ^a ±10.19	239.88 ^b ±20.15	254.33 ^a ±16.07	231.77 ^b ±18.54	221.86 ^b ±6.24	256.25 ^a ±6.94	251.03 ^a ±6.50
Mortality Rate %	6/48 12.50 ^b	10/72 13.60 ^a	4/48 8.33 ^f	8/72 11.11 ^c	5/48 10.42 ^d	7/72 9.72 ^e	15/144 10.42 ^b	23/216 11.48 ^a	13.05 ^a ±0.33	8.33 ^c ±1.73	10.07 ^b ±0.21
F. consumption (g/hen/day)	87.20 ^b ±7.25	76.56 ^d ±7.88	94.98 ^a ±8.51	83.62 ^c ±8.96	92.82 ^a ±10.05	82.40 ^c ±6.65	91.67 ^a ±12.11	80.86 ^b ±9.13	81.88 ^b ±3.12	87.55 ^a ±4.52	87.61 ^a ±2.83
F. Conversion (feed/mass)	4.38 ^a ±0.11	4.26 ^b ±0.09	4.19 ^c ±0.04	4.08 ^d ±0.03	4.25 ^b ±0.10	4.19 ^c ±0.09	4.27 ^a ±0.08	4.18 ^b ±0.05	4.32 ^a ±0.03	4.14 ^c ±0.04	4.22 ^b ±0.02
Egg laying rate (wk)	3.44 ^d ±0.09	3.10 ^e ±0.04	3.91 ^a ±0.06	3.53 ^c ±0.08	3.77 ^b ±0.07	3.39 ^d ±0.06	3.71 ^a ±0.07	3.34 ^b ±0.10	3.27 ^c ±0.09	3.72 ^a ±0.14	3.58 ^b ±0.11
Egg weight (g)	40.51 ^a ±0.16	40.58 ^a ±0.23	40.58 ^a ±0.31	40.62 ^a ±0.12	40.53 ^a ±0.20	40.56 ^a ±0.14	40.54 ^a ±0.24	40.59 ^a ±0.18	40.54 ^a ±0.02	40.60 ^a ±0.01	40.54 ^a ±0.02
Egg mass (g/hen/wk)	139.35 ^c ±10.33	125.80 ^d ±11.65	158.67 ^a ±10.13	143.39 ^c ±11.21	152.80 ^b ±12.05	137.50 ^c ±10.76	150.27 ^a ±12.21	135.56 ^b ±10.08	132.57 ^c ±3.95	151.03 ^a ±5.73	145.15 ^b ±4.23

a, b, ... f Means having different superscripts along a row in treatments or in overall means are significantly different (P<0.05).

Table (4): Egg quality factors of Dandrawy laying hens fed diet fortified with betaine or increasing protein level under different stocking density (LSM±S.E).

Treatments	Control		Protein level		Betaine		Protein level					
	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	Control	Protein level	Betaine	
Items												
Albumen weight %	52.74 ^a ±0.06	52.80 ^a ±0.05	52.67 ^a ±0.03	52.58 ^a ±0.04	53.20 ^a ±0.05	52.80 ^a ±0.05	52.87 ^a ±0.08	52.73 ^a ±0.06	52.77 ^a 0.57	52.76 ^a 0.57	53.00 ^a 0.58	
Shell weight %	14.11 ^a 0.21	14.02 ^a ±0.25	14.10 ^a ±0.24	14.09 ^a ±0.19	13.93 ^a ±0.18	14.10 ^a ±0.15	14.05 ^a ±0.20	14.07 ^a ±0.24	14.06 ^a 0.02	14.10 ^a 0.04	14.01 ^a 0.06	
Yolk weight %	33.05 ^a ±0.37	33.18 ^a ±0.49	33.23 ^a ±0.45	33.33 ^a ±0.41	32.87 ^a ±0.53	33.10 ^a ±0.55	33.05 ^a ±0.46	33.20 ^a ±0.53	33.11 ^a 0.05	33.28 ^a 0.04	32.98 ^a 0.08	
Haugh unit	80.78 ^b ±1.45	80.91 ^b ±1.61	81.55 ^a ±1.36	81.39 ^a ±1.89	80.63 ^b ±1.48	80.84 ^b ±1.59	80.98 ^a ±1.56	81.04 ^a ±1.61	80.84 ^b 0.15	81.47 ^a 0.26	80.73 ^b 0.20	
Yolk index	43.15 ^a ±0.48	43.55 ^a ±0.45	43.36 ^a ±0.52	43.41 ^a ±0.64	43.25 ^a ±0.37	43.24 ^a ±0.63	43.25 ^a ±0.59	43.40 ^a ±0.41	43.35 ^a 0.13	43.38 ^a 0.01	43.24 ^a 0.02	
Shell thickness (mm)	0.308 ^{cb} ±0.04	0.311 ^b ±0.05	0.319 ^a ±0.06	0.321 ^a ±0.07	0.302 ^c ±0.06	0.310 ^b ±0.04	0.309 ^a ±0.09	0.314 ^a ±0.06	0.309 ^b 0.01	0.320 ^a 0.00	0.306 ^b 0.00	
Shape index	74.50 ^a ±0.14	74.50 ^a ±0.22	74.80 ^a ±0.16	74.80 ^a ±0.12	74.70 ^a ±0.19	74.70 ^a ±0.11	74.67 ^a ±0.17	74.67 ^a ±0.14	74.50 ^a 0.23	74.80 ^a 0.05	74.70 ^a 0.11	
Yolk color	5.80 ^b ±0.16	5.80 ^b ±0.10	6.20 ^a ±0.10	6.60 ^a ±0.19	5.40 ^b ±0.09	5.40 ^b ±0.15	5.80 ^a ±0.12	5.93 ^a ±0.28	5.80 ^b 0.05	6.40 ^a 0.18	5.40 ^c 0.23	

a, b, c, Means having different superscripts along a row in treatments or in overall means are significantly different (P<0.05).

Table (5): Hatching characteristics of Dandrawy laying hens fed diet fortified with betaine or increasing protein level under different stocking density (LSM±S.E).

Treatments	Control		Protein level		Betaine		Overall mean				
	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	Control	Protein level	Betaine
Items											
Fertility %	87.40 ^a ±1.17	87.10 ^a ±1.60	87.50 ^a ±1.25	87.60 ^a ±1.70	86.90 ^a ±2.21	87.10 ^a ±1.75	87.27 ^a ±2.05	87.27 ^a ±1.69	87.25 ^a ±0.18	87.55 ^a ±0.20	87.00 ^a ±0.14
Hatchability %	85.10 ^a ±0.65	84.90 ^a ±0.82	85.07 ^a ±1.21	85.12 ^a ±0.73	84.80 ^a ±0.61	84.95 ^a ±0.54	84.99 ^a ±1.15	84.99 ^a ±1.03	85.00 ^a ±0.23	85.09 ^a ±0.05	84.88 ^a ±0.20
Absolute Chick weight (g)	29.55 ^a ±1.19	29.78 ^a ±1.23	29.43 ^a ±1.26	29.85 ^a ±1.04	29.25 ^a ±1.15	29.36 ^a ±1.51	29.41 ^a ±1.32	29.66 ^a ±1.36	29.66 ^a ±0.06	29.64 ^a ±0.12	29.30 ^a ±0.03
Chick weight %	63.50 ^a ±0.15	63.71 ^a ±0.21	63.42 ^a ±0.49	63.35 ^a ±0.27	63.12 ^a ±0.13	63.25 ^a ±0.37	63.35 ^a ±0.23	63.44 ^a ±0.34	63.60 ^a ±0.19	63.38 ^a ±0.22	63.18 ^a ±0.11

Means having different superscripts along a row in treatments or in overall means are significantly different (P<0.05).

Table (6): Plasma biochemical parameters of Dandrawy laying hens fed diet fortified with betaine or increasing protein level under different stocking density (LSM±S.E).

Treatments	Control		Protein level		Betaine		Overall mean					
	8	12	8	12	8	12	8	12	Control	Protein level	Betaine	
Items	birds/m ²	birds/m ²	birds/m ²	birds/m ²	birds/m ²	birds/m ²	birds/m ²	birds/m ²	birds/m ²	birds/m ²	birds/m ²	birds/m ²
T ₃ (ng/dl)	147.15 ^a ±10.12	142.94 ^a ±12.05	146.20 ^a ±11.36	142.42 ^a ±10.15	145.10 ^a ±14.95	142.16 ^a ±10.70	146.15 ^a ±11.85	142.51 ^a ±10.96	145.04 ^c ±6.98	144.31 ^b ±6.86	143.63 ^a ±6.62	
Total protein (g/dl)	4.28 ^b ±0.24	4.22 ^b ±0.28	4.30 ^b ±0.40	4.27 ^b ±0.11	4.49 ^a ±0.17	4.47 ^a ±0.22	4.36 ^a ±0.18	4.32 ^a ±0.19	4.25 ^b ±0.04	4.29 ^a ±0.01	4.48 ^a ±0.014	
Albumin (g/dl)	2.47 ^b ±0.06	2.44 ^b ±0.07	2.47 ^b ±0.03	2.41 ^b ±0.08	2.56 ^a ±0.02	2.57 ^a ±0.10	2.50 ^a ±0.09	2.47 ^a ±0.11	2.45 ^b ±0.041	2.44 ^a ±0.02	2.56 ^a ±0.03	
Globulin (g/dl)	1.81 ^b ±0.04	1.78 ^b ±0.06	1.83 ^b ±0.03	1.80 ^b ±0.04	1.93 ^a ±0.02	1.90 ^a ±0.06	1.86 ^a ±0.07	1.83 ^a ±0.05	1.79 ^b ±0.019	1.81 ^a ±0.01	1.91 ^a ±0.01	
(ALT) (U/L)	1.36 ^a ±0.01	1.33 ^a ±0.02	1.39 ^a ±0.04	1.31 ^a ±0.03	1.33 ^a ±0.02	1.28 ^a ±0.06	1.36 ^a ±0.05	1.31 ^a ±0.03	1.34 ^a ±0.01	1.35 ^a ±0.01	1.30 ^a ±0.04	
(AST) (U/L)	11.25 ^a ±0.99	11.02 ^a ±1.14	11.12 ^a ±1.11	10.88 ^a ±1.02	11.20 ^a ±1.20	11.50 ^a ±1.09	11.19 ^a ±1.13	10.98 ^a ±1.08	11.13 ^b ±0.35	11.00 ^{ab} ±0.35	11.35 ^a ±0.49	
Glucose (mg/dl)	42.10 ^a ±3.20	41.80 ^a ±2.05	42.20 ^a ±4.20	41.90 ^a ±5.72	41.83 ^a ±1.51	41.89 ^a ±5.12	42.04 ^a ±3.61	41.86 ^a ±4.67	41.95 ^a ±0.37	42.05 ^a ±0.12	41.86 ^a ±0.50	

a, b, c, Means having different superscripts along a row in treatments or in overall means are significantly different (P<0.05).

Table (7): Blood hematological parameters of Dandrawy laying hens fed diet fortified with betaine or increasing protein level under different stocking density (LSM±S.E).

Treatments	Control		Protein level		Betaine		Overall mean				Protein level	Betaine	
	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	Control	Protein level			Betaine
Items													
Erythrocytes (x10 ⁶)	1.43 ^c ±0.04	1.61 ^a ±0.06	1.55 ^b ±0.06	1.62 ^a ±0.08	1.45 ^c ±0.04	1.58 ^{ab} ±0.06	1.48 ^b ±0.10	1.69 ^a ±0.08	1.52 ^b ±0.05	1.58 ^a ±0.02	1.51 ^b ±0.04		
Leukocytes (x10 ³)	11.0 ^d ±0.92	14.0 ^{bc} ±1.02	13.0 ^c ±0.71	17.0 ^a ±0.82	12.0 ^{cd} ±0.56	15.0 ^b ±0.75	12.00 ^b ±0.87	15.33 ^a ±0.98	12.50 ^c ±1.04	15.00 ^a ±1.55	13.50 ^b ±0.81		
Heterophil (H)	33.0 ^b ±0.0	40.0 ^a ±0.0	32.0 ^b ±0.0	37.0 ^a ±0.0	32.0 ^b ±0.0	39.0 ^a ±0.0	32.33 ^b ±0.0	38.67 ^a ±0.0	36.50 ^a ±2.101	34.50 ^b ±1.82	35.50 ^{ab} ±1.04		
Lymphocytes (L)	65.0 ^b ±0.0	60.0 ^c ±0.0	70.0 ^a ±0.0	70.0 ^a ±0.0	65.0 ^b ±0.0	61.0 ^c ±0.0	66.67 ^a ±0.0	63.67 ^b ±0.0	62.50 ^b ±2.174	70.00 ^a ±0.57	63.00 ^b ±1.55		
H / L ratio	0.51 ^b ±0.0	0.66 ^a ±0.0	0.45 ^c ±0.0	0.52 ^b ±0.0	0.49 ^{cb} ±0.0	0.64 ^a ±0.0	0.48 ^b ±0.0	0.60 ^a ±0.0	0.58 ^a ±0.043	0.48 ^b ±0.02	0.57 ^a ±0.02		
Monocyte	-----	2.00	-----	-----	-----	-----	-----	2.00	2.00	-----	-----		

a, b, c, Means having different superscripts along a row in treatments or in overall means are significantly different (P<0.05).

Table (8): Economical efficiency per square meter stocking density of Dandrawy laying hens fed diet increasing protein level or fortified with betaine from 24 to 36 weeks of age.

Treatments	Control		Protein level		Betaine		Overall mean				
	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	8 birds/m ²	12 birds/m ²	Control	Protein level	Betaine		
total feed intake (Kg/H)	7.85	6.89	8.55	7.53	8.35	7.42	8.25	7.28	7.37	8.04	7.89
total feed intake (Kg/m ²) ¹	62.8	82.68	68.4	90.36	66.8	89.04	66.00	87.36	72.74	79.38	77.92
feed price / kg (L.E) ²	2.19	2.19	2.27	2.27	2.20	2.20	2.22	2.22	2.19	2.27	2.20
total feed Cost / m ² (L.E) ³	137.53	181.07	155.27	205.11	146.96	195.89	146.59	194.02	159.3	180.19	171.42
total eggs number / H	41.28	37.2	46.92	42.36	45.24	40.68	44.48	40.08	39.24	44.64	42.96
total eggs number / m ² ⁴	330.24	446.4	375.36	508.32	361.92	488.16	355.84	480.96	388.32	441.84	425.04
total eggs return / m ² (L.E) ⁵	165.12	223.2	187.68	254.16	180.96	244.08	177.92	240.48	194.16	220.92	212.52
net revenue (L.E) ⁶	27.59	42.13	32.41	49.05	34.00	48.19	31.33	46.46	34.86	40.73	41.10
economic efficiency ⁷	0.20	0.23	0.21	0.24	0.23	0.246	0.213	0.238	0.215	0.225	0.24
relative economic efficiency (%) ⁸	100	100	105	104.35	115.68	106.96	100	111.74	100	104.65	111.62

¹Total feed intake (Kg/m²) = Total feed intake (Kg/H) x number of birds / m² (8 or 12).
²Feed Price / Kg (L.E) as shown in table 2
³Total feed Cost / m² = Total feed intake (Kg/m²) x Feed Price / kg.
⁴Total Eggs number / m² = Total Eggs number / H x number of birds / m² (8 or 12).
⁵Eggs Return / m² = Total Eggs number / m² x Price of egg (0.50, L.E). (Price of each egg = 0.50 L.E at the time of experiment).
⁶Net revenue = Eggs Return / m² - Total feed cost / m²
⁷Economic efficiency = Net revenue / Total feed Cost / m²
⁸Relative economic efficiency (%), assuming the Control treatments = 100%.

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الملخص العربي

زيادة كثافة الدجاج البياض المصري باستخدام

3- زيادة مستوى البروتين & إضافة البيتاين

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أجريت هذه الدراسة لزيادة كثافة الدجاج البياض المصري (الندراوى) من 8 طائر/م² الي 12 طائر/م² ولمعرفة قدرة زيادة مستوى بروتين العليقة أو إضافة مركب البيتاين علي تخفيف أو إزالة إجهاد زيادة الكثافة علي الأداء الإنتاجي والفيولوجي للدجاج البياض. تم استخدام عدد 330 دجاجة و 30 ديك من نوع مصري (الندراوى) في عمر (20) أسبوع حيث وزعت الطيور عشوائيا إلى ستة مجموعات متساوية في الأوزان تقريبا. تم إسكان الثلاث مجموعات الأولى بكثافة 8 طائر / م² بمعدل 1250 سم² / طائر من مساحة الأرضية 0 أما الثلاث مجموعات الثانية تم إسكانها بكثافة 12 طائر / م² بمعدل 834 سم² / طائر من مساحة الأرضية 0 تم تربيتهم تحت ظروف بيئية متماثلة مع تغذية كل تقسيم أو تصنيف ل لكثافة علي عليقة المقارنة بها 16% بروتين خام أو عليقة تجريبية بها 18% بروتين خام أو مضاف إليها 4 جزء في المليون من مركب البيتاين علي الترتيب. وذلك في ثلاث مكررات تم جمع وتحليل بيانات مكررين فقط والمكرر الثالث كان احتياطيا لتعويض الطيور النافقة من المكررين تحت التجربة للحفاظ علي الكثافة (عدد الطيور / م²). استمرت التجربة لمدة 4 أشهر 20 - 36 أسبوع من العمر 0 أوضحت النتائج الأتي :-

- 1 - أدت الكثافة العالية الي انخفاض معنوي ($P < 0.05$) لكل من وزن الجسم المكتسب وإنتاج البيض (معدل وكتلة) و العلف المأكول و علي العكس كان هناك زيادة معنوية ($P < 0.05$) في معدل النفوق و كرات الدم الحمراء وكرات الدم البيضاء (monocyte and heterophil) وكذلك النسبة بين كرات الدم البيضاء المتعادلة والليمفوية (lymphocyte ratio / heterophil). وكذلك تحسنت الكفاءة التحويلية معنويا بمستوي ($P < 0.05$). بينما كان البروتين الكلى والألبومين والجلوبيولين ومستوى هرمون تراهي أيودوثيرونين (T_3) و ALT, AST والجلوكوز في بلازما الدم لم تتأثر معنويا 0
- 2 - أدت زيادة بروتين العليقة أو إضافة مركب البيتاين إلي تحسن معنوي ($P < 0.05$) في وزن الجسم المكتسب والعلف المأكول واللفظة تحويل الغذاء وعدد البيض وكتلته ومعدل إنتاج البيض وفي معدل النفوق وذلك في مجموعات الكثافة العالية (12 طائر / م²) أو في مجموعات الكثافة العادية (8 طائر / م²) عند المقارنه بمجموعات الكنترول وهذا دليل علي أن المعاملات التجريبية خففت معنويا من تأثير إجهاد الكثافة العالية
- 3 - أدت زيادة بروتين العليقة إلي تحسن معنوي ($P < 0.05$) في صفات جودة البيضة (لون الصفار وسمك القشرة و Haugh units) أما مع إضافة مركب البيتاين لم يتغير معنويا أي من صفات جودة البيضة.
- 4 - لم تؤدي زيادة بروتين العليقة أو إضافة مركب البيتاين إلي أي تحسن معنوي في نسبة التفريخ والخصب أو وقيم أوزان الكتاكيت المفرخة المطلقة أو النسبية.

- ٥ - أدت إضافة مركب البيتاين إلى زيادة معنوية ($P < 0.05$) في البروتين الكلى والألبومين والجلوبولين. أما قيم هرمون تراسي أيدوثيرونين (T_3), وكذلك إنزيمي AST, ALT , كوظائف للكبد و مستوى الجلوكوز لم تتأثر معنويا . بينما جميع القياسات السابقة لم تتأثر معنويا مع زيادة بروتين العليقة.
- ٦ - أدت زيادة بروتين العليقة أو إضافة مركب البيتاين إلى إنخفاض معنوي ($P < 0.05$) لنسبة خلايا (Heterophil(H) and Lymphocytes (L) (H / L ratio) وذلك دليل علي أن الإضافات المستخدمة خففت معنويا من haematological stress الناتج من تأثير إجهاد الكثافة العالية. أما إضافة مركب البيتاين أدي إلى إنخفاض عددي فقط
- ٧ - أدت زيادة بروتين العليقة إلى زيادة معنوي ($P < 0.05$) لنسبة خلايا Leucocytes وذلك يرجع إلى زيادة معنوي ($P < 0.05$) في خلايا الـ lymphocytes وذلك دليل علي أن الإضافات المستخدمة زادت من مناعة الطيور وخففت معنويا من haematological stress الناتج من تأثير إجهاد الكثافة العالية . أما إضافة مركب البيتاين أدي إلى زيادة عديدة فقط
- ٨ - أدت زيادة بروتين العليقة أو إضافة مركب البيتاين إلى إخفاء خلايا Monocyte وذلك دليل علي أن الإضافات المستخدمة خففت معنويا من haematological stress الناتج من تأثير إجهاد الكثافة العالية.
- ٩ - يستخلص من هذه النتائج إمكانية التوصية بزيادة بروتين العليقة إلى 18% بدلا من 16% أو إضافة مركب البيتاين إلى علائق الدجاج البيض بكثافة 12 طائر لكل متر مربع بدلا من 8 طائر لزيادة إنتاجية البيض من وحدة المساحة دون حدوث أثار جانبية ظاهرة على الأداء الإنتاجي أو الفسيولوجي أو أي تدهور في صفات جودة البيض 0 فضلا عن أن التطبيق الجديد (زيادة الكثافة بنسبة 50% مع الإضافات العلفية المستخدمة) يسبب إنخفاض 50% في تكاليف الإسكان والأجور والعمالة والأدوات لكل طائر أو لكل وحدة مساحة وبالتالي تعظيم الأرباح وزيادة العائد الاقتصادي لمزارع إنتاج البيض . والنتائج تؤكد علي إختيار زيادة الكثافة إلى 12 طائر لكل متر مربع وأن الإضافات المستخدمة قادرة علي تخفيف أو إزالة إجهاد زيادة الكثافة.