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IMPACT OF FEED REGIMES AND MICROORGANISM STRAINS ON EGG QUALITY, FERTILITY, HATCHABILITY PARAMETERS AND ECONOMICAL EFFICIENCY OF LAYING HENS.

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ABSTRACT:

This study was conducted to investigate the effects of dietary feed regimes (FR) and microorganism strain (MS) on egg quality traits, semen quality, fertility and hatchability per total or fertile eggs as well as economic efficiency of Inshas hens (Egyptian local developed strain) during laying period.

A total number of 240 laying pullets + 24 cockerals, 24 weeks old were randomly taken to be similar in body weights (1463.15±5.57), which were randomly divided into eight experimental groups, (30 pullets + 3 cockerals in each). Each group was contained three replicates (10 pullets +1 cockeral in each). The experimental groups involved a 2x4 factorial arrangement, 2 diet groups feed regimes (ad -libitum, mash diets and restricted mash diets (110g diet/bird/day) and 4 microorganism Bacillus strains as feed additives, which were 0.5 % Bacillus subtilus (10^9CFU/gm), 0.5 % Bacillus licheniformis (10^9CFU/gm) and 0.5 % Bacillus amyloliquefaciens (10^9CFU/gm)), respectively, during the experimental period lasted four month from 24 to 40 weeks of age.

***The obtained results showed that** feeding regimes in laying hens' significantly (P<0.05 and P<0.01) caused to improve in Haugh unite and shell weight percentage and fertility percentage and significantly (P<0.01) decrease of albumin index when compared to ad libitum group. While, egg weight, shape index, sell thickens, yolk index, yolk weight %, albumen weight % and hatchability of egg set and fertile eggs % were not significant effected. However, the effect of differences between FR show at 110g diet/bird/day an increase of net revenue (NR) and economic efficiency than the fed ad libitum. Concerning effect of microorganism Bacillus strains supplementation in layer diets caused to increase improved (P≤0.01) significantly of yolk index, Haugh units, yolk weight and albumen weight percentages, sperm motility, dead spermatozoa, sperm abnormalities, sperm cell concentration and fertility values* *when compared to control group. However, the effect of MS supplementation showed the best of (EEF) when treated with B. subtilus.*

The interaction effects between FR and MS supplementation were significant ($P \leq 0.05$) and ($P \leq 0.01$) in egg shape index, albumen index and albumen weight and shell weight percentage percentages were significantly ($P<0.05$) while, semen quality fertility and hatchability per total or fertile eggs values were insignificantly effect. The higher average of relative EEf (41.52, %) was found in the interaction between 110g dietary consumed and dietary supplementation B. subtilus, whereas, the lower one (14.00%) was shown in the interaction between add- libitum dietary consumed without any additives,

Conclusively, *it can be concluded that, feeding layer diets at 110g with supplementation MS at level 0.5% were more effective for improving of yolk index, Haugh units, albumen index, shell weight (%), semen quality, fertility and hatchability per total or fertile eggs values as well as recorded the highest (EEF) when compared to other treatment groups of Inshas laying hens.*

Keywords: *Feed regimes, microorganism strain, egg quality, fertility, hatchability parameters, economical efficiency laying hens.*

INTRODUCTION

Feed management practices aiming to improve poultry industry without increasing production cost **(Mateos *et al.*, 2012**) Quantitative feed restriction is one of the possible ways to control body weight of hens during laying period and metabolic rate to some extent as well as improving feed conversion and reducing feed cost. Therefore, hatching egg producers use feed restriction programs to prevent birds from getting over weighed, to delay sexual maturity, to avoid reproductive dysfunction, and to increase the production of settable eggs (**Renema and Robinson, 2004**).

Recently, published results have reported that the 75% feed restriction program employed during the rearing stage provides the best performance and reproductive traits response of broiler breeder hens reared on floor pens (**Carneiro *et al*., 2019**).Moreover, **Moreira *et al.* (2012**) observed that laying hens can be submitted to 5% feed restriction with the supply of hay *ad -libitum* without significant changes on the performance of the hens and egg quality. In the metabolism of chickens either to produce more desirable end- products (**Leesons and Summers, 2008**).

Feed restriction is a widely used method in the poultry industry for reasons including controlling body weight (BW), flock uniformity and performance (**Lu *et al.*, 2021; Scott *et al.*, 1999)**, and improving egg quality (**Tolkamp *et al.*, 2005)**, feed efficiency, increased profitability (**Ewa *et al.*, 2008; Olawumi, 2014)** and disease management (**Han and Smyth, 1972**).

There are many ways to restrict feed, including quantitative measures such as reducing the feed allowance provided several times a day (**Taherkhani *et al.*, 2010)**, non-daily feeding (**Wilson *et al.*, 2018)**, and time-restricted feeding (**Saibaba *et al.*, 2021)**, in addition to specific methods that allow birds to access different allocations of nutrients (protein, energy or amino acids) in diets (**Ghazanfari *et al.*, 2010)** or dilute feed with components of low nutritional value (**Rezaei and Hajati, 2009; Röhe *et al.*, 2018)**.

Recently, **Carneiro *et al.*, (2019)** evaluated the effect of different feed restriction programs applied, during rearing period on performance and reproductive traits of broiler breeder pullets reared on floor pens and found that 3/4 program could be more efficient than 2/5 program in fertility and hatchability percentages.

Wesam Ibrahim *et al* (2021) & Wesam Ibrahim (2024) reported that feed restriction had significantly (P<0.01) on hatchability of set and fertile eggs, which were significantly increased at *ad libtum* as compared to consumption of 90% and 80% *ad libitum* during the different experimental.

There are many definitions of probiotics, the most important of which is the joint report between the Food and Agriculture Organization. In 2002, the World Health Organization published that probiotics is a farmer of microorganisms that may be unilateral or mix. It has positive effects on the health of the host. There are many studies dealt with this aspect, as it emphasized the role of probiotics in improving production efficiency from the rate of body weight gain, the rate of nutritional conversion and immune responses, as well as the rate of mortality and many positive effects within the body of the bird (**Higgins *et al.*, 2007; Mountzouris *et al.*, 2007; Awad *et al.*, 2009; Blajman *et al.*, 2015).**

Certainly, egg quality characteristics considered one of the most important measurements that used in the evaluation of bird response to feed additives. In this context, a lot of research has confirmed the positive effects of the use of probiotic as additions to feed, especially the white chicken, which was evident in improving the shell thickness, albumin, and yolk well as the Haugh units **(Ashayerizadeh *et al*., 2011; Ray, 2018 (Panda *et al*., 2003; Panda *et al.*, 2008)**

Xu *et al.*, (2006) studied supplemented dried 500, 1, 000, or 1, 500 mg of *B. subtilis* culture that affect egg quality of 25-wk-old Lohmann Brown laying hens. They conveyed that increases in eggshell thickness, yolk color, and Haugh unit, and decreases in yolk cholesterol concentration (P < 0.05).In addition, **Li *et al.*, (2006)** found that dried *B. subtilis* cultures supplementation on laying hen increased eggshell thickness.

Different types of bacteria are present in the intestines of birds, which may negatively affect the quality and fertility of semen (**Triplett *et al.*, 2016;**

dos Santos *et al.*, 2018a, b). In contrast, feeding diets containing *B. subtilis* KATMIRA1933 and *B. amyloliquefaciens* B-1895 to roosters resulted in increased sperm concentration and reduced abnormal sperm counts, as well as increased fertility (**Mazanko *et al.*, 2018)**.

Therefore, the aim of this study to evaluate feeding regimes (FR) and supplementation of microorganism *Bacillus* strains in the diet on some productivity, egg production, egg quality traits, fertility and hatchability per total or fertile eggs and economic parameters of Inshas (Egyptian local developed strain) laying hens..

MATERIALS AND METHODS

Birds, management and experimental design:

This study was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt.

A total number of 240 laying pullets + 24 cockerals, 24 weeks old were randomly taken to be similar in body weights (1463.15±5.57), which were randomly divided into eight experimental groups, (30 pullets + 3 cockerals in each). Each group was contained three replicates (10 pullets +1 cockeral in each). The experimental groups involved a 2x4 factorial arrangement, 2 diet groups feed regimes *(ad -libitum*, mash diets and restricted mash diets (110g diet/bird/day) and 4 microorganism Bacillus strains as feed additives, which were 0.5 % *Bacillus subtilus* (109CFU/gm), 0.5 % *Bacillus licheniformis* (10^9CFU/gm) and 0.5 % *Bacillus amyloliquefaciens* (10^9CFU/gm)), respectively, during the experimental period lasted four month from 24 to 40 weeks of age.

All birds were housed individually in layer's rooms and maintaining in similar managerial and conditions environment with a photoperiod length of 17 h daily. Feed and water were provided *ad libitum* throughout, the experimental period (24-40 weeks of age). Experimental diets were formulated to be *is nitrogenous* and *iso- caloric* to cover the nutrients requirements as recommended by **Agriculture Ministry Decree (1996)** as shown in Table 1.

Preparation of Bacillus strains as dietary probiotic bio-additives in layer feed.

The three selected Bacillus strains were isolated from different sources according to their National Center for Biotechnology Information (NCBI) accession number **(El-Masry, 1997)** as shown in Table 2.

Measurements studied:

Total feed intake (TFE) Kg were recorded weekly in each replicate, during the experimental periods (24 - 40 weeks of age). Egg quality parameters

Table (1): Composition and calculated chemical analysis of the experimental diets

Ingredients	Basal diet, %
Yellow corn	66
Soybean meal (44%)	24
Limestone	7.59
Di-calcium phosphate	1.71
Sodium chloride	0.3
Vit.& Min. Mixture***	0.3
DL.Methionine	0.1
Total	**100**
Calculated analysis	
Metabolizable energy (kcal/kg)	**2750**
Crude Protein, %	**16.43**
Crude fiber, %	**3.20**
Ether extract, %	**2.70**
Calcium, %	**3.33**
Available phosphate, %	**0.45**
Lysine, %	**0.86**
Methionine, %	**0.39**

Each 3 kg of Vitamins and Minerals mixture * contains: vit.A, 10000 IU; D_3, 2000 IU; Vit.E, 10mg; Vit.K_3,1mg; vit.B_1, 1mg; vit. B_2, 5mg; vit.B_6, 1.5mg; vit. B_{12}, 10mcg; Niacin, 30mg; Pantothenic acid, 10mg; Folic acid, 1mg; Biotin, 50µg; Choline, 260mg; Copper, 4mg; Iron; 30mg; Manganese, 60mg; Zinc, 50mg; Iodine, 1.3mg; Selenium, 0.1mg; Cobalt, 0.1mg.
** According To Egyptian feed composition Tables (2001)
***According To AOAC (1998)
****According To NRC (1994).

Table (2): Method of isolating selected Bacillus strains

Bacillus strain	Source	Accession number
Bacillus subtilis MASRY R strain	isolated from soil	KY952907
Bacillus licheniformis MASRY R strain	isolated from the cecum of a healthy rabbit	OP764001
Bacillus amyloliquefaciens MSRY F strain	isolated from buffalo dung	OP762997

were determined at 32, 36 and 40wk of age. Six eggs in each experimental group (2 in each replicate) were randomly taken to measure egg quality traits. Egg dimensions (width and length) were measured using digital dernier caliper for shape index (%). Eggs were broken on a flat glass plat for measuring yolk, albumen indices according to **Amer (1972).** Albumen and yolk heights were measured to the nearest millimeter by triple micrometer for Haugh units and albumen and yolk indices according to **Ismail (2009).** Shell membrane thickness was obtained after measured shell thickness and then after washed and cleaned carefully from membranes. Shell membrane is the difference between shell thickness with membrane and shell thickness without membrane. Relative weights of each egg component to whole egg weight (shell, yolk and albumin weight) were then calculated according to **Amer (1972)**.

Fertility (%) was calculated as the percentage of the number of fertile eggs relative to the number of total eggs. Hatchability (%) per total set eggs and fertile eggs was estimated, while chick weight produced at hatching was measured at 32, 36 and 40 weeks of age.

Semen characteristics:

At 32 weeks of age semen samples collected using massage technique according to **Lake and Stewart (1978),** and the ejaculated volume were measured by graded tube then samples of each treatment were mixed before applying artificial insemination. Both motility % and dead sperm % were determined according to **Hackett and Macpherson (1965),** while the percentage of abnormal morphological characterizations sperms was determined as described by **Blom (1983).** Sperm cell concentration ($x10^9$/ml) was determined according to **Lake and Stewart (1978)**. Acrosomal damage (%) was determined according to **Watson (1975).** Total sperm output ($x10^9$/ejaculate) was calculated by multiplying both the ejaculated volume sperm cell concentration.

Statistical analysis.

The experiment data were statistically examined by analysis of variance according to **Snedecor and Cochran (1982)** using ANOVA procedures of **SAS (SAS, 2011).**The statistical model was used as follows:

$$Yijk = \mu + Si + Fj + (SF)ij + eijk$$

Where:, Yij = an observation, μ = overall mean , Si = Effect of the feed additives groups, (i= 1, 2, 3 and 4), Feeding regimes(j=1, 2), (SF)ij= Interaction effect (ij= 1, 2 ….+8), eij = residual "random error".

Mean treatment differences were obtained by Duncan's multiple range tests (**Duncan ,1955)** an**d** values are presented as means ± SEM. All the analyses were considered to be statistically significant at $P < 0.05$. The percentage values were subjected to be arcsine transformation before performing the analysis of variance. Means were presented after recalculated from the transformed value to percentages.

RESULTS AND DISCUSSION

Egg quality parameters:

The effect of dietary feeding regimes (FR) and dietary supplementation of different microorganism types (MS)and their interaction on egg quality parameters(external egg quality, internal egg quality indices and component of egg weight percentages) of laying hens for the whole experimental period (24-40 weeks of age) are shown in Table 3.

Feeding regimes with 110g was significantly (P<0.05 and P<0.01) caused an improve in Haugh units and shell weight percentage and significantly (P<0.01) decrease of albumin index when compared to *ad libitum* group. Egg weight, shape index, sell thickens, yolk index, yolk weight %, albumen weight% were not significant effect (Table 3).

This finding agree with **Olawuni *et al*, (2014)** who stated that treatments 90, 80 or 70 *ad libtum* feeding caused significant linear ($P < 0.05$) increases in albumen index and Haugh unit values. **Baloch *et al*. (2001)** found no significant differences in egg weight as a result of starvation or feeding regime. **Ukachukwu and Akpan, (2007)** reported that the egg quality was not significantly affected by the different feeding regimes in chickens. **Zukiwsky *et al.* (2021)** reported that, EW of feed-restricted birds increased up to 29 wk of age, which coincided with this time BW began to increase. EW may have been similar across feed-restricted of broiler breeders because of frequent meals that provided a sufficient amount of nutrients throughout the day. **Hasnath (2002)** found insignificant differences in yolk index, albumen index and shell thickness, of fayomi hens between laying hens fed *ad libitum* and 80% of *ad libitum.* **Weesam Ibrahim *et al.* (2021) &Weesam Ibrahim (2024)** illustrated that egg weight quality (g) shape index and yolk index while, significant (P<0.01) effect of on albumen % and egg shell thickness of Mandarah laying hens had insignificantly affected by feed restriction during the different ages.

Ukachukwu and Akpan, (2007) reported that the egg quality was not significantly affected by the different feeding regimes in chickens.

These results agree with the result of **Olawumi, (2014).** who reported that albumen and egg Haugh units (HU) with Brown laying hen had higher (P<0.01) mean values than with Black laying hens. Haugh unit is a measure of internal egg quality, the higher value, meaning higher nutritive value of an egg.

Data present in Table 3 show that egg weight, sell thickness, yolk index, albumen index, and cell weight percentage were insignificantly affected by MS as compared to control group. But, egg shape index, Haugh units and yolk weight and albumen weight percentages were significantly ($P<0.05$ and $P<0.01$). These results is agree with **Bothina El-Kheshin *et al* (2021) & Bothina El-Kheshin (2024)** reported that synbiotic supplementation in egg weight quality were not significantly.

Regarding the effect of interaction between FR and MT in egg weight quality, sell thickness, yolk index, Haugh units and yolk weight percentage were insignificantly affected by MT as compared to control group. But, egg shape index, albumen index and albumen weight and shell weight percentage percentages were significantly ($P<0.05$) are present in Table 3.

In confirmation to our findings several researchers also observed no significant difference in shape index due to supplementation of either probiotics or prebiotics in diet of layers (**Zarei *et al.*, 2011 and Yosefi and Karkoodi (2007**). In contrast to the present results, **Swain *et al.*, (2011)** reported that shape index was increased ($P\leq0.05$) due to probiotic and yeast supplementation 0.5 or 1.5 or 2.0g/Kg of diet.

These results is agree with **Obianwuna *et al* (2023**) showed that; eggshell quality was increased by PRO ($P \leq 0.05$) and albumen indices (Haugh units, thick albumen content, and albumen height) were enhanced by PRO, PRE and SYN ($P \leq 0.05$). **Bothina El-Kheshin *et al* (2021) & Bothina El-Kheshin (2024)** indicated that the difference synbiotic supplementation in (AI) were significantly ($P\leq0.01$) improved by increasing levels as compared with control group during all the experimental periods.

The results indicated that the difference MS supplementation in shell thickness were not significantly during the experimental period when compared to control grouping (Table 4). **Mahdavi *et al.*, (2005),** and **Mohebbifar *et al.*, (2013),** found no considerable effects from inclusion of probiotic in the layers' diet on egg quality whereas **Sheoran *et al.*, (2017),** reported increase in eggshell thickness from 0.348 to 0.374 mm when feed was supplemented with probiotics and prebiotics. It was assumed that the beneficial effect on eggshell quality was associated with the stimulating effect of pro- and prebiotics on metabolic events and utilization of calcium (**Abdelqader *et al.*, (2013** and **Li *et al.*, 2017**).

These results are in the same line with those obtained by **Neijat *et al.* (2019)** who reported that *B. subtilis* supplementation led to an improvement in albumen quality and nesting units of laying hens. **Bothina El- Kheshin (2024)** reported that the difference between synobiotic were not significantly in AW%, during all the experimental periods.

The egg is known to normally consist of water (74%), proteins (12%), lipids (12%), carbohydrate (<1%) as well as vitamins and minerals **(Li-Chan and Kim, 2008)**.

Table (3): Effect of feed regimes and Microorganism strain as feed additives on egg quality parameters of Inshas layers.

Treatments \ Parameters		External egg quality			Internal egg quality indices			Component of egg weight (%)		
		Egg weight (g)	Shape index (%)	Sell thickens (mm	Yolk index (%)	Albumen index (%)	Haugh unit (score)	Yolk weight (%)	Albumen weight (%)	Shell weight (%)
Feeding regimes (FR)										
ad libitum		49.41	76.99	36.48	42.22	72.8^{a}	87.9^{b}	31.57	56.51	11.9^{b}
110g/hen/day		48.04	76.84	36.33	42.75	69.9^{b}	89.8^{a}	31.38	56.38	12.2^{a}
MSE		**0.51**	**0.53**	**0.23**	**0.34**	**0.36**	**0.58**	**0.25**	**0.29**	**0.9**
Sig. test		**NS**	**NS**	**NS**	**NS**	**	*	**NS**	**NS**	**
Microorganism types (MT)										
Non-supplemented		48.50	76.92^{ab}	36.32	42.71	71.35	87.82^{bc}	30.87^{a}	57.26^{a}	11.89
B. subtilus		48.82	78.25^{a}	36.23	43.40	71.88	91.51^{a}	32.49^{a}	55.48^{b}	12.05
B. licheniformis		49.00	76.50^{b}	36.67	41.09	70.67	90.05^{ab}	31.91^{a}	55.82^{b}	12.29
B. amyloliquefaciens		48.56	76.00^{b}	36.40	42.73	71.64	86.15^{c}	30.57^{b}	57.21^{a}	12.17
MSE		**0.71**	**0.34**	**0.32**	**0.48**	**0.82**	**0.76**	**0.34**	**0.38**	**0.12**
Sig. test		**NS**	*	**NS**	**NS**	**NS**	**	**	**	**NS**
interactions										
FR	**MT**									
ad libitum	**Non-supplemented**	49.09	77.45^{ab}	36.00	42.54	70.39^{bc}	86.62	31.28	56.83^{ab}	11.90^{b}
	B. subtilus	49.12	77.43^{ab}	36.47	43.76	75.05^{a}	90.75	32.80	55.17^{cd}	12.04^{b}
	B. licheniformis	49.51	77.47^{ab}	36.42	40.78	72.52^{abc}	89.65	31.41	56.75^{abc}	11.86^{b}
	B.samyloliquefaciens	49.91	75.60^{b}	37.05	41.80	73.46^{ab}	84.61	30.78	57.28^{ab}	11.96^{b}
110g/ hen/day	**Non-supplemented**	47.91	76.39^{b}	36.64	42.88	72.32^{abc}	89.07	30.45	57.69^{a}	11.87^{b}
	B. subtilus	48.52	79.06^{a}	360.4	43.04	68.71^{c}	92.27	32.18	55.78^{bcd}	12.06^{b}
	B. licheniformis	48.49	75.53^{b}	36.93	41.39	68.81^{c}	90.44	32.41	54.88^{d}	12.72^{a}
	B. amyloliquefaciens	47.22	76.39^{b}	35.75	43.67	69.81^{bc}	87.69	30.50	57.14^{ab}	12.37^{ab}
MSE		**1.03**	**0.47**	**0.46**	**0.63**	**1.32**	**1.02**	**0.49**	**0.56**	**0.17**
Sig. test		**NS**	*	**NS**		*	**NS**	**NS**	*	*

^{a,b} Means having different letters in the same column differ significantly ($P \leq 0.05$).
NS= Not significant ; * = ($P \leq 0.05$);

Probiotic supplements to poultry feed improve egg and qualities especially eggshell. The increase in eggshell thickness is associated with the production of short sequential fatty acids due to fermentation later reduces the lymphine pH **(Scholz-ahrens *et al.*, 2007)**.In this connection, **Mazanko *et al.* (2018)**reported that inclusions of *B. subtilis* KATMIRA1933 and Bacillus *amyloliquefaciens* B-1895 as a nutritional supplement resulted in an increase in egg quality.

Semen quality

Data of semen quality are presented in Table (4). It could be noted that feed restricted at 110g per cock/ day had significantly ($p \leq 0.01$) higher values for sperm abnormality percent and decrease of sperm cell concentration) when compared with *ad -libitum* of Inshas cocks. On the other hand, ejaculate volume, hydrogen-ion concentration, sperm motility, dead spermatozoa percent's were not significantly differed between the two FR (Table 4).

This result shows that adequate nutritional enveronment is essential to maitain the breeding flock in good reprodutive condition. **Pana *et al* (2000)** reported that Cornish broiler cocks whose daily feed onsumoion Limited to 130 g produced ejuclates whose concenration did not differ significantly from their full- feed counterparts. Reducing the daily feed intake by (30 to 50%) was found not adversely affect semen production and semen quality attributes of Rod Island cocks (**Kabir *et al* 2007)**

Regarding the effect of microorganism strain (MS) supplementation of *B subtilus*, *B licheniformis* or *B amyloliquefaciens* (0.5%) in cocks diets improved significantly ($P<0.05$) in sperm motility, dead spermatozoa, sperm abnormalities percent's and sperm cell concentration as compared to non-supplemented group. While, the ejaculate volume and hydrogen-ion concentration were not significantly differed between the MT groups (Table 4).

Different types of bacteria present in the intestines of birds may negatively affect the quality of semen **(Triplett *et al.*, 2016; dos Santos *et al.*, 2018a, b).** On the contrary, and in line with the current study, many studies have recorded the positive effect of probiotics on the semen quality of roosters **(Mazanko *et al.*, 2018; dos Santos *et al.*, 2018a, b; Prazdnova *et al*., 2019)**.

When feeding roosters with *Bacillus subtilis* KATMIRA1933 and *Bacillus amyloliquefaciens B-1895* supplements, semen concentration and fertility increased while the number of abnormal sperm decreased **(Mazanko *et al.*, 2018)**.

Also, adding *Bacillus subtilis KATMIRA1933* and *Bacillus amyloliquefaciens B-1895* to the diet of roosters' improved testicular development and antioxidant status by raising vitamin E and A concentrations as well as reducing DNA damage, thus reducing the percentage of abnormal sperm and the percentage of abnormal sperm. Promote sperm concentration and fertility **(Prazdnova *et al.*, 2019)**.

Table (4): Evaluation of semen quality strain of Inshas layers as affected by feeding regimes (FR) and dietary supplementation of different microorganism types (MT), at 32 weeks of age**.**

Treatments \ Parameters		Ejaculate volume (ml)	Hydrogen-ion concentration (pH)	Sperm motility (%)	Dead spermatozoa (%)	Sperm abnormalities (%)	Sperm cell concentration ($X10^9$/ml)
Feeding regimes (FR)							
ad libitum		0.26	7.26	88.34	11.75	9.00^{b}	3.11^{a}
110g/hen/day		0.26	7.22	85.00	13.75	12.00^{a}	2.74^{b}
MSE		**0.02**	**0.03**	**0.87**	**0.69**	**1.05**	**0.13**
Sig. test		**NS**	**NS**	**NS**	**NS**	*****	*****
Microorganism types (MT)							
Non-supplemented		0.26	7.17	83.34^{bc}	14.50 ^{a}	14.17 ^{a}	2.76^{b}
B. subtilus		0.26	7.25	91.677^{a}	12.34^{ab}	11.50^{ab}	2.85^{b}
B. licheniformis		0.25	7.24	90.00^{ab}	10.00 ^{b}	7.50^{c}	3.37^{a}
B. amyloliquefaciens		0.26	7.30	81.67 ^{c}	14.17^{a}	8.84^{bc}	2.73^{b}
MSE		**0.08**	**0.04**	**0.61**	**0.97**	**0.98**	**0.19**
Sig. test		**NS**	**NS**	*****	*****	*****	*****
Interactions							
FR	**MT**						
Ad libitum	***Non-supplemented***	0.27	7.20	85.00	14.00	13.00	2.89
	B. subtilus	0.25	7.14	81.67	15.00	15.34	2.63
	B. licheniformis	0.25	7.24	93.34	11.67	9.34	3.08
	B.amyloliquefaciens	0.27	7.27	90.00	13.00	13.67	2.61
110g/ hen/ day	***Non-supplemented***	0.25	7.27	91.67	9.67	7.67	3.3
	B. subtilus	0.25	7.20	88.34	10.34	7.34	3.43
	B. licheniformis	0.25	7.34	83.34	11.67	6.00	3.16
	B. amyloliquefaciens	0.27	7.27	80.00	16.67	11.67	2.30
MSE		**0.05**	**0.05**	**3.32**	**1.68**	**1.44**	**0.21**
Sig. test		**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

[a,b] Means having different letters in the same column differ significantly ($P \leq 0.05$).
NS= Not significant ; * = ($P \leq 0.05$); ** = ($P \leq 0.01$)., MSE; means of the standard error.

Concerning the effect of interaction between feeding regimes and dietary supplementation of different microorganism types showed insignificant effect on semen quality of cocks at period studied (Table 4).

2. *Fertility and hatchability traits*

Feed regimes had significantly ($P<0.01$) effect on fertility percentages, while hatchability of total egg sett and fertile eggs which had not affected significantly by feed regimes which, was increased by feeding *ad libitum* as compared with those consumed 110g/ day feeding during the different experimental ages, (Table 5). These results agree with **Carneiro *et al*., (2019)** who evaluated the effect of different feed restriction programs applied during rearing on the performance and reproductive traits of broiler breeder pullets reared on floor pens and found that ¾ of *ad libitum* could be more efficient than 2/5 of *ad libitum* for fertility and hatchability percentages. The obtained results agree with those indicated by **Crouch *et al.* (2002)** who showed that cumulative mean hatchability of fertile eggs was significantly greater in turkeys that were restricted feed during the rearing phase than in turkeys that were fed *ad libitum* during the same rearing period. The same authors found turkeys that were shifted from restricted feeding during the rearing phase to *ad libitum* feeding during the laying phase had a significantly higher embryonic mortality and hence a lower hatching percentage compared with other treatments. **Hassan *et al* (2003)** reported that feed restriction at 70 and 85% of *ad libitum* intake did not significantly decrease fertility between 6 and 13 wks of quail age.

Regarding the effect of microorganism strain (MS) supplementation of *B subtilus*, *B licheniformis* or *B amyloliquefaciens* (0.5%) in layer diets improved insignificantly hatchability traits, except fertility percentage which, was significantly (P<0.05) by supplementation of *B subtilus*, *B licheniformis* as compared with un-supplemented and supplemented of *B amyloliquefaciens.* These results are agreement with **Elham Darsi and Zaghari (2021)** reported that the *Bacillus subtilis PB6* supplementation in the broiler breeder diet increased eggs hatchability.

Age and diet can influence embryogenesis and hatchability of broiler breeder eggs (**Peebles *et al.* 2000**). Our results were in accordance with those of **Mojgani *et al.* (2020)** who reported that quails fed probiotics (10^8 colony forming units /ml *B. megaterium*) significantly (P < 0.05) improved H% by about 12% and reduced embryonic mortality by about 10% compared with the control. Furthermore, **Beck *et al.* (2019)** observed that the Bifido bacterium animalis treatment significantly reduced the percentage of piped eggs compared to the control. This indicates the possibility of injecting B. animalis into the amnion of an embryo at the 18th of embryonic development with a potential to improve hatching performance.

On the contrary, **Ayasan (2013)** found that dietary supplementation of commercial probiotics (protexin) with levels of 0.05 and 0.10%/kg diet did not affect F% and H% from the fertile eggs of Japanese quail layers compared to the control.

Table (5): Fertility and hatchability traits of Inshas layers as affected by feeding regimes (FR) and dietary supplementation of different microorganism strain (MS), during the experimental periods

Treatments \ Parameters		Fertility (%)	Hatchability of total egg (%)	Hatchability of fertile eggs (%)
Feeding regimes (FR)				
ad libitum		86.37[a]	72.18	83.59
110g/hen/day		83.19[b]	70.69	85.07
MSE		**0.73**	**1.15**	**1.23**
Sig. test		**	**NS**	**NS**
Microorganism types (MT)				
Non-supplemented		83.46[bc]	68.65	82.33
B. subtilus		85.93[ab]	74.58	86.77
B. licheniformis		86.76[a]	74.38	85.82
B. amyloliquefaciens		82.97[c]	68.15	82.38
MSE		**1.08**	**1.52**	**1.66**
Sig. test		*	**NS**	**NS**
Interactions				
FR	**MT**			
ad libitum	**Non-supplemented**	75.56	62.23	74.05
	B. subtilus	82.97	68.15	82.38
	B. licheniformis	88.44	77.61	87.78
	B.amyloliquefaciens	83.42	71.54	85.77
110g/hen/day	**Non-supplemented**	88.15	69.14	84.91
	B. subtilus	85.38	73.94	86.72
	Ba. licheniformis	84.94	67.17	79.37
	B. myloliquefaciens	80.99	74.82	85.40
MSE		**2.23**	**2.61**	**2.94**
Sig. test		**NS**	**NS**	**NS**

a,b,c: Means in each classification in the same column with different superscripts, differ significantly (P<0.05).
N.S: Not Significant, * P < 0.05, ** P< 0.01. MSE: Mean of standard error.

The interaction between feeding regimes and dietary supplementation of different microorganism types showed insignificant effect in fertility, hatchability of set and fertile eggs (%) of Inshas laying hens at period studied (Table 5).

4. *Economic efficiency*

Economic efficiency of egg production of Inshas laying hens as affected by FR, MS and their interactions, during period from 24 to 40 weeks of age are shown in Table 6. Results obtained revealed that the 110g dietary consumed recorded the higher (best) relative EEf percentage being 46.32 % as compared with the group fed *ad libitum* which was recorded 31.65%. These results agreement with **Olawumi, (2014)** found that 90% *ad libitum* was better and efficient than *ad libitum* and 80% *ad libitum* recorded the highest net returns and economic efficiency of laying hens. **Hassan *et al* (2020).** reported that the quantitative feed restriction (100 or 110g /hen /day) was employed to control growth by feeding predetermined amount of balanced diet in order to achieve a good production during laying period as well as, enhance the economic efficiency of laying hens. **Wesam Ibrahim *et al* (2021) &Wesam Ibrahim (2024)** reported that the 90 % dietary consumed recorded the higher (best) relative EEf percentage when compared with 80% dietary and *ad libitum*.

The diet supplemented with *B. subtilus* attained the higher relative EEf value, being 33.20 %.but the lowest relative EEf value 17.17% in control group (Table 6). **Cristina *et al.* (2010)** reported that, synbiotic feed additive usage in laying hens feeding also influenced certain economic factors, through feeding expenses and value of produced eggs. Thus, feed expenses across the entire experimental period reached 114.12 RON in control group and 116.81 RON in E group. These were influenced by the overall feed intake values and by feed price/kg, which was different and related to additive inclusion proportion. **Riad *et al.*, (2010)** who indicated that both net revenue and economic efficiency increased in probiotic additives treatments than control ones. However, dietary supplementation at a level of 1g probiotics *(Saccharomyces cervisiae)* +1g prebiotic/kg diet showed an improvement by about 14.70 % in relative economic efficiency% more than control group on broiler chicks at 0-42 day of age.

In addition, **Patel *et al*., (2015)** reported that the return over feed cost was highly significant ($P \leq 0.01$) for dietary supplementation probiotic 100 mg/kg diet as compared to 50 mg/kg and control group of Broilers. **Mohammed *et al*., (2016)** reported that probiotics, prebiotic, synbiotic, organic acids and enzymes had a positive effect on economic performance of broiler chick. **Bothina El Kheshin *et al* (2023) & Bothina El Kheshin (2024)** reported that the effect of synbiotic levels supplementation showed the best of (EEF) recorded, when treated with 0.2% synbiotic but the lowest value recorded in control group.

The higher average of relative EEf (41.52, %) was found in the interaction between 110g dietary consumed and dietary supplementation *B. subtilus*, whereas,

Table (6): Economic efficiency % of Mandarah hens as affected by feed restriction, different microorganism strain (MS), and the interactions between them, during period from 24 to 48 week at age.

Treatments		Egg number	Price/ egg (LE)	Total revenue eggs (LE)1	Total feed intake (kg)	Price /Kg feed (LE)	Total Feed cost (LE)2	Fixed cost (LE)	Total cost (LE)3	Net revenue (LE)4	(EEf)5 (%)
Feeding regimes (FR)											
ad libitum		52.84	4.20	221.93	13.80	12.00	165.57	3.00	168.57	53.36	31.65
110g/hen/day		52.44	4.20	220.25	12.29	12.00	147.52	3.00	150.52	69.73	46.32
Microorganism types (MT)											
Non-supplemented		49.04	4.20	205.97	13.04	13.25	172.78	3.00	175.78	30.19	17.17
B. subtilus		55.40	4.20	232.68	12.96	13.25	171.69	3.00	174.69	57.99	33.20
B. licheniformis		53.96	4.20	226.63	12.97	13.25	171.80	3.00	174.80	51.83	29.65
B. amyloliquefaciens		52.20	4.20	219.24	13.08	13.25	173.36	3.00	176.36	42.88	24.31
Interactions											
ad libitum	Non-supplemented	50.40	4.20	211.68	13.79	13.25	182.69	3.00	185.69	25.99	14.00
	B. subtilus	54.88	4.20	230.50	13.65	13.25	180.86	3.00	183.86	46.64	25.36
	B. licheniformis	53.52	4.20	224.78	13.92	13.25	184.42	3.00	187.42	37.36	19.94
	B. amyloliquefaciens	52.56	4.20	220.75	13.88	13.25	183.86	3.00	186.86	33.89	18.14
110g/hen/day	Non-supplemented	47.68	4.20	200.26	12.30	13.25	163.01	3.00	166.01	34.24	20.63
	B. subtilus	55.88	4.20	234.70	12.29	13.25	162.84	3.00	165.84	68.85	41.52
	B. licheniformis	54.40	4.20	228.48	12.29	13.25	162.90	3.00	165.90	62.58	37.72
	B. amyloliquefaciens	51.84	4.20	217.73	12.29	13.25	162.86	3.00	165.86	51.87	31.27

1. Total revenue hen (LE) = Egg Number * Price of one egg (LE)
2. Total feed cost / hen (LE)= Total feed in take / hen (Kg) * Total feed cost / hen (LE)
3. Total cost hen (LE) Total feed cost / hen (LE) + fixed hen (LE)
4. Net revenue / hen (LE) = Total revenue hen (LE) – Total cost hen (LE), Economic efficiency (EEF) = Net revenue/hen.

the lower one (14.00%) was shown in the interaction between *add- libitum* dietary consumed without any additives as shown in Table 6.

Conclusively, it can be concluded that, feeding layer diets at 110g with supplementation MS at level 0.5% were more effective for improving of yolk index, Haugh units, albumen index, shell weight (%), semen quality, fertility and hatchability per total or fertile eggs values as well as recorded the highest (EEF) when compared to other treatment groups of Inshas laying hens.

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تأثير أنظمة التغذية وسلالات الكائنات الحية الدقيقة على جودة البيض والخصوبة ومعايير الفقس والكفاءة الاقتصادية للدجاج البياض.

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أجريت هذه الدراسة للتحقيق في تأثير أنظمة التغذية الغذائية (FR) وسلالة الكائنات الحية الدقيقة (MS) على صفات جودة البيض وجودة السائل المنوي والخصوبة ومعدل الفقس لكل بيضة إجمالية أو مخصبة وكذلك الكفاءة الاقتصادية لدجاج إنشاص (سلالة محلية مصرية) خلال فترة وضع البيض.(٢٤-الى ٤٠اسبوع).

تم أخذ إجمالي عدد ٢٤٠ دجاجة بياضة + ٢٤ ديك، بعمر ٢٤ أسبوعًا بشكل عشوائي لتكون متشابهة في أوزان الجسم (١٤٦٣.١٥ ± ٥.٥٧)، والتي تم تقسيمها عشوائيًا إلى ثماني مجموعات تجريبية (٣٠ دجاجة + ٣ ديك في كل منها). احتوت كل مجموعة على ثلاث مكررات (١٠ دجاجات + ديك واحد في كل منها). شملت المجموعات التجريبية ترتيب عاملي ٢x ٤، ومجموعتين غذائيتين من أنظمة التغذية

(علائق غذاء حتى الشبع، واخرى علائق محددة التغذية (١١٠ جرام علف/طائر/يوم) و٤ سلالات من الكائنات الحية الدقيقة Bacillus كمضافات غذائية، والتي كانت ٠.٥٪ *Bacillus subtilus* (10^9CFU/جم)، ٠.٥٪ *Bacillus licheniformis* (10^9CFU/جم) و ٠.٥٪ *Bacillus amyloliquefaciens* (10^9CFU/جم))، على التوالي، خلال فترة التجربة التي استمرت أربعة أشهر من ٢٤ إلى ٤٠ أسبوعًا من العمر.

أظهرت النتائج المتحصل عليها أن أنظمة التغذية في الدجاج البياض أدت بشكل ملحوظ ($P < 0.05$) و ($P < 0.01$) إلى تحسن في نسبة وزن الوحدة وقشرة هاوج ونسبة الخصوبة وانخفاض ملحوظ ($P < 0.01$) في مؤشر الألبومين عند مقارنتها بالمجموعة حسب الطلب. في حين لم يكن هناك تأثير معنوي لوزن البيضة ومؤشر الشكل وسماكة البيع ومؤشر الصفار ونسبة وزن الصفار ونسبة وزن البياض ونسبة فقس البيض ونسبة البيض المخصب. ومع ذلك، فإن تأثير الاختلافات بين FR يظهر عند ١١٠ جرام من العليقة / طائر / يوم زيادة في صافي العائد (NR) والكفاءة الاقتصادية مقارنة بالتغذية حسب الرغبة. فيما يتعلق بتأثير مكملات سلالات الكائنات الحية الدقيقة Bacillus في علائق البياض تسبب في زيادة تحسن كبير ($P \leq 0.01$) في مؤشر الصفار ووحدات Haughونسبة وزن الصفار ووزن البياض وحركة الحيوانات المنوية والحيوانات المنوية الميتة وتشوهات الحيوانات المنوية وتركيز الخلايا المنوية وقيم الخصوبة عند مقارنتها بمجموعة التحكم. ومع ذلك، أظهر تأثير مكملات MS أفضل (EEF) عند المعاملة بـ*B. subtilus*.

كان تأثير التفاعل بين مكملات FR و MS معنويا ($P \leq 0.05$) و ($P \leq 0.01$) في مؤشر شكل البيض ومؤشر الألبومين ونسبة وزن الألبومين ووزن القشرة معنويا ($P < 0.05$) في حين كانت جودة السائل المنوي والخصوبة ونسبة الفقس لكل إجمالي أو قيم البيض المخصب غير معنوية. وقد وجد أعلى متوسط لـ EEf النسبي (٤١.٥٢%) في التفاعل بين ١١٠ جرام من الغذاء المستهلك والمكملات الغذائية من نوع*B. subtilus* ، في حين ظهر المتوسط الأدنى (١٤.٠٠%) في التفاعل بين الغذاء المستهلك حسب الرغبة بدون أي إضافات،

التوصية:، يمكن أن نستنتج أن تغذية الدجاج البياض على علائق بوزن ١١٠ جرام مع مكملات MS عند مستوى ٠.٥% كانت أكثر فعالية في تحسين مؤشر الصفار ووحدات هاو ومؤشر البياض ووزن القشرة (%) وجودة السائل المنوي والخصوبة وقابلية الفقس لكل إجمالي أو قيم البيض المخصب وكذلك سجلت أعلى (EEF) عند مقارنتها بمجموعات المعاملة الأخرى لدجاج إنشاص البياض.

الكلمات المفتاحية: نظم التغذية، سلالة الكائنات الحية الدقيقة، جودة البيض، الخصوبة، معايير قابلية الفقس، الكفاءة الاقتصادية لدجاج البياض.