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Biochemical, Immunological Blood Parameters and Bacterial Counts using Different Beneficial Microbes as Feed Additives for Improving Performance of Egyptian Local of Developed Laying Hens

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Abstract

This study conducted to investigate the effects of dietary feed regimes and microorganism types on productive and reproductive performance; blood constituents blood constituents and intestinal bacteriology of Inshas (Egyptian local developed strain) laying hens. A Total number of 240 laying hens + 24 cocks, 24 weeks old were randomly taken to be similar the body weight in (1463.15±5.57) the study. Birds were randomly divided into eight experimental groups,(30 hens + 3 cocks in each group) and each group was contained three replicates(10hens+1cock /replicate).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 types as feed additives Bacillus 0, 0.5 % *Bacillus subtitles* (10^9CFU/gm), 0.5 % *Bacillus licheniformis* (10^9CFU/gm) and 0.5 % *Bacillus amylolique faciens* (10^9CFU/gm)). Respectively, during the experimental period lasted four month from 24 to 40 weeks of age. The obtained results showed that laying hens fed of 110g diet recorded the improve (P<.01) significantly in daily feed intake (DEM, g) and feed conversion ratio (FCR) and kg feed /number eggs as compared with *ad-libitum*. Addition of 110g diet per day to laying hens' might improve FC, IgG, IgM, TAOC total aerobic count and E. *coli*. Hens received (Bacillus 0, 0.5 % *Bacillus subtitles*, 0.5 % *Bacillus licheniformis*, *Bacillus amyloliquefaciens* at level 0.5%% can improve (P<.01) significantly of productive performance, semen quality, fertility%, IgG, IgM, TAOC and bacterial count. It can be concluded that, feed regimes at 110 g per day is not negative effect of productive and reproductive performance, while supplemental layer diets with Microorganism types were more effective for improving productive performance traits, biochemical, immunological blood parameters and bacterial count of Inshas laying hens.

Keywords: *Biochemical blood, immunological parameters, bacterial counts, different beneficial microbes*

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1. Introduction

Feed restriction is a management technique widely used in the poultry industry to control body weight, improve nutrient utilization of layers by reducing the amount of feed provided to birds and thus flock uniformity and performance, and improve egg quality, feed efficiency, herd profitability and disease management [1-2]. Quantitative measures such as reducing the feed allowance provided several times a day, non-daily feeding, and time-restricted feeding, as well as qualitative methods that allow birds to access to different rations of nutrients (protein, energy or amino acids) in the diet or diluting feed with ingredients of low nutritional value, are well-established methods of restricting feed consumption in the Poultry industry [3-4]. Various feeding regimens enhance the generation of viable eggs under heat-stress conditions, and applying an 8-hour restricted feeding schedule had a beneficial impact on Japanese quails' body weight (BW), fertility, hatchability, egg production, egg-specific quality, and ovo-positional time [5]. Because there is a link between excess body fat and decreased egg production, fertility, and hatchability, FR techniques are, in general, used to prevent excessive fat deposition [6]. [7] reported that reducing the amount of feed provided to hens (100g/hen/day) a good production during laying period as well as,

enhance economic efficiency of laying hens. [8-9] illustrated that restricted at 90 % dietary consumed recorded higher (best) relative EEf percentage when compared with feeding *ad- libitum*.

Despite many benefits that accrue too many, whether producers or consumers, from increasing animal production, this has led to creation of two main problems. The first problem is the excessive use of antibiotics as growth promoters on a large scale in animal feed, which scientists have become aware of their risks to human health; hence, it has been banned in many countries, including European Union, because of potential for development of antibiotic resistance in microbial populations associated with human and animal diseases. The second problem is food-borne zoonotic diseases such as campylo bacteriosis, salmonellosis, pathogenic Escherichia coli infection, and others, which are dangerous because they relate to public health throughout world and can cause serious economic losses [10]. Direct-fed Microbial are now being researched and used more widely in the laying hens because of the benefits to limit food-borne pathogens [11-12]**,** increase egg production and quality parameters [13-16]. And increase the ability of laying hens to utilize nutrients more efficiently due to a more diverse and healthy gastrointestinal system [17-18]. Therefore, the aim of this study to evaluate of feed regimes and supplementation of microorganism types in diet on productive performance traits, biochemical, immunological blood parameters and bacterial counts of Egyptian local of developed laying hens strain.

2. Materials and Methods

2.1. Birds, management and experimental design

The present study was carried out at the Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt. A total number of 240 Mandarah (Egyptian local developed strain) laying hens+ 24 cocks, 24 weeks old were randomly taken from the farm flock, to be similar in body weight. Birds were randomly divided into eight treatment groups (30 hens + 3 cocks in each group) and then each treatment group was divided into three replicates (10 hens+ 1 cock /replicate). The experimental groups involved a 2 x 4 factorial arrangement, 2 diet groups)*ad libitum*, mash diets and restricted mash diets (110g diet/bird/day) and 4 feed additives Bacillus 0, 0.5 % *Bacillus subtitles* (10^9CFU/gm), 0.5 % *Bacillus licheniformis* (10^9CFU/gm), *Bacillus amyloliquefaciens* (10^9CFU/gm)). Respectively, during the experimental period lasted four month from 24 to 40 weeks of age was fed a balanced basal diet, during the experimental period lasted four month. 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, experimental period (24-40 weeks of age). Experimental diets formulated to be *is nitrogenous* and *iso- caloric* to cover nutrients requirements as recommended by [19] as shown in Table 1.

2.2. 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 [20]. Bacillus strains selected based on microscopic characteristics including growth rate, identification, and characterization for secretion of celluloses and amylase production, survivability in low pH, bile salts, the growth rate of spores, and susceptibility to antibiotics assessed in vitro. The bacterial strain was grown in a nutrient medium (g/L: tryptone 10; meat extract 5; sodium chloride 5; pH medium 7.2 ±2 before autoclaving) and incubated in a shaker-incubator (200 rpm) at 37º C for 24 h in aerobic conditions as reported previously by [21-24].The inoculum was analyzed by serial 10-fold dilutions using phosphate buffered saline solution (PBS), and then, 1 mL from $10^{-10}$to $10^{-12}$was placed on nutrient agar medium (g/L: tryptone 5; meat extract 3; bacteriological agar 5; distilled water).

To determine the viability of spores-forming, the vegetative cell was inactivated by thermal treatment (80º C, 10 min). Serial dilutions in PBS on nutrient medium agar followed by incubation at 37 ºC in an aerobic atmosphere for 24 h. The biomass surviving spores were collected by centrifugation (5,000 rpm, 10 min, 4º C), washed twice, and then suspended in PBS solution. The strain had the capacity to sporulate 1 x 10^{11} CFU spores/mL. In our study, the initial spores count was adjusted at 5 x 10^{11} CFU/mL and kept at 4º C until utilization in layers feed. For starting the experiment, the strain biomass, was adjusted at 5 x 10^{9} CFU spores g^{-1} feed (10 ml of 5 x 10^{11} CFU/mL for each kilogram feed as 1% bio-additive feed) and included and blended with diets every week. After mixing, the diets supplemented with Bacillus strains analyzed for spore counts weekly.

2.3. Measurements

Body weight (BW) of bird at 24 and 48 weeks of age and change body weight (%) recorded. Daily and total egg number and egg weight (g) recorded for each hen/in each group, while daily and total feed intake recorded, during experimental periods. Egg production rate (%) calculated for four weeks intervals, during production periods as egg number/hen/period for each replicate and calculated average of whole experimental period. Egg mass calculated by multiplying egg number X average egg weight. Feed conversion (g feed /g mass and the Kg feed/ eggs) calculated as Kg feed consumption produced number of eggs for four weeks intervals and whole experimental period. At 40 weeks of age, three hens slaughtered from each treatment 1-2 hours post-ova-position (at 2 pm) in order to take some blood samples from jugular vein, during slaughter and then placed in two groups of heparinized tubes. First tubes group used to perform some blood hematological measurements. White blood cells ($\times 10^3$) accounted using improved Neuberger hem cytometer (Brand, Wertheim, Germany) according to [25]**.**

Micro hematocrit centrifuge at 12.000 rpm for 5 minute and micro hematocrit capillary tubes using to evaluate percentage packed cell volume [26]**.** To separate the blood plasma, the second group of blood samples were centrifuged at 3000 rpm for 15 minutes, then stored at -20 °C to determine total protein [27], albumin [28]**.**, globulin (subtracting albumin values from the corresponding total protein total value), and plasma activities transaminases: aspartine aminotransferase (AST) and alanine aminotransferase (ALA) according to [29]**.** On the other hand, the concentrations of some immunoassays have been estimated as IgG and IgM by ELISA as described in [30]**.** In addition to total antioxidant capacity according to [31]**.** At the end of experiment, the same three birds slaughtered were chosen for intestinal bacterial count studies. All viscera were

carefully removed by hand from the carcass under sterile conditions, and one gram of the intestinal content from ileo-cecal junction portion was transferred to a sterile test tube containing nine ml of 1% sterile peptone water (first dilution 10-1) and vortexed for 1 min to homogenize.

The homogenate was diluted serially from 10-1 dilution to 10-8. For each dilution 0.1 ml was plated onto sterile selective medium agar for enumeration of tested bacteria groups. MRS agar (Oxoid, Uk) for enumerating total aerobic count and lactic acid bacteria, brilliant green agar (Fischer scientific, USA) for enumerating Salmonella ssp., Violet red bile glucose agar (Sigma-Aldrich, UK) for enumerating *Escherichia coli*. After preparing media according to manual descriptions, it poured in Petri dishes previously sterilized at 180 °C for 3 hours, and left to hardening at room temperature (28 ± 2°C). Then 0.1 ml of each dilution planted (duplicate) for each microbial group and left to dry. Dishes then incubated at 37 °C for 24 hours for Salmonella (Pink or colorless colonies with a red halo), 72 hours for E. coli (purple – pink´) and 48 hours for LAB in anaerobic jar with GAS Pack (Oxoid, UK), The number of colonies then counted to determine colony forming units (CFU). CFU per gram of fresh caecal content then expressed on logarithms [32].

2.4. Statistical analysis

The experiment data were statistically examined by analysis of variance according to [33] using ANOVA procedures of [34]. The statistical model was used as follows:

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

Where:

Y_{ijk} = an observation;μ = Overall mean; Si = Effect of the feed additives groups (i= 1, 2, 3 and 4, Fj : 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 and values are presented as means ± SEM. All the analyses were considered to be statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Productive performance traits

The effect of dietary feeding regimes (FR) and dietary supplementation of different microorganism types (MT) and their interaction on productive performance traits of laying hens for the whole experimental period (24-4 weeks of age) are shown in Table 3. Feed restricted (110g) was significantly (P<0.01) caused to improve in daily feed intake (DEM, g) and feed conversion ratio (FCR) and kg feed /number eggs as compared to hens in receiving *ad- libation* groups. While, total egg umbers (TEN), egg weight (EW) and daily egg mas (DEM) were insignificantly affect by FR. Similar results were obtained by [8-9] that the best feed conversion ratio, egg number and egg production for laying receiving 105 gram/ hens compared with 90 or 120 gram/ hens from 24 to 48 weeks of age. [35] Found that feed restriction improved the body weight gain due to improvement in feed conversion of broiler chickens. During the three periods of lay, egg production level in the *ad libitum* feeding was less than that at restricted feeding [36]**.**

[37] Reported that feed restriction delays the onset of egg production. These results agreed with [38] who found that the poultry production in the free-range system to be feasible should be directed to the use of alternative feeding and pastures, in the free-range system, the feeding of birds with exclusively commercial diet may cause losses, even selling the eggs with price higher than the recommended for eggs produced industrially, the consumption of forage by birds is low, and balanced, supplementary diet is undoubtedly necessary to maintain a good health and high levels of poultry production. Concerning effect of microorganism types (MT) supplementation of *B. subtitles*, *B licheniformis* or *B amyloliquefaciens* (0.5%) in layer diets improved significantly (P<0.01) in TEN, EP, DEM and FCR (kg feed/eggs) as compared to non-supplemented group (Table 3). The improvement in productive performances may be due to increased efficiency of digestion and nutrient absorption processes due to presence of probiotic bacteria [39]**.**

These results agreed with those reported by [40] who founded that diet supplemented with 0.5 g probiotic /kg diet had higher LBW compared with the control group, then by hens fed supplemented 1.0 g probiotic /kg diet a positive effect on LBW of breeder hens of line K–White Plymouth rock. [41] Who reported that the addition of Bactocell® (*Pediococcus acidilactici*) at the dose of 10^9 UFC/kg of feed was improved the EPR (+2.39%) of Hy-line laying hens. [42] found that the broiler breeder Ross 308 study for a period of 48 -64 weeks of probiotic, prebiotic and symbiotic for 0.15/ kg diet caused highly significant increase in egg production. Regarding the interaction, it could be shown that FBW, BWC, EW, TFI, DFI and FCR (kg feed/number eggs) were significantly (P<0.01) influenced by the supplementation with FR and MT, while the other traits of TIN, EP EM, with no significant, during 24-40 weeks of age as shown in Table 3.

3.2. Some hematological and liver functions

Results of feed regimes (FR) or dietary supplementation of different microorganism types **(**MT**)** and their interaction on some hematological and liver functions; it could be seen in Table 4. There were insignificant differences in (WBCs and PCV) and blood liver of function, except ALT, which was significantly (P≤0.05), decreased affected by FR diet at 110g as compared with *ad- ibitum*. These results agree with the results reported by [43]**,** who indicated that total protein and albumin significantly decreased by feed restriction 85 or 70 % of were *ad libitum* feeding. However, at 35 d of age these blood traits were higher in the qualitative FR groups. However, [44-45] reported that early feed restriction had no significant effect on serum ALT, the AST, proteins, albumin, and globulin. Concerning effect of (MT) supplementation in layer diets caused significantly (P≤0.05 and P≤0.01) in PCV, Total protein, Globulin, the ALT and AST values. While, WBCs and albumin were not significant affect (Table 4). The Synbiotic supplementation at different levels was positive effect on the plasma total protein and globulin may be belonged to immune stimulant effect of these feed additives in poultry [46].

These results were in concord with, [47] who observed that feeding broiler chickens on a prebiotic supplemented diet, increased serum total protein and globulin. Similarly, [48] revealed that prebiotic inclusion in quail's diet caused to increase significant (P<0.05) in concentration of total plasma protein and total globulin. On other hand, these results were in contrast to those of [39-49]**.**, where they revealed that synbiotic had no significant effect on blood total protein, albumin, globulin and albumin /

globulin ratio in chickens [50] Indicated that synbiotics supplementation did not effect on serum total protein, albumin, globulin and glucose, except packed cell volume, increased in additive treatments with restriction at end of experiment. Moreover, [51-52] indicated that supplementing broiler diet with probiotics or prebiotics did not any effect on each of total protein, albumin, globulin and albumin to globulin ratio. He effect of interaction between the FR and MT in WBCs, PCV and liver function showed no significant affect except, the AST which significantly (P≤0.05) increased by supplemented *Bacillus amyloliquefaciens* to laying hens feeding *ad- libbitum* and *Bacillus licheniformis* to laying hens feeding 110g per day than other treatment groups.

3.3. Immunological response, total antioxidant capacity and mortality rate

Effect of FR or MT and their interaction their interaction on immunological response, antioxidants mortality rate are showed in Table 5. Significantly (P≤0.01) increased of IgG, IgM and significantly (P≤0.01) decreased of T-AOC values by feeding *ad- labium* as compared with 110g group. However, mortality rate value was insignificant affected by FR. This results agreement with [53] showed that a feed restriction of 15 and 30% of *ad libitum* feed intake had a low influence on the immunity of broiler chicken. [54] found that chickens during d 1 to 35 of age were fed either 100 or 80 % of the daily amount of feed consumed by the control group, feed restriction significantly increased plasma albumin but decreased total cholesterol while, globulin, total antioxidant capacity (TAC) were not affected. [8-9] illustrated that feed restriction on antioxidant total antioxidants capacity (TAC), super oxide dismutase (SOD), glutathione peroxidase (GPX) of Mandarah laying hens had significantly (P<0.01).

Regarding the effect of microorganism types (MT) supplementation of the *B subtilus*, *B licheniformis* or *B amyloliquefaciens* (0.5%) in laying diets improved significantly (P<0.05) and insignificantly (P≤0.01) of the IgM, TAOC, IgG and mortality rate as compared with control group(un- supplemented. The findings of [55] revealed that T-AOC in broiler chickens was significantly increased in both the *Lactobacillus fermentum* and the *Enterococcus faecium* supplemented groups, but the activities of antioxidant enzymes were not determined. The discrepancy among these studies is likely due to the different animals, physiological status, diet compositions, the probiotic source, and their application levels. Altogether, our results demonstrated that supplementation of the *B. licheniformis* has no impact on improving the antioxidant enzymes activities in laying hens. The concentrations of the serum immunoglobulin theIgA and IgM were significantly increased by the *B. subtilis* in the diet [56]. Concerning the interaction it could be notice IgM and the T-AOC were significantly (P≤0.01), while the IgG and mortality rate were insignificantly affected by the FR and MT.

3.4. Bacterial count

The effect of FR at different levels on lactic acid bacteria count, total aerobic count, the *E. coli* and the salmonella count, was noted in Table 6**.** Significantly (P≤0.01) decreased in total aerobic and *E. coli* by feeding 110 g as compared with feeding at *ad-libitum*, while, lactic acid and salmonella were insignificantly by FR. Similar results were obtained by [57] reported that the feed restriction programs had a statistically significant (P≤0.05 or (P≤0.01)) effect on the Total aerobic count, the *Escherichia coli*, while the *lactobacilli* and Salmonella count (SC) were not significant. Similar results were reported in broiler by [8-9-58-59], as well as in other species. Concerning the effect of microorganism types (MT) supplementation of the *B subtilus*, *B licheniformis* or *B amyloliquefaciens* (0.5%) in laying diets increased significantly (P≤0.01) of lactic acid, while it was decreased significantly (P≤0.01) and (P≤0.05) of total aerobic count, the *E. coli* and the salmonella as compared to control group (Table 6). These results are in agreement with the findings of [16-49] demonstrated that the addition of the synbiotic (Biomin Imbo) reduced the *Escherichia coli* and the total coliform populations in the intestines of broiler chickens.

On the contrary they added that different levels of symbiotic increased the numbers of *Lactobacillus* in the intestine of broiler chickens. [60] Showed that the addition of synbiotic to the diet resulted in a decrease of caecal coliform organism counts, which could be positive effects of synbiotic on gut microbial ecology, but differed from the results reported by [61]**.** Moreover, [62] reported that the challenges with nutritional interventions for Salmonella control were variable depending on the nutritional management and Salmonella status of the flock. Synbiotic supplementation had limited efficacy on decreasing SE colonization, although it was not certain that the microorganisms present in these products failed to colonize the enteric microenvironment. Furthermore, it is necessary to consider the composition of the commercial products, their dosage, the route of administration (feed or water) and the farm sanitary conditions. All these factors are able to influence the efficacy of the products [63]**.** It is possible that synbiotic could balance the intestinal micro eco-system by controlling pathogenic bacteria via a competitive exclusion, which improve the count of beneficial bacteria**.**

Previous studies have indicated that probiotics and prebiotics as the synbiotic could regulate the intestinal micro ecological environment in different ways [16-64-65]. The use of (prebiotic, probiotic) as feed additives for pathogen control and performance enhancement in poultry production has gained attention recently due to the increasing restriction of antibiotics as growth -promoting agents [66]**.** According to [67] the prophylactic and curative use of antibiotics to control Salmonella is not recommended for three reasons), which were antibiotic resistant salmonella (and other) strains have emerged; there is a concern about the presence of antibiotic residues in meat and most antibiotics fail to eliminate the Salmonella from animals, although some decreased contamination from this pathogen in animals has been observed. An interaction effect between the FR and TM it could be notice the Lactic acid bacteria count, total aerobic count and salmonella count improved (P≤0.01) significantly, while E. *coli* was insignificantly affected by FR and MT (Table 6).

Table 1. Composition and calculated chemical analysis of the experimental diets

Ingredients	**Basal, %**
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.
**A According to [21], ***According to [22], ****According to [23].

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

Table 3. Effect of feeding regimes (FR) and dietary supplementation of different microorganism types (MT) on productive performance parameters of Inshas layers from 24 to 40 weeks of age.

Items		Productive performance parameters							
		TEN	**EW, g**	**E P, %**	**EM / day**	**TFI, kg**	**DFI, g**	**FC (g feed/g egg)**	**FC (kg feed/ number eggs)**
Effect of feeding regimes (FR)									
ad libitum		52.84	46.39	47.16	21.88	13.84^{a}	123.2^{a}	3.82^{b}	5.66^{a}
110g/hen/day		52.44	46.12	46.82	21.59	12.32^{b}	109.7^{b}	4.26^{a}	5.10^{b}
MSE		**0.84**	**0.13**	**0.15**	**0.38**	**0.02**	**0.18**	**0.01**	**0.11**
Sig.		**NS**	**NS**	**NS**	**NS**	**NS**	**	**	**
Effect of microorganism types (MT)									
Non-supplemented		49.04^{c}	46.30	43.77^{c}	20.27^{c}	13.08^{a}	116.46^{a}	3.75^{c}	5.76^{a}
Bacillus subtilus		55.40^{a}	46.29	49.44^{a}	22.89^{a}	13.00^{b}	115.78^{b}	4.26^{a}	5.08^{bc}
Bacillus licheniformis		53.96^{ab}	46.53	48.16^{a}	22.40^{ab}	13.12^{a}	115.77^{b}	4.12^{ab}	5.23^{bc}
Bacillus amyloliquefaciens		52.20^{b}	45.89	46.60^{ab}	21.38^{b}	13.12^{a}	116.83^{a}	3.98^{b}	5.45^{b}
MSE		**0.73**	**0.16**	**0.69**	**0.35**	**0.09**	**3.11**	**0.07**	**0.18**
Sig. test		**	**NS**	**	**	**	**	**	**
Effect of interactions (FR)x(MT)									
ad libitum	Non-supplemented	50.40	46.37^{a}	44.98	20.85	13.80^{b}	123.09^{ab}	3.65^{c}	5.93
	Bacillus subtilus	54.88	46.26^{a}	48.99	22.67	13.68^{c}	121.87^{c}	4.01^{b}	5.40
	Bacillus licheniformis	53.52	46.83^{a}	47.77	22.37	13.92^{a}	124.27^{a}	3.85^{b}	5.58
	B. amyloliquefaciens	52.56	46.10^{a}	46.91	21.62	13.88^{ab}	123.91^{ab}	3.79^{b}	5.75
110g/hen /day	Non-supplemented	47.68	46.23^{a}	42.56	19.68	12.32^{d}	109.83^{d}	3.87^{bc}	5.59
	Bacillus subtilus	55.84	46.33^{a}	49.89	23.12	12.32^{d}	109.69^{d}	4.53^{a}	4.76
	Bacillus licheniformis	54.40	46.23^{a}	48.55	22.44	12.32^{d}	109.77^{d}	4.41^{a}	4.90
	B. amyloliquefaciens	51.84	45.67^{b}	46.28	21.14	12.32^{d}	109.76^{d}	4.21^{ab}	5.15
MSE		**96.4**	**0.28**	**0.93**	**0.46**	**0.02**	**0.28**	**0.02**	**0.15**
Sig. test		**NS**	*	**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. SEM: Mean at standard error.
TEN=Total egg number, EW, g = Egg weight, EP, % = Egg production, DEM / day = Daily egg mass, TFI, kg= Total feed intake, FC= Feed conversion (Kg feed/ eggs).

Table 4. Some hematological parameters and liver functions $(\overline{X} \pm SE)$ of Inshas layers as affected by feeding regimes (FR) and dietary supplementation of different microorganism types (MT), at the end of experimental period.

Treatments \ Parameters		Some hematological parameters		Liver functions				
		WBC's ($\times 10^3$)	**PCV (%)**	**Total protein (g/dl)**	**Albumin (g/dl)**	**Globulin (g/dl)**	**ALT (U/l)**	**AST (U/l)**
Feeding regimes (FR)								
ad libitum		118.64	28.85	5.98	3.59	2.40	13.05^{a}	236.50
110g/hen/day		120.92	29.95	6.07	3.45	2.62	11.92^{b}	237.75
MSE		**2.62**	**0.55**	**0.14**	**0.10**	**0.14**	**0.52**	**7.41**
Sig.		**NS**	**NS**	**NS**	**NS**	**NS**	*****	**NS**
Microorganism types (MT)								
Non-supplemented		123.77	28.99^{b}	5.91^{b}	3.61	2.30^{b}	12.09^{ab}	216.84^{b}
Bacillus subtilus		116.82	29.37^{b}	6.54^{a}	3.59	2.9^{a}	13.74^{a}	249.84^{a}
Bacillus licheniformis		114.14	27.62^{b}	5.81^{b}	3.45	2.37^{b}	13.52^{a}	233.67^{ab}
Bacillus amyloliquefaciens		124.39	31.60^{a}	5.82^{b}	3.42	2.40^{b}	10.59^{b}	248.17^{a}
		3.93	******	**0.18**	**0.15**	**0.13**	**0.63**	**9.34**
Sig.		**NS**	**NS**	*****	**NS**	*****	******	******
Interactions (FR)x(MT)								
FR	**MT**							
ad libitum	Non-supplemented	127.10	28.04	5.86	3.49	2.37	13.34	228.67^{bc}
	Bacillus subtilus	120.44	29.94	5.97	3.73	2.24	10.84	205.00^{c}
	Bacillus licheniformis	11.04	28.24	6.43	3.64	2.97	14.14	228.67^{bc}
	Bacillus amyloliquefaciens	122.60	30.50	6.65	3.71	2.94	13.34	271.00^{a}
110g/hen/day	*Non-supplemented*	111.10	27.37	5.93	3.70	2.24	14.04	221.34^{bc}
	Bacillus subtilus	117.17	27.87	5.70	3.20	2.50	13.00	246.00^{ab}
	Bacillus licheniformis	125.30	31.74	5.69	3.69	2.00	10.67	267.34^{a}
	amyloliquefaciens	123.47	31.47	5.96	3.16	2.80	10.50	229.00^{bc}
MSE		**4.62**	**0.86**	**0.16**	**0.18**	**0.14**	**0.73**	**8.76**
Sig.		**NS**	**NS**	**NS**	**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. SEM: Mean at standard error.
WBC': White blood cells, PCV (%): Packed Cell Volume**, ALT:** Alanine Aminotransferase, **AST:** Aspartine Aminotransferase

Table 5. Immunological response, total antioxidant capacity and mortality rate of Inshas layers as affected by interactions b/w feeding regimes dietary supplementation of different microorganism types during experimental periods from 24-40 weeks of age.

Items		Immunological response		Total antioxidant capacity (mM/L)	Mortality rate (%)[1]
		IgG (ug/ml)	IgM (ng/ml)		
Effect of feeding regimes (FR)					
ad libitum		14.22[a]	5.25[a]	1.65[b]	3.33
110g/hen/day		12.34[b]	3.10[b]	2.05[a]	0.83
MSE		**0.42**	**0.23**	**0.15**	**-**
Sig. test		****	****	*	**NS**
Effect of microorganism types (MT)					
Non-supplemented		12.94±0.34	3.59±0.26 [b]	1.60±0.18 [b]	5.00
Bacillus subtilus		13.49±0.98	4.32±0.51[ab]	2.08±0.23[ab]	1.67
Bacillus licheniformis		13.22±0.35	4.77±0.63 [a]	1.52±0.16 [b]	1.67
Bacillus amyloliquefaciens		13.47±0.68	4.04±0.48[ab]	2.21±0.20 [a]	0.00
MSE		**0.71**	**0.53**	**0.22**	**-**
Sig. test		**NS**	*	*	**NS**
Effect of interactions (FR)x(MT)					
FR	**MT**				
ad libitum	Non-supplemented	13.40	4.07[cd]	1.27[c]	6.67
	Bacillus subtilus	12.47	3.10[d]	1.92[ab]	3.33
	Bacillus licheniformis	15.10	5.40[ab]	1.84[b]	0.00
	Bacillus amyloliquefaciens	11.87	3.24[d]	2.32[a]	0.00
110g/hen/day	Non-supplemented	13.90	6.50[a]	1.45[c]	3.33
	Bacillus subtilus	12.54	3.04[d]	1.60[bc]	0.00
	Bacillus licheniformis	14.47	5.04[bc]	2.05[ab]	3.33
	amyloliquefaciens	12.47	3.04[d]	2.37[a]	0.00
MSE		**0.87**	**0.28**	**0.0.24**	**-**
Sig. test		**NS**	*	*	**NS**

[1]These values of mortality rate were analysis by using Chi-Square Means are bearing different letters, differ significantly (P0.05). a, b Means having different letters in the same column differ significantly ($p \leq 0.05$). NS= Not significant; * = ($P \leq 0.05$).

Table 6. Bacterial count $(\bar{X} \pm SE)$ of Inshas layers as affected by feeding regimes (FR) and dietary supplementation of different microorganism types (MT), during the experimental periods from 24 to 40 weeks of age.

Parameters / Treatments		Lactic acid bacteria count	Total aerobic count	E. *coli*	Salmonella count (SC)
Effect of feeding regimes (FR)					
ad libitum		46.56	17.35^{b}	2.34^{a}	1.8083
110g/hen/day		48.71	36.99^{a}	1.73^{b}	1.9167
Sig. test		**NS**	**	**	**NS**
Effect of microorganism types (MT)					
Non-supplemented		31.54 ^{c}	43.62^{a}	2.15^{a}	2.4167^{a}
Bacillus subtilus		51.67^{ab}	24.42 ^{b}	1.67^{b}	1.7167^{b}
Bacillus licheniformis		59.84^{a}	22.29 ^{b}	1.50^{b}	1.6333^{b}
Bacillus amylolique faciens		47.50^{b}	18.34^{c}	1.65^{b}	1.6833^{b}
Sig. test		**	**	**	*
Effect of interactions (FR)x(MT)					
FR	**MT**				
ad libitum	Non-supplemented	31.70^{e}	20.07^{c}	2.35	2.33^{a}
	Bacillus subtilus	45.97^{cd}	16.30^{cd}	2.28	1.70^{b}
	Bacillus licheniformis	73.74^{a}	15.54 ^{d}	1.98	1.60^{b}
	Bacillus amyloliquefaciens	34.84^{de}	17.47^{cd}	1.93	1.60^{b}
110g/hen/day	Non-supplemented	31.37^{e}	67.17^{a}	1.84	2.500^{a}
	Bacillus subtilus	57.37^{bc}	32.54^{b}	1.45	1.733^{b}
	Bacillus licheniformis	45.94^{cd}	29.04^{b}	1.52	1.667^{b}
	Amylolique faciens	60.17^{b}	19.20^{cd}	1.07	1.767^{b}
Sig. test	**MT**	**	**	**NS**	*

a, b, c Means having different letters in same column differ significantly ($p \leq 0.05$) NS= Not significant; * = ($P \leq 0.05$); ** = ($P \leq 0.01$), X ± SE= Average ± Standard error.

4. Conclusions

It can be concluded that, feed regimes at 110 g per day is not negative effect of productive and reproductive performance, while supplemental layer diets with Microorganism types were more effective for improving productive and reproductive performance traits, biochemical, immunological blood parameters and bacterial count of Inshas laying hens.

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