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EFFECT OF PROPOLIS AND ZINC METHIONINE SUPPLEMENTATION ON IMPROVEMENT OF PRODUCTIVE, REPRODUCTIVE AND IMMUNITY PERFORMANCE OF LOCAL DEVELOPED INSASH STRAIN UNDER EGYPTIAN SUMMER CONDITIONS.

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ABSTRACT: The objective of this study was to evaluate the impact of propolis and zinc methionine (Zn– Met) supplementation on improvement of productive, reproductive and immunity performance of local developed Insash strain under Egyptian summer conditions. A total number of 180 laying hens and 36 cocks of Inshas chicken at 24 weeks of age was used and divided into equal 6 experimental groups (30 hens + 6 cocks), each of three replicates (10 hens +2 cock in each replicate).

The obtained results revealed a significant (P<0.05) improvement in egg production percentage, egg mass and feed conversion ratio in the groups fed propolis at the level of 100 or 300 mg plus 80 mg Zn–Met or at 300 mg propolis only /kg diet as compared with the control group. Percentage of fertile eggs, serum ALT, dead spermatozoa, sperm abnormalities, were significantly improved at the level of 100 or 300 mg propolis either alone or plus 80 mg Zn–Met /kg diet. While, percentage of hatchability, sperm motility, sperm cell concentration, were significantly increased in the group received 300 mg propolis only or plus 80 mg Zn–Met /kg diet. Serum total protein, globulin and cholesterol, acrosomal damage, immune response after injection of phytohemoaglutinine (PHA–P), serum IgG and IgM esteems were significantly (P<0.05) enhanced in all supplemented groups compared with the contrasted control. Digestibility coefficients of dry matter (DM), crude protein (CP) and organic matter (OM) were significantly (P<0.05) improved at the level of 100 or 300 mg propolis plus 80 mg Zn–Met /kg diet or at 300 mg propolis /kg diet alone, while AST and ether extract (EE) were significantly (P<0.05) improved at the level of 300 mg propolis /kg diet.Therefore, it could be concluded that, supplementation of 300 mg propolis either single or plus 80 Zn-Met /kg diet is recommended for improving most of productive and reproductive, traits including egg production, semen quality, fertility, hatchability and serum biochemical traits as wellas immune response.

Keywords: Propolis-zinc methionine-laying hens-egg production-reproduction-immunity.

INTRODUCTION

Heat stress is a great hazard to poultry production and has a deleterious impact on fowls health and productivity. High environmental temperature causes substantial economic losses to the poultry business due to low nutrient intake, high mortality rate , and low meat quality (Chiang et al., 2008). The dietary utilization of normal antioxidant is considered as a possible strategy to reduce the deleterious consequence of heat stress in birds (Patra et al., 2011). Bongiovanni et al., (2007) examined the impacts of natural antioxidants on stress including heat stress and finished that the dietary consideration of flavonoids may give security against intense heat stress. Therefore, propolis may be utilised in poultry diet because of its antistress effects (Tatli- Seven et al., 2008). It protects the bird from the adverse effect of lipid peroxidation and free radical formation during heat stress (Tatli- Seven et al., 2009).

Propolis is known for its pharmaceutical properties, for example, expanding cell resistance to hyperthermia, in view of its antioxidants impacts (Belloni et al. (2015). Propolis is rich of substantive elements, including Mg, Cu, Fe, Mn, Ni and Ca, that might also be responsible for reactivating antioxidant enzymes (Farooqui and Farooqui, 2010). Propoilis, has a vehement antioxidant properties, as fine as, it can enhance the growth performance and egg quality in laying hens raised under shrilling surrounding temperatures (Seven et al., 2011). Also, Propolis has hard antibacterial (Bankova et al., 2000) antioxidant (Banskota et al., 2000), antiviral (Vynograd et al., 2000) and mitigating (Sforcin, 2007), antifungal and immunostimulatory properties (Bankova et al., 2000). Propolis invigorates the body immune system and thus it may enhance the growth performance and health position of broiler and laying hens. (Cetin et al., 2010). Seven et al. (2011) found that constituent of propolis in the diet of laying hens has led to significantly lessening adverse effects of heat stress on performance, nutrient digestibility and egg shell quality.

Zinc is important microelement in supporting bone and tissue advancement (Sahraei et al., 2012), egg shell characteristics (Zhang et al., 2017), as wellspring as in legitimate working of the immune system (Jarosz et al., 2017; Perez et al., 2017). Increment zinc methionine in the diet of laying hens exposed to heat stress significantly improved egg shell weight and thickness either at level of 80 mg/kg (Moreng et al., 1992) or at level of 100 mg/kg (Batnave and Zhang 1993). Supplementation of natural zinc in diet of quails during heat stress at levels of 30 and 60 mg /kg diet brought about status in egg production, egg quality, feed intake and efficiency; linearly increased digestibility of dry matter, organic matter, crude protein and ether extract (Sahin and Kucuk, 2003). Bealish et al.(2010) noted that Inshas cocks fed 105 mg Bioplex Zn / Kg diet showed the highest sperm cell concentration and lowest dead sperm percent as compared with their controls.

MATERIALS AND METHODS

Birds, diet and treatments:

The experimental work was carried out at Inshas Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Giza,

Egypt, at the period from June, to Augusts, 2017.

A total number of 180 laying hens and 36 cocks of Inshas chicken at 24 weeks of age (At 44 % egg production) was used and divided into equal 6 experimental groups (30 hens + 6 cocks), each of three replicates (10 hens +2 cock in each replicate) with similar body weight and egg production rate in a completely randomized design to investigate the effects of propolis and zinc methionine (Zn–Met) supplementation on improvement of productive, reproductive and immunity performance of local developed Insash strain under Egyptian summer conditions. Birds in each replicate per treatment group were kept in single cage in an open system house. The basal diet was formulated to meet the NRC (1994) recommendations as shown in Table 1 and the calculated analysis was according to Feed Composition Tables for Animal and Poultry Feedstuffs used in Egypt (2001). The experimental groups were arranged as follows: : the 1st group fed a basal diet with no dietary supplementation (as a control), the 2nd group was fed the basal diet supplemented with 80 mg Zn–Met diet, where the 3rd up 4th groups were fed the basal diet supplemented with 100 and 300 mg propolis /kg diet, respectively, while the 5th and the 6th groups were fed the basal diet supplemented with 80 mg Zn – Met / kg diet and 100 or 300 mg propolis/kg diet, respectively. Birds were fed ad libitum and fresh water was continuously provided. Birds were submitted to the same managerial conditions in a window house with light cycle regimen of 16 hours light: 8 hours darkness. The birds were inspected against illnesses and treated with anti-microbials and immunizations to keep them healthy. The average minimum and maximum of ambient temperature during the experimental period ranged between 27 and 37.6^{0}C, relative humidity ranged between 22.2 to 82.4 % and temperature-humidity index (THI) from 23.93to 36.35 under Inshas, Sharkia Governorate, Egypt as shown in Table 2. THI was estimated according to the following formula:

THI=db ^{o}C-{(0.31-0.31 RH) (db ^{o}C - 14.4)}, where db ^{o}C = bulb temperature in Celsius and RH= RH%. The THI values indicate the following: <22.2 = absence of heat stress; 22.2 to <23.3 = moderate heat stress: 23.3 to <25.6 = severe heat stress and 25.6 and more = Extreme severe heat stress under environmental conditions in Egypt (Marai et al., 2000).

Laying performance traits:

The body weight changes of laying hens calculated by the difference between final and initial weight, while, the egg number (EN) and egg weight (EW) were daily recorded. Feed consumption (FC) was weekly recorded. The egg production rate (EP) was calculated during the experimental period where:

Egg production rate = Egg number / hen/ x 100 and egg mass (EM) were calculated during the whole experimental period from 24-36 weeks of age. Feed conversion (g feed/g egg) (FCR) was also calculated. The mortality rate was daily recorded for each treatment from 24 weeks of age until the finish of the experiment (36 weeks of age).

Hatching traits:

After production of the primary egg, all females were artificially inseminated twice times every week with a blend of semen collected from the same group of

cocks. At 36 weeks of age, around 60 eggs from every treatment group were gathered and hatched. Subsequent to hatching, the chicks were counted and non-hatched eggs were broken to decide the rates of fertility and hatchability. Fertility was calculated as the percentage of fertile eggs from the total number of set eggs, while the hatchability was expressed as the chicks hatched from fertile eggs and from total eggs.

Immune response evaluation:

1-Evaluation of cell-mediated immune response using Phyto-hemagglutinin-p (PHA-p) injections:-

On day 70 of age, twenty birds from inshas strain were injected intra-dermally in the right wattle with 100 µl of 1 mg/ml phytohemagglutinin PHA-P (Sigma L 9017, St. Louis, Mo, USA) dissolved in phosphate buffered saline (**Lee et al., 2005**). Then, as a control, the left wattle of the same bird was injected moments later with 0.1 ml of phosphate buffered saline (PBS). The thickness of the right wattle of each bird was measured using tension calipers before PHA-p injection, as well as, at 24, 48 and 72 hours post injection. The Wattle Swelling was determined as the distinction between the thickness of wattle when infusion.

2- **The immunoglobulin IgG and IgM** in blood were determined using a commercial ELISA kit from Bethyl Laboratories (Montgomery, AL, USA), as the technique depicted by Gao et al. (2008).

Blood samples:

At the finish of the trial period, 3 hens arbitrarily browsed every treatment and blood tests were acquired from the brachial vein into tubes. Blood serum was isolated by centrifugation of the blood at 3000 rpm for 15 min and was then kept at -20°C for investigation. The frozen samples were permitted to defrost at room temperature before examination. Total protein, albumin, cholesterol and liver enzymes (AST and ALT) were evaluated using business Kits delivered by Bio-demonstrative, Egypt. Globulins were evaluated by subtraction of albumin value from total protein value.

Semen quality:

Semen was collected at three times amid the trial time frame at 28, 32 and 36 weeks of age from 6 cocks in each treatment which were randomly chosen using the massage method. Immediately after semen collection, semen-ejaculate volume (ml) was measured utilizing graduate collecting tubes and hydrogen-ion concentration (pH) was measured by Universal Indicator Paper and Standard Commercial Stain. A drop of semen with the guide of a micro-pipette was set on a pre warmed microscope slide, which was then covered with a glass cover slip and inspected at a magnification of ×400. Motility of semen was evaluated as the level of motile spermatozoa having moderate to fast dynamic motility according to Ommati et al., (2013). No less than 10 minuscule fields were assessed for each semen test. Eosin-Nigrosine stains were used to detect the percent of morphologically sperm variations from the sperm abnormalities and dead spermatozoa. For sperm cell concentration (X 109/ml) a droplet of diluted semen (1:200 in refined water) was delicately put on the two committees of a Neubauer hemocytometer and the quantity of spermatozoa was resolved minutely (Ommati et al., 2013). Acrosomal damage of spermatozoa (%) was determined according to Waston (1975). Not less than 10 minuscule fields were inspected for every semen test.

Digestibility trials:

At the end of experimental period, sixty birds were put into individual battery cages and distributed into six treatments, 10 birds each, for assurance of nutrient digestibility. Digestibility of nutrients was estimated by evaluating the feed consumed and by gathering excreta two times per day. The excrement samples were oven-dried at 60°C for 48 hours then ground for chemical analyses. Biochemical analyses of experimental samples were determined according to AOAC (2000). Moisture was analyzed by oven drying at 105°C for 24 h. Crude protein was determined by the Kjeldahl method and multiplying by a factor of 6.25. Crude ash was determined using a muffle furnace at 550°C for 24 h. Crude fibre was determined by sample digestion with H2SO4 and NaOH. Lipids were extracted from samples using the chloroform: methanol mixture (Bligh and Dyer, 1959).

The economic efficiency (EEF):

The economic efficiency (EEF) was evaluated as follows:

EEF = (Net revenue/hen / Total cost hen) X 100.

Statistical analysis:

Data were analyzed by the least square analysis of variance according to Snedecor and Cochran (1982) using the General Linear Model Procedure (SAS, 2004) at the 5% level of significance as the following model:

$Y_{ij} = \mu + N_i + e_{ij}$

Where: Y_{ij} = Any observation, μ = Overall mean, N_i = Effect of treatment (i = 1….6)., Rj= Replicates(j1,2,3), eij = Experimental random error. All percentage data were transferred to percentage angle using arcsine equation before subjected to statistical analysis. Significant differences among means were tested using Duncan Multiple New Range Test (Duncan**,** 1955).

RESULTS AND DISCUSSION

Productive performance:

As shown in Table (3) body weight, feed intake, egg weight and viability percentage were insignificantly affected by dietary supplementation of Zn – Met or propolis either alone or together in the diet of Inshas strain under Egyptian summer as compared to the control group. However, egg production percentage, egg mass and feed conversion ratio were significantly ($P<0.05$) improved in the hens received propolis at the level of 100 or 300 mg plus 80 mg Zn-Met or at 300 mg propolis only /kg diet as compared to the control group. However, the other treated groups were numerically improved than the control. The best values of productive performance were accomplishment in all groups received propolis while the worst were of the control and Zn-Met groups. This positive effect on the performance traits of laying chickens due to propolis supplementation may be because it improves intestinal enzymes (glucase, oxidase, catalase and peroxidase) that may be associated with better digestibility of different nutrients (Khojasteh and Shivazad, 2006). Also, propolis has strong antioxidants and stimulates the body immunity process, this may increase the growth performance and health status of laying hens that reared under high ambient temperature (Cetin et al., 2010). The results obtained here are in concurrence with that acquired by Drumus et al. (2004) who found that

expansion of zinc at various levels had no significant effect on egg production rate, egg weight, feed conversion ratio and viability. On the contrary, Seven et al. (2011) found a significant improvement of egg production, egg mass and feed conversion ratio by dietary supplements of 3 g extract propolis /kg in the diet of layer hens during heat stress. Likewise, Abdel-Kareem and El-Sheikh (2015) also observed the same effect by inclusion of propolis in the diet of layer at level of 250 or 500 mg/kg diet. These results are not in accordance with those reported by Belloni et al. (2015) who found a significant reduction in egg production, egg weight and feed conversion ratio of layer hens that fed a diet supplied with propolis at level of 1, 2 and 3% under hot climatic environment as contrasted with the control group.

Fertility and hatchability percentages:

The results in Table (4) indicated that dietary supplementation at 100 or 300 mg propolis /kg diet either alone or plus Zn-Met resulted in a significant ($P<0.05$) increase in percentage of fertile eggs, while hatchability of total eggs was significantly ($P<0.05$) increased at 300 mg propolis /kg diet either alone or with Zn-Met and insignificant hatchability of fertile eggs as compared with the control group. However, the group of Zn-Met alone had no significant effect on these traits, the highest values were achieved at the level of 300 mg propolis only or with Zn-Met. The improvement in fertility may be attributed to propolis that provides prevention of infecundity by increasing the sterioidogenesis and hence testosterone production (Yousef and Salama, 2009). These results are in agreements with the findings of El-Neny et al. (2014) and Shreif and El-Saadiny (2016) who reported that the addition of propolis to the diet of laying hens resulted in a significant increase in hatchability and fertility. Also, dietary supplementation of 100 or 150 mg zinc Methinine/kg diet (Kout EL-Kloub et al. (2004) resulted in a significant increase in fertility and hatchability rates. On the other hand, percentages of fertility and hatchability were not significantly different in laying hens fed 60 mg organic zinc/kg diet (Sahin and Tasdemir 2017).

Immune response:

Cell–mediated immune response as measured by phytohemoaglutinine PHA–P stimulation (wattle) is illustrated in Figure (1). The data revealed that wattles thickness were significantly ($P<0.05$) higher at 24 ,48 and 72 hr after injection of PHA–P in the all experimental groups compared with their control. These results agree with the findings of Galal et al. (2008) who reported that wattles response of PHA–P injections in the hens fed 100 or 150 mg propolis/kg diet. Propolis demonstrates to be viable against an assortment of microbes, viruses, fungi and molds. Histological, PHA–P is emphatically mitogen to t-lymphocytes and intradermali infusions evoke macrophage penetration and thick per vascular aggregation of lymphocytes 24 post infusion in birds (McCorkle et al., 1980). The increased infiltration by basophils hypersensitivity response (Stadeckerm et al. 1977). For, serum IgG and IgM values were significantly ($P<0.01$) higher in all experimental groups that fed propolis or Zn-Met either alone or together as compared to the control group (Figure 2). The improvement in

immunological status may be to propolis contains several biological and pharmacological properties such as antimicrobial, anti-inflammatory, immunomodulatry and antioxidant effects (Newairy et al., 2009 and Orsatti et al., 2010), Moreover, artepillin C which is one of propolis components has been described to activate the immune system by increasing phagocytic activity as well as number of lymphocytes (Kimoto et al., 1998). Wang et al. (2011) announced that Zn initiates generation of Zn-metallothionein, which is an effective scavenger for hydroxyl radical and providing protection against immune-mediated free radical attack. Addition of zinc-methionine to the diet of laying hens may improve the immune system and augment disease resistance (Kidd et al., 1996). Same exact results, serum IgG and IgM levels were significantly increased by inclusion of propolis in the diet of laying hens at level of 3 g/kg /diet (Cetin et al., 2010) and 250 and 450 mg/kg (Shreif and El-Saadny 2016). Also, Hudson et al. (2004) found similar cell reaction to PHA and protein titres against the Newcastle infection in broiler breeders fed diets supplemented with organic zinc. Sunder et al. (2008) reported that body substance and cell – interceded immune responses iwere significantly higher enhanced with 80 mg/kg than those enhanced with under 80 mg/kg of zinc. Bartlett and Smith (2003) revealed that dietary Zn supplementation enhanced lymphoid organ weights, essential and optional protein reactions, substantial cell capacity of macrophages, absolute IgM and IgG immunizer titres in male broilers raised up in high ecological temperature.

Serum biochemistry parameters:

Results in Table 5 reveal that total protein and globulin were significantly higher and cholesterol was significantly ($P<0.01$) lower by supplementation of Zn – Met or propolis either alone or together as compared to the control group. While, no significant differences were detected in serum albumin between the experimental groups.. The increase in total protein could be credited to enhancement of protein absorption due to the supplemented propolis to the diet. Giurgea et al. (1981) showed that supplying propolis to chicken changed the blood concentration of total protein, amino acid and cholesterol. Propolis invigorates protein biosynthesis (Gabrys et al., 1986). While increase of globulin might be expected may be an indication of increased immunity in the rabbits since the liver will be able to synthesize enough globulins for immunologic action as mentioned by Sunmonu and Oloyede (2007). Similar results reported by different authors (Galal et al., 2008 , Abdel –Kareem and El – Sheikh 2015, Shreif and El –Saadny 2016) that ,supplement of propolis to the diet of laying hens enhanced blood concentrations of total protein and globulin. The decreasing value of cholesterol might be credited to propolis. Uyanik et al. (2001) demonstrated that Zn supplementation decreased serum cholesterol concentration of broilers (Wang et al., 2011). In regard to,AST enzyme, it was significantly ($P<0.05$) decreased at 100 or 300 mg propolis/kg diet. However, ALT enzyme significantly ($P<0.05$) reduced at 100 mg or 300 mg propolis either alone or plus 80 Zn-Met as compared with the control group.

Semen traits: Results of semen traits presented in Table 6 show that ejaculate experimental treatments when compared with the control group. On the other hand, percentage of sperm motility was significantly ($P<0.05$) increased by addition of propolis at level of 300 mg /kg diet either alone or plus Zn-Met when compared with the control group, while the remaining treatments did not result in a significant increase of this parameter compared with the control. In regard to the percentages of dead spermatozoa and sperm abnormality, there were significant ($P<0.05$) decrease in the groups received propolis either alone or plus Zn-Met compared with the control, however, these traits were slightly improved in the group that received Zn –Met alone. Sperm cell concentration was significantly ($P<0.05$) higher in the group received 300 mg propolis only or plus 80 mg Zn-Met /kg, however, the other treatments did not differ significantly from the control. Percentage of acrosomal damage was significantly ($P<0.01$) improved in all applied experimental treatments compared with the control group. Characteristics of semen quality achieved the best values at the level of 300 mg proplis either alone or plus Zn-Met followed by the level of 100 mg propolis, while the group received Zn –Met showed the lowest value. This enhancement in semen quality might be ascribe to propolis useful impact in reducing the adverse effect of lipid peroxidation and free radical formation during heat stress (Tatli- Seven et al., 2009), propolis is rich in folavonoids, phenolic acid and terpenoid content (Prytzyk et al., 2003) that might shield unsaturated fats from oxidation in cell membranes (Havesteen 2002). Our outcomes are in agreement with the findings of Yousef et al. (2010) in rabbit, El-Neny et al. (2014) and Sherif and El-Saadny (2016) in cocks. However, Jian-bin et al. (2007) did not prove any significant difference in survival state, live index, and the density of sperm either alone or in combination with Zn-Met under prevailing summer conditions in Egypt.

volume (ml) and hydrogen ion concentration (pH) insignificantly affected by dietary

when fed 60 mg Zn/kg diet, whereas, it was significantly lower than that fed 180 mg Zn/diet.

Nutrients digestibility coefficients:

Results in Table (7) show that incorporation of Zn – Met or propolis either alone or together in diets had no significant effect on digestion coefficients of ash and crude fiber (CF). However, the digestibility coefficients of dry matter (DM), crude protein (CP) and organic matter (OM) were significantly ($P<0.05$) improved at level of 100 or 300 mg propolis /kg diet plus 80 mg Zn – Met /kg diet or at 300 mg propolis /kg diet alone compared with the control group. Whereas, the digestibility coefficients of ether extract (EE) was significantly ($P<0.05$) improved with 300 mg propolis /kg diet. These results are in agreement with those of Tatli-Seven et al. (2008) who reported that digestibility of dry matter, organic matter, crude protein and ether extract were enhanced with expanding of both dietary vitamin C and propolis ($p<0.05$). Seven et al. (2011) found that supplementations of layer diet with propolis have significantly diminished the negative impacts of heat stress on nutrient digestibility (dry matter, crude proteins and organic matter).

Economic efficiency (EEf):

Data presented in Table (8) reveal that Inshas layers received 300 mg Propolis fed diet recorded the highest net revenue and best economic efficiency followed by those fed 80 mg Zn Met +100 mg Propolis / kg diet, however; the control group had the lowest net revenue and economic efficiency.

CONCLUSION,

the results proved that supplementation of propolis either alone or plus Zn-Met in the diet of Inshas layers strain leading to a significant improvement in productive,reproductive and immunity performance, the best values has been acheived at the level of 300 mg propolis

Table (1): Composition and calculated analysis of the basal diet.

Ingredients	**%**
Yellow corn	61.80
Soybean meal (44% CP)	15.10
Wheat bran	8.28
Limestone	8.10
Corn gluten meal (60% CP)	4.75
Dicalcium phosphate	1.35
Vegetable oil	0.00
Salt	0.30
Vit + Min. premix*	0.30
DL-Methionine	0.02
Total	100
Calculated analysis :(NRC, 1994)	
Crude protein (CP); %	16.07
ME; kcal/kg	2691
Ether extract	2.94
Crude fiber	3.43
Calcium	3.47
Av. Phosphorus	0.304
Lysine	0.65
Methionine	0.31
Methionine + cystine	0.61

*Vitamin and mineral premix: added to the 1 kg of diet including Vit. A 10000 I.U; Vit. D3 2000 I.U; Vit. E 15 mg; Vit. K3 1 mg; Vit. B1 1mg; Vit. B2 5 mg; Vit. B12 10 µg; Vit. B6 1.5mg; Niacin 30mg; Pantothenic acid 10mg; Folic acid 1mg; Biotin 50 µg; Choline 300 mg; Zinc 50mg; Copper 4mg; Iodine 0.3 mg; Iron 30mg; Selenium 0.1mg; Manganese 60mg; Cobalt 0.1mg.

Table (2): Means of air temperature (^{0}C), relative humidity (RH %) and temperature-humidity index (THI) during the experimental period (Source: The Egyptian Meteorological Authority)

Parameter / **Month**	**^{0}C**		**RH (%)**		**THI**	
	Min*	**Max****	**Min***	**Max****	**Min***	**Max****
June	25.5±0.32	37.14±0.45	18.44±1.03	79.04±2.6	22.69	35.66
July	27.55±0.24	38.36±0.43	22.04±1.48	84.62±1.67	24.37	37.21
August	27.78±0.21	37.33±0.24	26.2±1.18	83.65±0.9	24.72	36.17
Average	26.95±0.26	37.61±0.38	22.23±1.23	82.44±1.73	23.93	36.35

Table (3): Effect of dietary supplementation of zinc methionine and/or propolis on productive performance $\left(\overline{X} \pm SE\right)$ of Insash laying hens

Parameter / Treatment	Body weight gain (g.)	Feed intake (g. / hen/ day)	Feed conversion (g. feed/ g. egg mass)	Egg production %	Egg weight (g.)	Egg mass (g./hen)	Viability, %
Control	203.47±27.32	104.59±2.41	5.08±0.16 [a]	47.27±0.83 [c]	43.60±0.26	20.61±0.44[c]	93.33
80 mg Zn Met	200.39±24.29	105.25±2.4	4.87±0.07 [ab]	48.42±1.57 [bc]	44.69±0.38	21.64±0.78[bc]	93.33
100 mg Propolis	263.34±35.01	104.81±3.08	4.59±0.18 [abc]	51.34±1.46 [abc]	44.57±0.32	22.87±0.49[abc]	100.00
300 mg Propolis	277.86±31.14	106.46±2.56	4.22±0.25 [b]	56.67±2.85 [a]	44.78±0.50	25.40±1.51[a]	96.67
80 mg Zn Met +100 mg Propolis	229.65±31.25	107.67±2.31	4.42±0.17 [bc]	55.34±1.77 [a]	44.17±0.62	24.47±1.11[ab]	96.67
80 mg Zn Met +300 mg Propolis	235.72±38.04	106.5±2.14	4.47±0.24 [bc]	53.67±2.03 [ab]	44.62±0.45	23.95±0.96[ab]	96.67
Sig.	NS	NS	*	*	NS	*	NS

Means having different superscripts in the same row, differ significantly. * = (P<0.05); ** = (P<0.01); NS= Not significant.

Table(4): Effect of dietary supplementation of zinc methionine and/or propolis on hatchability traits $\left(\overline{X} \pm SE\right)$ of Insash laying hens.

Parameter Treatment	Fertile Eggs, %	Hatchability /Total eggs, %	Hatchability/ Fertility eggs, %
Control	82.79±1.87 [c]	75.76±2.47 [c]	91.55±2.89
80 mg Zn Met	83.84±2.03 [bc]	78.79±1.75 [c]	94.09±3.08
100 mg Propolis	89.40±1.65 [ab]	79.31±2.22 [bc]	88.80±3.29
300 mg Propolis	91.19±1.50 [a]	85.72±0.39 [a]	94.07±1.86
80 mg Zn Met +100 mg Propolis	88.92±2.27 [ab]	79.62±1.46 [bc]	89.73±3.77
80 mg Zn Met +300 mg Propolis	90.57±1.83 [a]	85.18±1.95 [ab]	94.08±1.78
Sig.	*	*	NS

Means having different superscripts in the same row, differ significantly. * = (P<0.05); ** = (P<0.01); NS= Not signifi

Table (5): Effect of dietary supplementation of zinc methionine and/or propolis on some blood parameters $(\overline{X} \pm SE)$ of Insash laying hens

Parameter / Treatment	Total protein (g/dl)	Albumin (g/dl)	Globulin	AST (mmol/l)	ALT (mmol/l)	Cholesterol (mg/dl)
Control	4.80±0.18 [b]	2.95±0.06	1.86±0.20 [b]	0.78±0.019 [a]	0.59±0.011 [a]	153.03±8.63 [a]
80 mg Zn Met	5.42±0.20 [a]	2.79±0.13	2.64±0.29 [a]	0.72±0.020 [abc]	0.56±0.015 [ab]	130.08±5.67 [b]
100 mg Propolis	5.52±0.29 [a]	2.85±0.17	2.67±0.27 [a]	0.71±0.017 [bc]	0.53±0.016 [b]	126.41±7.13 [b]
300 mg Propolis	5.68±0.25 [a]	3.08±0.1	2.60±0.22 [a]	0.69±0.015 [c]	0.52±0.018 [b]	119.09±5.60 [b]
80 mg Zn Met +100 mg Propolis	5.91±0.19 [a]	2.96±0.14	2.96±0.18 [a]	0.75±0.018 [ab]	0.52±0.008 [b]	124.27±5.90 [b]
80 mg Zn Met +300 mg Propolis	5.82±0.15 [a]	2.98±0.17	2.85±0.20 [a]	0.73±0.013 [abc]	0.53±0.018 [b]	120.91±6.02 [b]
Sig.	*	NS	*	*	*	*

Means having different superscripts in the same row, differ significantly. * = (P<0.05); ** = (P<0.01); NS= Not significant.

Table (6): Effect of dietary supplementation of zinc methionine and/or propolis on semen quality $(\bar{X} \pm SE)$ of Insash laying hens.

Parameter Treatment	Ejaculate volume (ml)	Hydrogen-ion concentration (pH)	Sperm motility (%)	Dead spermatozoa (%)	Sperm abnormalities (%)	Sperm cell concentration (X 10^9/ml)	Acrosomal damage (%)
Control	0.16±0.04	7.00±0.07	80.00±2.24^{c}	18.71±1.41^{a}	13.09±1.90^{a}	2.74±0.19^{b}	14.84±1.02^{a}
80 mg Zn Met	0.26±0.06	7.03±0.07	81.50±2.08^{bc}	16.17±1.38^{ab}	9.00±1.79^{ab}	3.15±0.13^{ab}	11.34±0.96^{b}
100 mg Propolis	0.25±0.04	7.03±0.16	82.92±1.64^{bc}	13.84±1.28^{bc}	7.25±1.44^{b}	3.25±0.21^{ab}	10.17±0.84^{bc}
300 mg Propolis	0.28±0.02	7.12±0.04	87.09±1.64^{ab}	11.75±1.43^{c}	6.30±1.39^{b}	3.47±0.15^{a}	8.17±0.80^{c}
80 mg Zn Met +100 mg Propolis	0.27±0.07	7.05±0.10	84.17±2.72^{abc}	14.21±1.25^{bc}	7.67±1.48^{b}	3.24±0.15^{ab}	8.00±1.00^{c}
80 mg Zn Met +300 mg Propolis	0.20±0.01	7.11±0.10	90.00±2.59^{a}	10.50±1.24^{b}	6.92±1.30^{b}	3.53±0.24^{a}	7.84±0.71^{c}
Sig.	NS	NS	*	**	*	*	**

Means having different superscripts in the same row, differ significantly. * = (P<0.05); ** = (P<0.01); NS= Not significant.

Table (7): Effect of dietary supplementation of zinc methionine and/or propolis on nutrients digestibility coefficients $\left(\overline{X} \pm SE\right)$ of Insash laying hens.

Parameter / Treatment	DM	Ash	CF	EE	CP	OM
Control	90.35±0.32[b]	25.76±1.16	14.52±0.88	64.21±0.57 [b]	74.00±0.88 [b]	73.98±0.21 [b]
80 mg Zn Met	90.53±0.28[b]	28.16±0.93	15.65±0.11	64.49±0.58 [b]	75.78±0.74 [ab]	74.34±0.32 [b]
100 mg Propolis	90.70±0.26[b]	28.92±1.35	15.34±1.09	64.91±0.74 [b]	75.80±0.61 [ab]	74.20±0.43 [b]
300 mg Propolis	92.03±0.31[a]	30.98±1.81	17.02±1.25	67.87±0.84 [a]	77.82±1.20 [a]	76.58±0.83 [a]
80 mg Zn Met +100 mg Propolis	91.35±0.47[ab]	29.07±1.82	15.23±0.77	64.62±0.82 [b]	77.01±0.67 [a]	75.70±0.65 [ab]
80 mg Zn Met +300 mg Propolis	91.97±0.40[a]	30.29±1.71	16.8±0.73	65.50±0.75 [b]	77.87±0.58 [a]	76.45±0.67 [a]
Sig.	*	NS	NS	*	*	*

Means having different superscripts in the same row, differ significantly. * = (P<0.05); ** = (P<0.01); NS= Not significant.

Table (8): Economic efficiency of dietary supplementation of zinc methionine and/or propolis at the end of the experimental period

Treatment Item	Control	80 mg Zn Met	100 mg Propolis	300 mg Propolis	80 mg Zn Met +100 mg Propolis	80 mg Zn Met +300 mg Propolis
Egg production	47.27	48.41	51.33	56.67	55.33	53.67
Price/egg (LE)	1.25	1.25	1.25	1.25	1.25	1.25
Total revenue hen (LE)	59.09	60.52	64.17	70.83	69.17	67.08
Total feed intake/ hen(kg)	8.79	8.84	8.80	8.94	9.04	8.95
Price/Kg feed (LE)	5.250	5.300	5.375	5.625	5.425	5.675
Total feed cost/ hen (LE)	46.12	46.86	47.32	50.30	49.07	50.77
Fixed costs/hen (LE)	3.00	3.00	3.00	3.00	3.00	3.00
Total cost hen (LE)	49.12	49.86	50.32	53.30	52.07	53.77
Net revenue/hen (LE)	9.96	10.66	13.84	17.53	17.10	13.31
Economical efficiency (E.Ef.)	20.29	21.38	27.51	32.89	32.85	24.76
Relative E.Ef.	100.00	105.39	135.63	162.14	161.92	122.08

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الملخص العربي

تأثير إضافة البروبوليس والزنك ميثيونين على تحسين الأداء الإنتاجي والتناسلي والمناعي لسلالة انشاص المستنبطة محليا تحت ظروف الصيف المصري

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الهدف الرئيسي من هذه الدراسة هو دراسة تأثير إضافة البروبوليس والزنك ميثونين (Zn - Met) على تحسين الأداء الإنتاجي والتناسلي والمناعة لسلالة أنشاص المحلية المستنبطة تحت ظروف الصيف المصرية. تم استخدام عدد 180 دجاجة و 36 ديك من دجاج انشاص عمر 24 أسبوعًا (عند 44٪ إنتاج بيض) وتم تقسيمها إلى 6 مجموعات تجريبية متساوية (30 دجاجة + 6 ديوك) ، كل منها بها ثلاث مكررات (10 دجاجة + 2 ديك) متماثلة في وزن الجسم وإنتاج البيض في تصميم عشوائي تام. وتم ترتيب المجموعات التجريبية على النحو التالي: المجموعة الأولى تغذت على عليقه أساسيه (كنترول) ، تم تغذية المجموعة الثانية علي عليقه أساسيه مضاف إليها 80 مجم زنك ميثونين ، و تم تغذية المجموعة الثالثة حتى الرابعة علي عليقه أساسيه مضاف اليها100 و 300 مليجرام بروبوليس / كجم عليقة ، على التوالي ، في حين المجموعة الخامسة إلى السادسة تم تغذيتهم عليقه أساسيه مضاف اليها 80 مليجرام زنك ميثونين + 100 أو 300 مليجرام بروبوليس / كجم عليقة على التوالي.

أظهرت النتائج تحسن معنوي في معدل أنتاج البيض ومعامل التحويل الغذائي وكتلة البيض في المجموعات التى تناولت البروبليس عند مستوى 300 ملجم بروبوليس بمفردة وعند مستوى 100 ملجم بروبوليس + 80 مليجرام زنك ميثونين/ كجم عليقه عند المقارنة بمجموعه الكنترول. تحسنت معنويا نسبة البيض المخصب وسيرم الدم لإنزيم ALT ونسبة الحيوانات الميتة والشاذة عند مستوي100 أو 300 مليجرام بروبوليس منفردا أو مع 80 مليجرام زنك ميثونين / كجم عليقه. بينما زاد معنويا نسبة الفقس بالنسبة للبيض الكلي و النسبة المئوية لحركة الحيوانات المنوية وتركيز الحيوانات المنوية الطبيعية ومعامل هضم المادة الجافة والعضوية عند مستوي300 مليجرام بروبوليس منفردا أو مع 80 مليجرام زنك ميثونين / كجم عليقة. وتحسن معنويا كلا من البروتين الكلي والجلوبيولين ومستوي الكولستيرول وتلف اكروسوم الحيوانات المنوية والاستجابة المناعية بزيادة سمك الدليات عند 24 و 48 و 72 ساعة بعد الحقن PHA – P والجلوبيولين المناعي IgM ، IgG لكل المجاميع التجريبية مقارنة بالكنترول. ووجد أن معامل هضم البروتين الخام تحسن معنويا عند مستوي100 أو 300 مليجرام بروبوليس مع 80 مليجرام زنك ميثونين / كجم عليقه أو 300 مليجرام بروبوليس فقط بينما أنزيم AST ومستخلص الأثير تحسن عند مستوي 300 مليجرام بروبوليس / كجم عليقه.

التوصيه: أوضحت النتائج أن إضافة 300 ملجم بروبليس منفردا أو مع80 مليجرام مع الزنك مثيونين يوصي بها لتحسن معظم الصفات الإنتاجية والتناسلية التي تتضمن البيض وجودة السائل المنوي والخصوبة والفقس وصفات الدم وتحسن معامل هضم المواد الغذائية والمناعة.