

INCREASING STOCKING DENSITY OF EGYPTIAN LAYING HENS BY USING: 2 – Enzymes & Tranquilizers

By

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Abstract: *A study was conducted to increasing stoking density of Egyptian laying hens from 8 to 12 birds / m². Also, to investigate the ability of supplemental Enzymes (Avizyme) or Tranquilizer (Diazepam) to alleviate the deleterious effects of increasing stocking density to 12 birds / m². A total of 330 pullets and 30 cocks of Dandarawy at 20 wks of age were randomly chosen. Birds were allocated into six groups. The first three groups were housed as 8 birds / m² to achieve hen density of 1250 cm²/bird. The other three groups were housed as 12 birds / m² to achieve hen density of 834 cm²/ bird. Every category was fed the basal diet, fortified with 1 gm avizyme / kg diet or 2 ppm diazepam, respectively.*

Results indicated that increasing stocking density (12 birds / m²) significantly reduced body weight gain, egg laying rate, total egg mass, feed consumption and lymphocyte cells number (L). Besides, mortality rate and erythrocyte cells were significantly increased but feed conversion was not affected. Conversely, in response to avizyme or diazepam supplements, live weight gain, mortality rate, feed consumption, feed conversion, egg laying rate and egg mass were improved ($P < 0.05$) in both densities or overall means, indicating that the two supplements alleviate the negative effects of increasing stocking density to 12 bird/m². Egg weights were not significantly influenced by either densities or dietary supplements. Except for yolk index which altered ($P < 0.05$) with enzyme supplementation, none of the egg quality parameters, fertility, hatchability, average values of relative hatched chick weight and absolute chick weight were affected by either dietary supplementations or both stocking densities. Diazepam supplementation reflected ($P < 0.05$) higher T₃ hormone and total protein as well as albumin and globulin, in both densities; however none of metabolic profile parameters were affected by the avizyme addition. A significant raise in heterophil cells (H) number, together with a corresponding significant reduction in lymphocyte cells (L) number also, significant increase in Heterophils / Lymphocytes ratio, and appearance of monocyte cells were

observed with increasing stocking density. Conversely, both types of supplementation caused ($P < 0.05$) decrease in Heterophil cells number, also ($P < 0.05$) increase in leucocytes which attribute to increase lymphocyte cells ($P < 0.05$), Heterophils / Lymphocytes ratio was significantly ($P < 0.05$) lower, and disappear of monocyte cells, indicating that the dietary supplementation could partially ameliorate haematological stress of increasing stocking density. The results would suggest that increasing stocking density from 8 to 12 birds / m^2 with avizyme or diazepam supplementation increased egg productivity and minimizing the physiological stress without worsen egg quality. Seemingly, the new aspects could be applicable to achieve sharp decreases (50%) in the housing, wages and labor, and equipment costs per hen subsequently maximizing profits and economic returns of layer farms and assurance the alternative of increasing stocking density to 12 birds / m^2 and that does not lead to excessively poor bird welfare.

INTRODUCTION

In poultry operations, stocking density has always been one of the major issues. Increase hen density has been a trend in the commercial layer corporations to minimize the housing, wages and equipment costs subsequently maximizes profits. However, it is claimed for higher mortality and lower egg production (Adams and Craig, 1984; Tauson, 1998; Bilcik and Keeling, 1999 Tollba *et al.*, 2006). It is well known that feed additives can be used safely in poultry diets to improve their performance.

Many enzymes have been found to be beneficial when added to poultry diets containing carbohydrate or protein sources, such as xylanase, protease, and amylase enzymes as consist of avizyme. Recent research has reemphasized that corn and soybean meal are incompletely digested by poultry (Pack and Bedford, 1997). Enzymes supplementation as a feed additive has become common since last four decades (Chesson, 1993). Moreover, their usage is especially common in European countries due to primarily their positive effects on animal performance as well as their lacking harmful effects on consumers (Dierck, 1989). Recently published studies suggested that enzymes may be beneficial in poultry diets (Wyatt, *et al.*, 1997; Zanella, *et al.*, 1999; and Douglas, *et al.*, 2000). Results reported by Ritz *et al.*, (1995 a, b); Marsman *et al.*, (1997); Lazaro *et al.*, (2003) and Wu *et al.*, (2005) showed that, on average, enzymes significantly improved cumulative: livability, eggs per hen house, and reduced feed per dozen eggs with no effect on feed per hen compared to laying hens fed diets with no enzyme supplementation.

Stocking density usually involve to increase competition, particularly for food, resulting in higher levels of aggression overall or directed at low ranked birds specifically (Hughes, *et al.*, 1997; Nicol, *et al.*, 1999; Newberry *et al.*, 2001; Estevez *et al.*, 2002). Diazepam is an anti-anxiety agent used primarily for short-term relief of mild to moderate anxiety and the associated nervousness, tension and muscle spasms. Diazepam may be involved in feed intake regulation because it has a role on the nervous system. Diazepam is effect by enhancing drug produces a calming effect by enhancing the action of the nerve transmitter gamma-amino butyric acid (G.A.B.A.) which in turn blocks the arousal of higher brain centers (James and Long, 1987). Diazepam is used to stimulate eating behavior (Shoukry, 1993). Recently published studies suggested that diazepam increased body weight gain and feed utilization efficiency (Singh and Sud, 1993; Sabry, 1998; and El-Wardany and El-Gendi (2000), T₃ (Saleem *et al.*, 1991), cumulative feed intake and decreased mortality rate (Demaziere *et al.*, 1992 and EL-Tantawy *et al.*, 2003). Rodriguez *et al.*, (1994) concluded that anxiolytic effects could account for an increase in feed intake.

Economic improvements related to avizyme or diazepam supplementations occurred on poultry was monitored. These beneficial effects might be more profound if birds were under stressful of increasing stocking density. Since, producers and researchers constantly search for new approaches to increase egg production and enhancing husbandry practices (increasing stocking density and feed supplementations) in order to improve the productivity indexes of laying hens. Therefore, the objective of this study was to assure the alternative of increasing stocking density from 8 to 12 birds /m² and possibility of reduce inhibition of layer performance and physiological aspects imposed by increasing stocking density by the addition of diazepam (tranquilizer agent) or avizyme (enzyme system) to the laying hens diet.

MATERIALS AND METHODS

This experiment was done at Mallawy Poultry Breeding Research Station, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Minia Governorate, Egypt.

Birds, diets, and management: Three hundred and sixty (330 females and 30 males), 20-wks old, Dandarawy pullets having nearly similar body weight were housed in open floor rooms (2m x 1.5m = 3 m² = 30000 cm²). Birds were divided into six treatment groups (Table, 1). Each of the first three groups was consisted of 48 birds in two replicates (22 pullets and 2

cocks each) and housed as 8 birds/ m² to achieve hen density of 1250 cm²/bird. While, each of the other three groups was included 72 birds in two replicates (33 pullets and 3 cocks each) and housed as 12 birds / m² to achieve hen density of 834 cm²/ bird. The first and fourth groups were fed the basal diet (Table, 2) without any supplementation and each of them kept as control. Also, the second and fifth groups were fed on basal diet plus 1 g Avizyme / kg of diet. Finally, the third and sixth groups were fed the basal diet plus 2 ppm diazepam as tranquilizer agent (7-chloro-1,3-dihydro-1-methyl-1-5-phenyl-2H-1,4-benzodiazepam-2-one). Avizyme¹⁵⁰⁰ "multi-enzyme" is a feed enzyme system specifically developed to improve the nutritional value of laying hen diets based on corn / soybean meal, marketed by Finnfeeds International. It contains enzymes produced by strains of bacteria *Trichoderma* and *Bacillus*, and has 800 u/g Xylanase, 6000 u/g protease, and 2000 u/g amylase. In each treatment, a third replicate was served as a reserve stock to compensate the dead birds. Similitude alive birds were substituted the sick 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, following convention methods, under natural environment prevails in Upper Egypt during winter season. The male to female mating ratio was 1:11 according to Campo and Davila (2002). The experimental work lasted until 36 weeks of age.

Sample collection and analytical procedure: Individual body weights were recorded at the beginning and at the end of the experiment 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 egg mass and laying rate throughout the experiment. Mortality was recorded daily. Egg quality measurements were determined at 36 weeks of age on 30 eggs per treatment laid on consecutive days. Egg shape index, yolk index and Haugh units were calculated. Shell thickness was determined at three locations on the egg (air cell, equator, and sharp end) by using a dial pipe gauge. At 36 wks old, four birds (two per replicate) were randomly chosen from each treatment group for slaughtering and their lymphoid organs were weighed (mg/100g body weight). Blood samples were collected during slaughtering in heparinized tubes, erythrocytes and leucocytes and its differential were counted. Some of blood samples were centrifuged, plasma was separated and stored at - 20 °C for analyzes. Tri-iodothyronine (T₃) (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. At

34 wks old, fifty eggs for hatching were collected from each treatment and set in an electric forced draft incubator to determine fertility and hatchability percents, the hatched chicks were individually weighed and relative weights were calculated.

Statistics: 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 their angles using arcsine equation before subjected to statistical analysis. Statistical significance was declared at ($P < 0.05$).

RESULTS AND DISCUSSION

1 – Laying performance:

1. 1 – Body weight gain and mortality rate

As expected, significant negative effects ($P < 0.05$) on live weight gain or mortality rate were observed in increasing stocking density groups due to treatments or in density overall means. Concurrent results were noticed by Anderson *et al.*, (2004); and Tollba *et al.*, (2006). However, hens fed diets supplemented with either avizyme or diazepam had significantly ($P < 0.05$) improved live weight gain or mortality rate comparing to those fed basal diet due to either density treatments or overall means (Table, 3), indicating that the two supplements alleviate the negative effects of increasing stocking density, however complete recovery was not achieved. Improvement in body weight gain of layers fed enzyme supplemented diet has been previously reported by Arscott and Rose (1960), Scheideler, *et al.*, (2005) and with broilers (Zanella *et al.*, 1999). Enzyme supplementation had a significant ($P < 0.001$) positive effect on metabolizable energy (Pan *et al.*, 1998), digestibility of crude protein (Jaroni *et al.*, 1999), and protein and calcium retention (Scheideler, *et al.*, 2005).

Results of diazepam treatment were in accordance with Singh and Sud (1993) Sabry (1998), El-Wardany and El-Gendi (2000) and EL-Tantawy *et al.*, (2003), they reported that growth may be stimulated when diazepam was added to the diet. Also, Allen and Wong (1993) found increasing body weight gain with male White Leghorn chicken fed diazepam supplemented diet.

1. 2 – Feed consumption and conversion:

The negative response of feed intake when laying hens incorporated into high density was marked ($P < 0.05$) contrast to the normal density due to treatments or in density overall means. Besides, none of the stocking

densities significantly altered feed conversion ratio due to treatments or in overall means. Sohail *et al.*, (2001) and Tollba *et al.*, (2006) found similar results. In response to avizyme or diazepam supplementations, feed intake decreased ($P<0.05$) with increasing stocking density due to treatments or in overall means. Also, feed conversion for hens fed the control diet was worse ($P<0.05$) than for hens fed the supplemental diets due to treatments or in overall means (Table, 3). The beneficial effects of enzyme supplementation on feed conversion in the present study are in agreement with previous reports of Pan *et al.*, (1998), Lazaro *et al.*, (2003) and Scheideler, *et al.*, (2005) in laying hens and Zanella *et al.*, (1999) in broiler.

Concerning results of diazepam, Singh and Sud (1993) indicated that diazepam increased body weight gain, stimulate feed intake and improved feed utilization efficiency. Rodriguez *et al.*, (1994) concluded that anxiolytic effects could account for an increase in feed intake. Diazepam promote feed intake by enhancing the transmitter of gamma-amino butyric acid (G.A.B.A.) which in turn blocks the serotonin which inhibit the feed intake of the bird, and as a result feed intake increase (Morley *et al.*, (1985).

1. 3 – Egg laying rate, egg weights and egg mass:

As can be seen from Table (3), the adverse effects of increasing stocking density on egg laying rate and egg mass were seemed ($P<0.05$) due to treatments or in density overall means. Similarly, Anderson and Adams, (1992), Sohail *et al.*, (2001) and Anderson *et al.*, (2004) reported that higher density had a significant ($P<0.01$) effect on hen-day egg production and ($P<0.05$) egg mass.

Moreover, supplementation of avizyme or diazepam to the diet improved ($P<0.05$) egg laying rate and egg mass due to treatment (in both densities) or in overall means, despite a dramatic effect of increasing stocking density. It was shown that the supplemental diets can be alleviated the adverse effects of increasing stocking density on laying performance. Significant improvement of enzyme supplementation on egg production agrees with the results of Nasi (1989), Lazaro, *et al.*, (2003) and Scheideler, *et al.*, (2005). Cone *et al.*, (1994) suggested that xylanase treatment improves nitrogen solubility in soybean meal by causing cell-wall degradation, leading to release of proteins as well as by some proteolytic activity in the crude enzyme preparation. Ritz *et al.*, (1995a,b) reported that a mixture of amylase and xylanase supplementations increased endogenous enzyme secretion and capacity of the gastrointestinal tract for digestion and absorption. Marsman *et al.*, (1997) have reported an increase in apparent digestibility of crude protein associated with protease. An enzyme mixture

containing xylanase, protease, and amylase activities significantly improved the digestibility of metabolizable energy and crude protein of corn/soybean based diet (Rafuse *et al.*, 2004).

Egg weights were not significantly influenced by either densities or dietary supplemental. Sohail *et al.*, (2001) concluded that cage density had no effect on egg weight. Pan *et al.*, (1998), Lazaro, *et al.*, (2003) and Scheideler, *et al.*, (2005) have found a lack of enzyme supplementation effects on average egg weight.

No relevant references could be found on the effect of diazepam supplementation to diet of laying hens so the significance of egg production, egg mass and egg weight could not be assessed. In general, some reports indicated that improvements in the egg production by incorporated tranquilizer agent in laying hens diet (Kondre *et al.*, 1964) or in laying quail diet (Abdel-Hakim *et al.*, 2005). Diazepam may release tranquilizing effects on birds by depressing their activities resulting in lowering body heat dissipation consequently better production.

1. 4 –Egg Quality factors:

No significant differences were noted in egg components (percents of shell, yolk and albumen) or egg quality (yolk index, shape index, yolk color, Haugh unit and shell thickness) of eggs produced by hens maintained in the both densities (Table 4). These results are matching completely with the result of Sohail *et al.*, (2001) and Tollba *et al.*, (2006).

As can be seen from Table (4), except for yolk index, none of the egg quality parameters was affected by the enzyme supplementation. Yolk index for hens fed the control diet was ($P < 0.05$) lower than hens fed the enzyme supplemented diets due to overall mean or both densities. Earlier works by Berg (1959, 1961) showed no changes in Haugh unit and yolk color in response to diets with supplemental enzyme. No alterations in inner and outer egg quality parameters in response to multi-enzyme supplementation were reported (Al Bustany and Elwinger, 1998). Enzyme supplementation had no effect on egg weight, yolk weight, shell weight, albumen height and weight or Haugh units values (Scheideler, *et al.*, 2005).

There is no information in the literature on the effect of diazepam supplementation in laying hens diet. In general, supplementing laying Japanese quail diets with reserpine as tranquilizer agent improved the external and internal egg quality aspects (Abdel-Hakim *et al.*, 2005).

1.5 – Fertility and hatchability percentage:

None of the treatments (densities or dietary supplementations) significantly altered the percent of fertility, hatchability (of fertile eggs), average values of relative hatched chick weight and absolute chick weight (Table 5), similar to Tollba *et al.*, (2006). The present results indicate that the supplementations can alleviate stress of increasing stocking density on laying performance.

2 – Relative weighs of some lymphoid and Physiological organs:

Lymphoid organs (spleen, thymus and liver) or Physiological organs (thyroid and heart) relative weighs of laying hens incorporated into high density were significantly ($P<0.05$) higher comparative to the normal density due to treatments or overall means. However, layers fed supplemental diets recorded significantly ($P<0.05$) lower relative weights of organs due to overall mean or in both densities (Table, 6). This result may reflect the possibility mentioned above that at lower stocking density or dietary supplementation hens were fatter and so if weights of organs remain constant across treatments the ratio will decrease because of increasing body weight; Aderemi *et al.*, (2006) reported that the result of the kidney or heart weight of layers recorded significantly ($P<0.05$) reduced, however the spleen did not have a particular trend with enzyme supplementation. Also, Allen and Wong (1993) and Sabry (1998) reported that diazepam supplementation decreased significantly liver weight percentage.

Productive performance results mentioned above show that the dietary supplementations have the potential to improve it under increasing stocking density, indicating that they can attenuate deleterious stress effects of the increasing stocking density.

3– Blood biochemical parameters:

None of the treatments (densities or avizyme supplementation) significantly altered T_3 hormone, total protein as well as albumin and globulin, ALT or AST as indicators to liver function, and glucose concentration due to treatments or in overall means (Table, 7). The results of enzyme supplementation are consistent with Yoruk *et al.*, (2004) who reported that the effect of multi-enzyme on metabolic profile was lacking. Also, Erisir and Erisir (2002) in laying quail and Tollba *et al.*, (2006) in laying hens reported that total protein and albumin were not affected significantly by increasing density.

Diazepam supplementation caused ($P<0.05$) higher T_3 hormone, total protein as well as albumin and globulin, in both densities or in overall

means (Table, 7). Similar results were reported by Saleem *et al.*, (1991), El-Wardany and El-Gendi (2000) and EL-Tantawy *et al.*, (2003). Besides, none of the ALT or AST activities as liver function and glucose concentration were altered by diazepam supplementation (Table, 7). These results are indicating that increasing density or dietary supplementations had no deleterious effect on blood biochemical parameters.

4 – Blood hematology

4.1 -Erythrocytes and Leucocytes cells:

Erythrocytes and Leucocytes number as well as Heterophil (H) of layers were significantly ($P<0.05$) higher by increasing stocking density with a corresponding lower ($P<0.01$) lymphocytes cells number. In response to dietary supplementations, Leucocytes were increased ($P<0.05$) which attribute to increase ($P<0.05$) lymphocytes as well as Heterophils (H) cells number comparing to their control due to treatments or in overall means (Table, 8), indicating that the dietary supplementations can ameliorate partially haematological stress of increasing densities. The alterations in heterophils, lymphocytes cells or H/L ratio suggest that hens of different stoking densities have a great effect on the stress response (Shini, 2003).

4.2 – Heterophils : Lymphocytes ratio (H/L ratio)

The H/L ratio were significantly ($P<0.05$) increased in high stocking density hens compared to normal density (Table, 8). This result suggests that high stocking density hens were more stressed. In response to supplementations, the H/L ratio did not differ significantly among treatments. However, a significant ($P<0.05$) decrease of H/L ratio were detected comparing to their control, in high stocking density (Table, 8). Insignificant and decreasing the H/L ratio indicated that the supplementations inclusion in layers diet had ameliorated the stress effects in high stocking density hens, despite a dramatic increasing in high density compared to normal density mentioned above. The significant reduction in stress levels, as indicated by the lower heterophil to lymphocyte ratio, observed in the hens supplemented with diazepam is in agreement with positive effects by decreased adverse effects of heat stress were reported by Singh and Sud (1993) or Scheideler *et al.*, (2005) with supplemented multi-enzyme. Several investigators have used H/L ratio as a very sensitive haematological indicator of stress response among chicken populations (Elston *et al.*, 2000 and Shini, 2003). The present results show that the dietary supplementations involved in adjustment the heterophil to lymphocyte ratio, indicating that they can ameliorate haematological stress of increasing stocking density.

4.3 – Monocyte cells

Monocyte cells were associated only with high stocking density (Table, 8). Shini, (2003) reported that in extreme stress, monocyte cells becomes evident in avian species. Tollba *et al.*, (2006) reported also that monocyte cells were evident on mild stocking density groups. However, both types of supplementations have responded on monocyte cells by disappearing it.

The blood hematology and biochemical results show that the dietary 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.

In conclusion, these supplements enhanced effect against increasing stocking density and may offer a potential protective management practices to ameliorate or minimize stocking density-related depression in the performance and haematological stress of laying hens. Consequently, it is of interest to strongly recommend the new aspects (increasing stocking density with the supplement) of management practices to maximize profit and sharply increase economic returns of laying hens and 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 randomly assigned to six groups, as follows:

Group	birds /m ²	birds / m ²	Avizyme	Diazepam
1	8	1250	0	0
2	8	1250	1 g / kg	0
3	8	1250	0	2 ppm
4	12	834	0	0
5	12	834	1 g / kg	0
6	12	834	0	2 ppm

Table (2): Composition and calculated analysis of the basal diet fed to experimental birds.

Ingredients	%
Yellow corn	64.00
Soybean meal 44 %	24.78
Limestone	7.91
Di-calcium phosphate	1.61
Wheat bran	1.00
Salt (NaCl)	0.30
Vit. & Min. Mixture *	0.30
DL-Methionine	0.10
Total	100.00
<u>Calculated analysis</u>	
Metabolizable energy (Kcal / Kg)	2713.00
Crude protein %	16.03
Crude fiber %	3.39
Crude fat %	2.84
Calcium %	3.34
Available phosphate %	0.42
Lysine %	0.89
Methionine %	0.39
Met + cystine %	0.66

* 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;

Table (3): Productive performance of Dandrawy laying hens fed diet supplemented with avizyme or diazepam under different stocking densities (LSM \pm S.E.)

Items	Control		Avizyme		Diazepam		Overall means				
	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	Without supplement	Avizyme supplement	Diazepam supplement
Body Weight gain (g)	219.10 ^b ± 10.06	191.35 ^d ± 17.15	243.17 ^a ± 7.23	210.50 ^c ± 10.11	239.28 ^a ± 9.10	208.57 ^c ± 12.04	233.85 ^a ± 7.46	203.47 ^b ± 6.71	205.22 ^b ± 11.37	226.83 ^a ± 12.33	223.90 ^a ± 12.85
Absolute Mortality Rate %	5/48 10.42 ^c	9/72 12.50 ^a	4/48 8.33 ^d	8/72 11.11 ^b	3/48 6.25 ^e	7/72 9.72 ^c	12/144 8.33 ^b	24/216 11.11 ^a	14/120 11.67 ^a	12/120 10.00 ^b	10/120 8.33 ^c
F. consumption (g/hen/day)	89.60 ^b ± 5.11	78.90 ^d ± 8.15	90.10 ^b ± 7.01	84.80 ^c ± 6.36	95.20 ^a ± 5.46	86.50 ^c ± 6.04	91.63 ^a ± 1.78	83.40 ^b ± 2.30	84.25 ^b ± 5.35	87.45 ^{ab} ± 2.65	90.85 ^a ± 4.35
F. conversion (feed/mass) (wk)	4.42 ^a ± 0.10	4.40 ^c ± 0.07	4.27 ^b ± 0.12	4.24 ^b ± 0.11	4.31 ^b ± 0.08	4.28 ^b ± 0.05	4.33 ^a ± 0.04	4.30 ^a ± 0.04	4.41 ^a ± 0.01	4.25 ^b ± 0.01	4.29 ^b ± 0.01
Egg laying rate (wk)	3.51 ^c ± 0.08	3.10 ^d ± 0.02	3.65 ^b ± 0.05	3.46 ^c ± 0.04	3.96 ^a ± 0.05	3.49 ^c ± 0.06	3.71 ^a ± 0.13	3.35 ^b ± 0.12	3.30 ^c ± 0.20	3.55 ^b ± 0.09	3.72 ^a ± 0.23
Egg weight (g)	40.43 ^a ± 0.22	40.49 ^a ± 0.31	40.46 ^a ± 0.23	40.51 ^a ± 0.28	40.48 ^a ± 0.14	40.57 ^a ± 0.32	40.45 ^a ± 0.01	40.52 ^a ± 0.02	40.46 ^a ± 0.03	40.48 ^a ± 0.02	40.52 ^a ± 0.04
Egg mass (g/hen/wk)	141.90 ^c ± 12.07	125.52 ^d ± 10.01	147.70 ^b ± 9.88	140.01 ^c ± 10.11	154.62 ^a ± 9.35	141.47 ^c ± 10.51	148.07 ^a ± 3.67	135.70 ^b ± 5.09	133.71 ^c ± 8.19	143.85 ^b ± 3.84	148.04 ^a ± 6.57

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 supplemented with avizyme or diazepam under different stocking densities (LSM \pm S.E.)

Treatments Items	Control		Avizyme		Diazepam		Overall means				
	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	Without suppleme nt	Avizyme suppleme nt	Diazepam supplement
Albumen weight %	52.95 ^a ± 0.02	52.70 ^a ± 0.03	53.10 ^a ± 0.02	52.75 ^a ± 0.02	53.10 ^a ± 0.04	52.70 ^a ± 0.02	53.05 ^a ± 0.05	52.72 ^a ± 0.10	52.82 ^a ± 0.12	52.92 ^a ± 0.17	52.90 ^a ± 0.20
Shell weight %	14.15 ^a ± 0.19	14.20 ^a ± 0.22	13.90 ^a ± 0.29	13.95 ^a ± 0.13	13.95 ^a ± 0.26	13.90 ^a ± 0.15	14.00 ^a ± 0.07	14.02 ^a ± 0.09	14.11 ^a ± 0.02	13.92 ^a ± 0.02	13.92 ^a ± 0.02
Yolk weight %	32.90 ^a ± 0.56	33.10 ^a ± 0.61	33.00 ^a ± 0.83	33.30 ^a ± 0.65	32.95 ^a ± 0.75	33.40 ^a ± 0.47	32.95 ^a ± 0.02	33.27 ^a ± 0.08	33.00 ^a ± 0.10	33.15 ^a ± 0.15	33.17 ^a ± 0.22
Haugh unit	81.08 ^a ± 1.45	80.57 ^a ± 1.61	80.92 ^a ± 1.48	80.24 ^a ± 1.59	81.55 ^a ± 1.36	80.62 ^a ± 1.89	81.18 ^a ± 0.25	80.48 ^a ± 0.11	80.82 ^a ± 0.25	80.58 ^a ± 0.34	80.43 ^a ± 0.19
Yolk index %	42.50 ^b ± 0.45	42.60 ^b ± 0.62	43.40 ^a ± 0.48	43.40 ^a ± 0.53	43.10 ^{ab} ± 0.71	43.10 ^{ab} ± 0.49	43.00 ^a ± 0.26	43.03 ^a ± 0.23	42.55 ^b ± 0.05	43.40 ^a ± 0.03	43.03 ^{ab} ± 0.23
Shell thickness (mm)	0.304 ^a ± 0.04	0.301 ^a ± 0.04	0.302 ^a ± 0.04	0.308 ^a ± 0.07	0.309 ^a ± 0.02	0.304 ^a ± 0.04	0.305 ^a ± 0.002	0.304 ^a ± 0.002	0.302 ^a ± 0.001	0.305 ^a ± 0.003	0.304 ^a ± 0.002
Shape index	74.70 ^a ± 0.16	74.60 ^a ± 0.20	74.90 ^a ± 0.21	74.70 ^a ± 0.29	74.70 ^a ± 0.15	74.80 ^a ± 0.24	74.77 ^a ± 0.06	74.70 ^a ± 0.05	74.65 ^a ± 0.05	74.80 ^a ± 0.10	74.75 ^a ± 0.05
Yolk color	5.20 ^a ± 0.10	5.40 ^a ± 0.19	5.10 ^a ± 0.13	5.30 ^a ± 0.10	5.10 ^a ± 0.12	5.20 ^a ± 0.12	5.13 ^a ± 0.03	5.30 ^a ± 0.05	5.30 ^a ± 0.10	5.20 ^a ± 0.10	5.15 ^a ± 0.05

a and b 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 supplemented with avizyme or diazepam under different stocking densities (LSM±S.E.)

Treatments Items	Control		Avizyme		Diazepam		Overall means				
	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	Without supplemen t	Avizyme supplemen t	Diazepam supplement
Fertility %	84.50 ^a ±1.57	85.10 ^a ±1.85	85.07 ^a ±2.61	85.32 ^a ±1.90	85.02 ^a ±2.05	84.44 ^a ±2.10	85.13 ^a ±0.18	84.95 ^a ±0.26	84.30 ^a ±0.20	85.19 ^a ±0.12	84.73 ^a ±0.29
Hatchability %	83.50 ^a ±0.48	83.90 ^a ±0.33	83.50 ^a ±0.39	83.70 ^a ±0.63	84.20 ^a ±0.91	83.60 ^a ±0.45	83.73 ^a ±0.08	83.73 ^a ±0.08	83.70 ^a ±0.20	83.60 ^a ±0.10	83.90 ^a ±0.30
Absolute Chick weights	29.03 ^a ±1.17	28.95 ^a ±1.61	28.85 ^a ±1.42	29.07 ^a ±1.92	29.05 ^a ±1.85	29.19 ^a ±1.75	28.98 ^a ±0.06	29.07 ^a ±0.06	28.99 ^a ±0.04	28.96 ^a ±0.11	29.12 ^a ±0.07
Chick weight %	63.31 ^a ±0.46	63.11 ^a ±0.67	63.27 ^a ±0.43	63.35 ^a ±0.55	63.20 ^a ±0.81	63.19 ^a ±0.24	63.26 ^a ±0.03	63.22 ^a ±0.07	63.21 ^a ±0.10	63.31 ^a ±0.04	63.19 ^a ±0.01

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

Table (6): Relative weights of some lymphoid and physiological organs (mg/ 100g B.W.) of Dandrawy laying hens fed diet supplemented with avizyme or diazepam under different stocking densities (LSM±S.E.)

Treatments Items	Control		Avizyme		Diazepam		Overall means				
	8 bird/m ²	12 bird/m ²	8 bird/m ²	12 bird/m ²	8 bird/m ²	12 birds /m ²	8 birds /m ²	12 birds /m ²	Without supplement	Avizyme supplement	Diazepam supplement
Spleen	99.46 ^a ±5.13	132.20 ^a ±8.15	95.44 ^a ±9.24	124.3 ^b ±11.02	96.62 ^a ±4.10	118.96 ^c ±9.05	97.17 ^b ±1.30	125.15 ^a ±3.84	116.00 ^a ±16.20	109.87 ^a ±14.33	107.09 ^b ±11.17
Thymus	43.90 ^a ±3.13	61.04 ^a ±3.02	56.09 ^a ±2.75	57.40 ^b ±3.06	37.17 ^c ±4.33	52.50 ^c ±3.98	40.02 ^b ±2.00	56.98 ^a ±2.47	52.47 ^a ±7.53	48.20 ^a ±9.20	44.83 ^a ±7.66
Liver	2896.52 ^a ±11.17	2957.8 ^b ±39.12	2781.1 ^d ±24.55	2958.4 ^b ±15.16	2776.8 ^d ±25.34	2967.3 ^a ±37.40	2802.73 ^b ±26.87	2961.16 ^a ±3.07	2904.16 ^a ±53.64	2869.75 ^b ±88.64	2872.08 ^b ±95.22
Lymphoid	4.84 ^a ±0.33	5.47 ^a ±0.51	4.25 ^a ±0.21	5.01 ^b ±0.40	4.22 ^d ±0.52	4.98 ^b ±0.39	4.44 ^b ±0.20	5.14 ^a ±0.14	5.13 ^a ±0.29	4.63 ^a ±0.38	4.60 ^b ±0.38
Heart	379.86 ^a ±11.81	401.12 ^a ±21.02	347.75 ^a ±18.55	391.15 ^a ±14.09	354.22 ^a ±23.15	397.02 ^b ±25.16	360.59 ^b ±9.78	396.43 ^a ±2.89	390.46 ^a ±10.66	369.95 ^b ±21.70	375.62 ^b ±21.40

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

Table (7): Plasma biochemical parameters of Dandrawy laying hens fed diet supplemented with avizyme or diazepam under different stocking densities (LSM \pm SE)

Treatments Items	Control		Avizyme		Diazepam		Overall means				
	8 bird/m ²	12 bird/m ²	8 bird/m ²	12 bird/m ²	8 bird/m ²	12 bird/m ²	8 bird/m ²	12 bird/m ²	Without supplemen t	Avizyme supplemen t	Diazepam supplement
T ₃ (ng/dl)	144.22 ^b \pm 11.35	149.40 ^b \pm 9.91	147.09 ^b \pm 12.41	145.53 ^b \pm 10.70	168.08 ^a \pm 13.01	162.36 ^a \pm 10.44	153.13 ^a \pm 7.52	152.43 ^a \pm 5.08	146.81 ^b \pm 2.59	146.31 ^b \pm 1.78	165.22 ^a \pm 2.86
Total protein (g/dl)	4.32 ^b \pm 0.19	4.28 ^b \pm 0.24	4.36 ^b \pm 0.33	4.34 ^b \pm 0.45	4.60 ^a \pm 0.30	4.56 ^a \pm 0.21	4.42 ^a \pm 0.08	4.39 ^a \pm 0.08	4.30 ^b \pm 0.02	4.35 ^b \pm 0.01	4.58 ^a \pm 0.02
Albumin (g/dl)	2.31 ^b \pm 0.04	2.26 ^b \pm 0.06	2.32 ^b \pm 0.05	2.28 ^b \pm 0.06	2.41 ^a \pm 0.03	2.39 ^a \pm 0.09	2.34 ^a \pm 0.03	2.31 ^a \pm 0.04	2.28 ^b \pm 0.02	2.30 ^b \pm 0.02	2.40 ^a \pm 0.01
Globulin (g/dl)	2.01 ^b \pm 0.04	2.02 ^b \pm 0.06	2.04 ^b \pm 0.08	2.06 ^b \pm 0.06	2.19 ^a \pm 0.03	2.17 ^a \pm 0.04	2.08 ^a \pm 0.05	2.08 ^a \pm 0.04	2.01 ^b \pm 0.01	2.05 ^b \pm 0.01	2.18 ^a \pm 0.01
(ALT) (U/L)	11.16 ^a \pm 0.65	10.22 ^a \pm 0.92	10.10 ^a \pm 1.11	10.90 ^a \pm 1.18	11.32 ^a \pm 1.05	11.12 ^a \pm 1.12	10.86 ^a \pm 0.38	10.75 ^a \pm 0.27	10.69 ^a \pm 0.47	10.50 ^a \pm 1.40	11.22 ^a \pm 1.10
(AST) (U/L)	40.10 ^a \pm 3.20	39.40 ^a \pm 2.05	40.81 ^a \pm 1.51	40.20 ^a \pm 9.12	40.50 ^a \pm 4.20	40.50 ^a \pm 5.72	40.47 ^a \pm 0.20	40.03 ^a \pm 0.32	39.75 ^a \pm 0.35	40.50 ^a \pm 2.30	40.50 ^a \pm 1.70
Glucose (mg/dl)	182.80 ^a \pm 9.15	178.53 ^a \pm 5.41	186.40 ^a \pm 10.33	181.23 ^a \pm 9.50	186.26 ^a \pm 6.45	184.40 ^a \pm 10.19	185.15 ^a \pm 1.17	181.39 ^a \pm 1.69	180.66 ^a \pm 2.13	183.81 ^a \pm 2.58	185.33 ^a \pm 4.93

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

Table (8): Blood hematological parameters of Dandrawy laying hens fed diet supplemented with avizyme or diazepam under different stocking densities (LSM \pm SE).

Items	Control		Avizyme		Diazepam		Overall means				
	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	Without supplement	Avizyme supplement	Diazepam supplement
Erythrocytes ($\times 10^6$)	1.47 ^b ± 0.05	1.58 ^a ± 0.08	1.48 ^b ± 0.04	1.57 ^a ± 0.06	1.48 ^b ± 0.07	1.58 ^a ± 0.06	1.48 ^b ± 0.01	1.58 ^a ± 0.03	1.52 ^a ± 0.05	1.52 ^a ± 0.04	1.53 ^a ± 0.05
Leukocytes ($\times 10^3$)	10.0 ^c ± 1.72	16.0 ^b ± 1.99	15.0 ^b ± 0.95	19.0 ^a ± 1.50	15.0 ^b ± 1.10	19.0 ^a ± 1.22	13.33 ^b ± 1.66	18.0 ^a ± 1.00	13.0 ^b ± 3.00	17.0 ^a ± 2.00	17.0 ^a ± 2.00
Heterophil (H)	31.0 ^c ± 0.0	33.0 ^b ± 0.0	33.0 ^b ± 0.0	34.0 ^a ± 0.0	33.0 ^b ± 0.0	34.0 ^a ± 0.0	32.34 ^b ± 0.66	33.66 ^a ± 0.67	32.0 ^b ± 1.00	33.5 ^a ± 1.00	33.5 ^a ± 1.00
Lymphocytes (L)	70.0 ^b ± 0.0	63.0 ^c ± 0.0	73.0 ^a ± 0.0	70.0 ^b ± 0.0	73.0 ^a ± 0.0	70.0 ^b ± 0.0	72.00 ^a ± 1.00	67.67 ^b ± 2.33	66.5 ^b ± 3.50	71.5 ^a ± 1.50	71.5 ^a ± 1.50
H / L ratio	0.44 ^c ± 0.0	0.52 ^a ± 0.0	0.45 ^c ± 0.0	0.48 ^b ± 0.0	0.45 ^c ± 0.0	0.48 ^b ± 0.0	0.45 ^b ± 0.01	0.50 ^a ± 0.01	0.48 ^a ± 0.04	0.47 ^a ± 0.02	0.47 ^a ± 0.02
Monocyte	-----	2.00	-----	-----	-----	-----	-----	2.00	2.00	-----	-----

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

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الملخص العربي

زيادة كثافة الدجاج البياض المصري باستخدام: ٢ - الإنزيمات & المهدئات

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أجريت هذه الدراسة لزيادة كثافة الدجاج البياض المصري (الدندراوي) من ٨ طائر/م^٢ الي ١٢ طائر/م^٢ ولمعرفة قدرة المستحضر الإنزيمي أفيزيم^{١٠٠} (xylanase, protease, and amylase) أو مركب الديازيبام كمستحضر مهدئ علي تخفيف أو إزالة إجهاد زيادة الكثافة علي الأداء الإنتاجي والفسيولوجي للدجاج البياض. تم استخدام عدد ٣٣٠ دجاجة و ٣٠ ديك من نوع مصري (الدندراوي) في عمر النضج الجنسي (٢٠) أسبوع حيث وزعت الطيور عشوائيا إلى ستة مجموعات متساوية في الأوزان تقريبا. تم إسكان الثلاث مجموعات الأولى بكثافة ٨ طائر/م^٢ بمعدل ١٢٥٠ سم^٢/ طائر من مساحة الأرضية. أما الثلاث مجموعات الأخرى تم إسكانها بكثافة ١٢ طائر/م^٢ بمعدل ٨٣٤ سم^٢/ طائر من مساحة الأرضية. تم تربيتهم تحت ظروف بيئية متماثلة مع تغذية كل تقسيم أو تصنيف للكثافة علي عليقة المقارنة أو مضاف إليها ١ جم/كجم عليقة من المستحضر الإنزيمي أفيزيم^{١٠٠} أو مركب الديازيبام بمعدل ٢ جزء في المليون علي الترتيب. وذلك في ثلاث مكررات تم جمع وتحليل بيانات مكررين فقط والمكرر الثالث كان احتياطيا لتعويض الطيور النافقة من المكررين تحت التجربة. استمرت التجربة لمدة ٤ اشهر (٢٠ - ٣٦) أسبوع من العمر. أوضحت النتائج الآتي :-

١- الكثافة العالية أدت الي انخفاض معنوي بمستوي (P<0.05) لكل من وزن الجسم المكتسب وإنتاج البيض (معدل وكتلة) و العلف المأكول وعلي العكس كان هناك زيادة معنوية في معدل النفوق أما الكفاءة التحويلية لم تتأثر معنويا. بينما البروتين الكلي والأليومين والجلوبيولين ومستوى هرمون تراي أيودوثيرونين (T₃) و AST, ALT والجلوكوز في

بلازما الدم لم يتأثر معنوياً. كرات الدم الحمراء وكرات الدم البيضاء (basophil, monocyte and Heterophil), وكذلك النسبة بين كرات الدم البيضاء المتعادلة والليمفاوية heterophil / lymphocyte ratio زادت معنوياً.

٢- الكثافة العالية أدت إلى زيادة معنوية بمستوي ($P < 0.05$) في الوزن النسبي لكل من غدة التيموسية والدرقية والطحال والكبد والقلب. أما الإضافات المستخدمة أدت إلى إنخفاضها معنوياً ($P < 0.05$)

٣- أدت إضافة الأفيزيم أو الديازيبام إلى تحسن معنوي بمستوي ($P < 0.05$) في وزن الجسم المكتسب والعلف المأكول وكفاءة تحويل الغذاء وعدد البيض وكتلته ومعدل إنتاج البيض وذلك في مجموعات الكثافة العالية (١٢ طائر/ م^٢) وهذا دليل على أن الإضافات المستخدمة خففت معنوياً من تأثير إجهاد الكثافة العالية. أما في الكثافة العادية حدث تحسن معنوي بمستوي ($P < 0.05$) في كل القياسات السابقة ماعدا العلف المأكول.

٤- لم تؤثر الإضافات المستخدمة أو زيادة الكثافة معنوياً على وزن البضة أو أي من صفات جودة البضة ما عدا دليل الصفار الذي تغير معنوياً مع إضافة الأفيزيم

٥- لم تؤثر الإضافات المستخدمة أو زيادة الكثافة معنوياً على أي من نسبة التفريخ والخصوبة وقيم أوزان الكتاكيت المفرخة المطلقة أو النسبية.

٦- أدت إضافة الديازيبام إلى زيادة معنوية بمستوي ($P < 0.05$) في البروتين الكلى والألبومين والجلوبيولين ومستوى هرمون ترائي أيودوثيرونين (T_3) في كل الكثافات المستخدمة بينما هذه القياسات لم تتأثر معنوياً مع إضافة الأفيزيم.

٧- أدت الإضافات المستخدمة مع زيادة الكثافة إلى إنخفاض معنوي ($P < 0.05$) لنسبة خلايا Heterophil(H) to Lymphocytes (L) وذلك دليل على أن الإضافات المستخدمة خففت معنوياً من haematological stress الناتج من تأثير إجهاد الكثافة العالية.

٨- أدت إضافة الأفيزيم أو الديازيبام إلى اختفاء خلايا Monocyte وذلك دليل على أن الإضافات المستخدمة خففت معنوياً من haematological stress الناتج من تأثير إجهاد الكثافة العالية.

يستخلص من هذه النتائج إمكانية التوصية بإضافة المستحضر الإنزيمي أفيزيم^{١٥٠٠} أو مركب الديازيبام كمستحضر مهدئ إلى علائق للدجاج البياض بكثافة ١٢ طائر لكل متر مربع بدلا من ٨ طائر لزيادة إنتاجية البيض من وحدة المساحة دون حدوث آثار جانبية ظاهرة على الأداء الإنتاجي أو الفسيولوجي أو أي تدهور في صفات جودة البيض. فضلا عن أن التطبيق الجديد (زيادة بكثافة مع الإضافات العلفية المستخدمة) يسبب إنخفاض كبير (٥٠ %) في تكاليف الإسكان والأجور والعمالة والأدوات لكل طائر أولكل وحدة مساحة وبالتالي تعظيم الأرباح وزيادة العائد الاقتصادي لمزارع إنتاج البيض. والنتائج تؤكد على اختيار زيادة الكثافة إلى ١٢ طائر لكل متر مربع وأن الإضافات المستخدمة قادرة على تخفيف أو إزالة إجهاد زيادة الكثافة