Performance of Egg Laying Japanese Quail Hens Fed Low Protein Diet Supplemented with Protease and/or Formic Acid.

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Abstract

This study aimed to decrease the cost of the laying rations by lowering protein as it's the most expensive constituent in the diet. So, the research stated to investigate the effect of incorporating protease and/or formic acid as feed additives into low protein isocaloric diet of laying Japanese quail hens on the productive performance, some egg quality parameters and economic efficiency. It was performed on 120 of 5–6-week-old healthy Japanese quail hens (weighed 268g±7.06) and randomly distributed into five groups (3 replicate per group). G1 (control with basal layer quail diet 20% protein), G2 (17% protein diet without protease enzyme or formic acid), G3 (17% protein diet + 0.05% protease enzyme), G4 (17% protein diet + 0.5% formic acid), G5 (17% protein diet + 0.05% protease enzyme + 0.5% formic acid). The experimental period lasted for 12 weeks with two weeks of adaptation before this period. For each replicate, daily egg number, daily egg weight and egg mass as well as weekly feed intake were recorded. At the end of each week, eggs were collected from each replicate to evaluate egg quality. Mean egg production, egg albumin %, egg shell % and egg yolk % in all groups do not differ significantly with control but G2 was numerically higher than control in egg production, egg mass, FCR, egg shell %, egg yolk % and feed intake. G2 significantly has the heavier egg weight among groups ($p<0.05$) as the average egg weights were 11.77g and 12.48g in control and G2 respectively. G3 was being the highest in feed intake and egg albumin percent than the other groups. G4 was being the highest in egg shell percent. Triglycerides per whole yolk were lower in all groups than the control. Cholesterol (mg)/whole yolk was low in G2 and G3. FCR was improved in G5. G2 had the highest net revenue and economic efficiency (48 LE&114.3%) in compare with control (46

LE&106.98%), while the values were 47.1LE&107.3%, 43.3LE&101.4% and 46.2 LE&105.5% for groups G3, G4 and G5 respectively. It could be concluded that, feeding laying Japanese quail hens on diet containing 17% protein without protease enzyme or formic acid not differ significantly than those fed on control diet containing 20% protein. Moreover, it showed the highest net revenue and numerical improvement than the control group regarding egg production, egg mass, FCR, egg shell %, egg yolk % and cholesterol in egg yolk.

Key words: laying quail hens, dietary protein level, egg production, protease, formic acid.

Introduction

Protein as an important nutrient for egg production, laying Japanese quail need 20% dietary protein for optimum production ***(NRC, 1994).*** However, ***Heywang et al. (1955)*** found that, 15 % protein was sufficient for egg production during hot weather and in one experiment it was noticed that 13 percent was adequate ***(Thornton and Whittet, 1960)***, instead of 17% recommended by ***(NRC, 1994)*** for laying hens.

Proteases have recently attracted attention as feed enzymes. In order to increase dietary protein hydrolysis and hence help to optimize nitrogen utilization with also decreasing the nitrogen content of manure which helps to reduce ammonia release, lower health risks and have a positive influence on the environment ***(Oxenboll et al., 2011)***.

Proteases are used as feed additives to increase the digestibility of proteins, improve amino acid absorption and reduce the cost of purchasing commercial synthetic amino acids ***(Toghyani et al., 2017).***

On the other hand, to prevent Salmonella spp. infection, minimize pathogen colonization and reduce of toxic metabolites, improve protein, Ca, P, Mg, and Zn digestibility, formic acid had been added to poultry diets which also act as substrates in the intermediary metabolism process ***(Lückstädt and Theobald, 2009; Ricke et al., 2020).***

It’s important point that we try to achieve maximal efficiency for laying quail performance with a lower cost of the diet formulated. Therefore, this study aimed to evaluate the effect of protease and⁄or formic acid supplementation to low protein diet, on the productive performance of laying quail, cholesterol level of egg yolk, some of internal and external egg quality parameters and serum biochemical parameters.

Martial and methods

1- Quail management: The current study was conducted at private farm in port said**.** A total of 120 female Japanese quails 5-6 week old (weighed 268g±7.06) apparently healthy were obtained from Mosa farm for quails at the road between Port Said and Damietta, used throughout this study. Hens were divided into five groups (each of three replicates of 8 birds) into layer cages 95cm lenghth×65cm width×1.60cm height for 12 weeks (experimental period) with two weeks adaption at the start of the experiment. The hens were allowed *ad libitum* access to feed and water. They were fed all dry mash layer diets. Water drinkers were thoroughly cleaned and clean water was replaced every day. Fecal trays were cleaned every day. Ventilation and temperature (22ºC-31ºC) were controlled to maintain bird comfort. Hens checked six times daily (at 5 am, 7am, 12 pm, 4 pm, 8 pm and10 pm) for food, water, manage light and egg collection or any mortality may occur. An artificial lighting program as 17 hr. light (L): 7 hr. dark (D) was followed throughout the experimental period ***(Yusuf et al., 2014)***.

2 Experimental design: The experimental groups were divided as follow: first group fed on the basal diet (corn-soybean meal based diet) as a control diet (20% CP& 2900 Kcal/kg) according to ***(NRC, 1994)*** **(**table 1). The feed ingredients were chemically analyzed according to ***(AOAC, 1990)*** before the ration formulation. The other groups fed on low protein isocaloric corn-soybean meal based diet (17% CP& 2900 Kcal/kg) (table 1). Group 2 (non-supplemented). Group 3 supplemented with protease enzyme (Ronozyme proact obtained by Eldakahlia Co., El-Mansoura-Egypt) at level 0.05% of diet. Group 4 supplemented with formic acid 85% (produced by El Nasr pharmaceutical chemicals Co., obtained from Elgomhouria Co. Port said-Egypt) at level 0.5% of diet. Group 5 supplemented with mixture of formic acid at level 0.5% of diet and protease enzyme at level 0.05% of diet.

3 productive performances: For each replicate, daily egg number, egg production and weekly feed intake were recorded according to ***(Geraldo et al., 2015)***. Daily egg weight and egg mass as well as FCR were recorded according to ***(Alagawany and Attia, 2015)***.

4 egg quality: At the end of each week, eggs were collected from each replicate to evaluate egg quality traits [shell weight (wt.) and %, egg yolk wt. and % and albumin wt. and %] according to ***Yusuf et al. (2014)***. Cholesterol of egg yolk ***(Rotenberg and Christensen, 1976)*** were evaluated at the end of the period.

5 Economic efficiency: It was measured according to ***Yusuf et al. (2014)***.

6 Statistical analysis

Duncan multiple ranges ***(Duncan, 1955)*** was used to compare mean among groups according to *(Snedecor and Cochran, 1989).* The estimation was made using SPSS 16 *(Coakes and Steed, 2009).*

Table (1): The determined composition and calculated values of the two experimental basal diets.

Ingredients%	Recommended diet 20%	Low protein diet 17%
Ground yellow corn (8.6%)[a]	63.8	69.3
Soya bean meal (45.6%)[a]	16.8	18
Corn gluten (60.7)[a]	11.3	4.7
Dical.Phosphate (22%Ca&19%P)	1.3	1.3
Limestone (38% Ca)	5.8	5.7
DL – Methionine (purity 99%)	0.15	0.1
L-Lysine (purity 99%)	0.25	0.3
Iodized sodium chloride	0.3	0.3
Minerals & vitamins premix[b]	0.3	0.3
Total	100	100
Calculated values:-		
Crude protein (%)	20%	17%
ME (Kcal per kg)	2900	2900
Calorie/ Protein ratio(C/P)	145	170.59
Calcium (%)	2.5	2.5
Available Phosphorus (%)	0.35	0.35

(a) Determined values.

(b) Each 3 kg contain the following vitamins and minerals: Vit. A 12 mIU, vit. D_3 3 mIU, vit. E 40000mg, vit. K_3 4000mg, vit. B_1 4000mg, vit. B_2 15000mg, vit. B_6 5000mg, vit. B_{12} 30mg, biotin 300mg, pantothinic acid 20000mg, nicotinic acid 60000mg, folic acid 3000mg, mangesium sulphate 1200000mg, zinc sulphate 100000mg, iron sulphate 80000mg, copper sulphate 30000 mg potassium iodide 3000 mg, sodium selenate 200mg, cobalt sulphate100mg, carrier(CaCO3) to 3kg. (High mix premix- Alpha Pharm Elasher, Egypt. patches No. 190827, production 9-2020).

Results

1 Productive performance.

Table (2): *productive performance of laying Japanese quail fed diets containing protease and/or formic acid as feed additives during the experimental period.*

Parameter / Groups	Average mean of these parameters from 1-12 week (total period)		
	egg production%	Feed intake (g) per hen/ day	FCR
G1	90.58 ± 0.99^{a}	38.58 ± 0.1^{b}	3.28 ± 0.1^{ab}
G2	92.37 ± 0.8^{a}	39.81 ± 0.3^{ab}	3.19 ± 0.01^{ab}
G3	92.27 ± 2.7^{a}	41.44 ± 1.2^{a}	3.44 ± 0.1^{a}
G4	88.27 ± 1.3^{a}	38.11 ± 0.5^{b}	3.37 ± 0.1^{ab}
G5	92.63 ± 2.1^{a}	38.36 ± 0.5^{b}	3.17 ± 0.04^{b}

Values are mean ± SE

Values in the same column different superscripts are significantly different at $p < 0.05$. G1: group fed basal layer diet. G2: group fed 17% protein diet. G3: group fed 17% protein +0.05%protease. G4: group fed 17% protein +0.5%formic acid. G5: group fed 17% protein + mixture of 0.5% formic acid and 0.05% protease.

2 Egg quality

Table (3): *egg quality parameters of laying Japanese quail fed diets containing protease and/or formic acid as feed additives during the experimental period.*

Parameters / Groups	Average throughout the all experimental period (1-12week)						
	Egg weight (g)	**Egg mass (g) per hen**	**Egg albumin %**	**Egg yolk %**	**Egg shell %**	**cholesterol (mg/whole yolk)**	**triglyceride (mg/whole yolk)**
G1	**$11.77\pm.3^{bc}$**	**272.22 ± 6.4^{a}**	**59.47 ± 0.4^{a}**	**31.74 ± 0.3^{a}**	**8.77 ± 0.03^{a}**	**82.42 ± 3.02^{a}**	**35.65 ± 1.3^{a}**
G2	**12.48 ± 0.1^{a}**	**285.45 ± 0.8^{a}**	**59.19 ± 0.2^{a}**	**32.03 ± 0.3^{a}**	**8.78 ± 0.1^{a}**	**63.44 ± 8.5^{b}**	**24.46 ± 2.5^{b}**
G3	**12.06 ± 0.1^{ab}**	**283.50 ± 9.3^{a}**	**59.52 ± 0.2^{a}**	**31.56 ± 0.1^{a}**	**8.92 ± 0.17^{a}**	**65.59 ± 1.2^{b}**	**24.33 ± 2.1^{b}**
G4	**11.31 ± 0.2^{c}**	**252.75 ± 2.9^{b}**	**59.49 ± 0.6^{a}**	**31.59 ± 0.6^{a}**	**8.97 ± 0.04^{a}**	**76.31 ± 2.2^{ab}**	**26.73 ± 2.2^{b}**
G5	**12.11 ± 0.2^{ab}**	**279.23 ± 6.96^{a}**	**59.07 ± 0.1^{a}**	**32.23 ± 0.2^{a}**	**8.70 ± 0.1^{a}**	**67.48 ± 5.2^{ab}**	**25.11 ± 1.5^{b}**

Values are mean ± SE

Values in the same column different superscripts are significantly different at $p < 0.05$. G1: fed basal layer diet. G2: fed 17% protein diet. G3: fed 17% protein +0.05%protease. G4: fed 17% protein +0.5%formic acid. G5: fed 17% protein + mixture of 0.5% formic acid and 0.05% protease.

3 Economic efficiency

Table (4): *The economical evaluation laying Japanese quail fed diets containing protease and/or formic acid as feed additives at the end of the experimental period.*

Parameters / Groups	G1	G2	G3	G4	G5
Price/hen (LE)	20	20	20	20	20
Feed intake/hen(g) (1-12 wk.)	3241	3344.1	3480.7	3201.1	3221.8
Feed cost*/hen(LE) (1-12 wk.)	22.7	21.7	22.6	20.8	20.9
Management/hen(LE) (1-12 wk.)	0.3	0.3	0.3	0.3	0.3
Feed additives cost/hen (1-12 wk.)	----	----	1	1.6	2.6
Total cost**/hen(LE) (1-12 wk.)	43	42	43.9	42.7	43.8
Selling price/hen (LE) At the end	15	15	15	15	15
Number of egg produced/hen (1-12 wk.)	74	75	76	71	75
Selling price/egg(LE)	1	1	1	1	1
Selling price/eggs produced/hen(LE) (1-12 wk.)	74	75	76	71	75
Total selling price/hen production(LE)***	89	90	91	86	90
Net revenue****(LE)	46	48	47.1	43.3	46.2
Economic efficiency (EE)*****	106.98 %	114.3 %	107.3 %	101.4 %	105.5 %

-feed additives price /kg feed =0.5 LE for formic and 0.3 LE for protease.
*Price of one kg of recommended diet =7 LE while for low protein diet=6.5 LE.
** Price of hen + management + feed cost + additive.
*** Selling price of eggs produced per hen plus the selling price of hen itself at the end.
**** (Selling price of eggs produced per hen + the selling price of hen itself at the end) – total cost / hen.
*****Net revenue /total cost*100.
G1: fed basal layer diet. G2: fed 17% protein diet. G3: fed 17% protein +0.05%protease. G4: fed 17% protein +0.5%formic acid. G5: fed 17% protein + mixture of 0.5% formic acid and 0.05% protease.

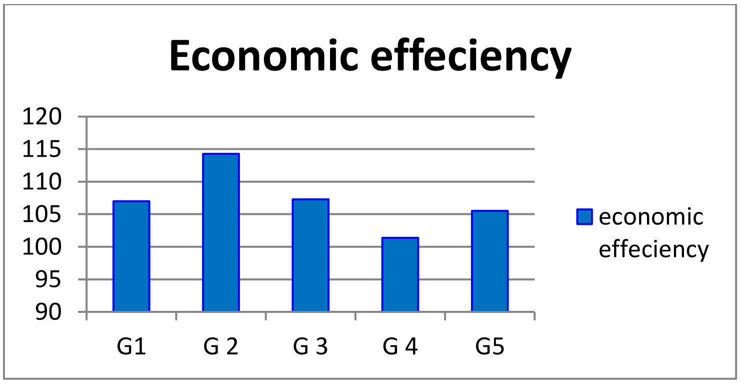

Figure (1): the economical evaluation laying Japanese quail fed diets containing protease and/or formic acid as feed additives at the end of the experimental period.

G1: fed basal layer diet. G2: fed 17% protein diet. G3: fed 17% protein +0.05%protease. G4: fed 17% protein +0.5%formic acid. G5: fed 17% protein + mixture of 0.5% formic acid and 0.05% protease.

Discussion

The results in (table 2) showed the impact of protease and organic acid on productive performance of laying Japanese quail hens.

G3 which fed on diet supplemented with protease had an increase in the average feed intake through the all experimental period, this results agreed with ***Ali et al. (2006)*** who used 0.1% of enzyme mixture contain protease 450u/g) into diet containing 50% wheat bran, and also agree with ***Barbosa et al. (2020)*** who studied the effect of adding proteases into diets of laying hens at peak production. But the results inconsistent with ***Koshy (2005)*** who studied the effect of addition of protease at level 0.02 and 0.04 percent into low protein diet (CP 18%) with the same level of metabolisable energy for laying Japanese quails on their production performance. The results revealed that feed intake per bird per day and feed efficiency was not significantly affected by the enzyme supplementation. And also disagreed with ***Yadav and Sah (2006)*** who found that when crude protein of laying hen diets lowered by 1% and supplemented with protease at level of 0.1%, feed consumption will be reduced.

Protease increased feed intake may be due to increase the digestibility of proteins and amino acids ***(Kocher et al., 2002)***.

Also there is a numerical increase in the feed intake through the all experimental period for low protein diet group (G2) than control but significantly there is no difference between two groups (table 2), this may be due to amino acid content into low protein diets may satisfy the requirements of the animals ***(Such et al., 2021)***, this result agreed with ***Yadav and Sah (2006)*** who said that, pullets consume more feeds when fed reduced crude protein diet, but there is no significant difference between the (control) and low protein group as with ***Torki et al. (2015)*** who used five crude protein(CP) levels: CP present in conventional layer diet containing 16.5 % CP(control) and low-CP diets containing 15.0, 13.5, 12.0, or 10.5 % CP. All diets were supplemented with crystalline amino acids, they found that, reductions in CP level of a laying hens' diet from 16.5 to 12.0 % had no negative effects on feed intake. But disagreed with ***Laudadio et al. (2012)***who said that, birds (broiler) feeding the low CP diet were associated with lowered feed consumption rather than with problems of nutrient digestibility.

In general protease and formic acid had not significantly affect on egg production through the all experimental period (table 2), this result agreed with ***Jaroni et al. (1999)*** who made a study which conducted to test the effects of wheat middlings with or without enzyme (xylanase and protease) supplementation at level of (0.1% and 0.2%) on late egg production (EP), egg yields, and egg composition in two strains of Leghorn hens. The results showed that egg production was not significantly affected by diet. Also agreed with ***Ipek et al. (2008)*** who studied the effect of supplementation of basal laying hen diet with 360 or 480 or 600 mg/kg of humic acid. The results showed that egg production not effected by any level of humic acid in all groups. But the results inconsistent with ***Kucukersan et al. (2005)*** who studied the effects of humic acid on feed consumption, egg production, feed efficiency, egg weight and external and internal egg quality of laying hens. Low protein group was not differ significantly than the control group in the egg production but numerically increased through the all experimental period (table2), this agreed with ***Penz Jr and Jensen (1991)*** who found that 16% CP group had 92.8% egg production but (13%) CP group had 93.2 % egg production of laying hens (there is no significant difference between the two groups). And with ***Keshavarz and Jackson (1992)*** who said that, egg production and egg weight of birds (laying hens) fed the level 14,13, and 12% of protein supplemented with same combination of amino acids or of the birds fed the level 15,14, and 13% of protein supplemented with methionine and lysine were not different from those that fed the positive control(basal). But inconsistent with ***Yadav and Sah (2006)*** who said that, reduction of the crude protein of the layer diet by 1% generally reduced the biweekly and overall average egg production of the pullets.

In average FCR through the all experimental period the result showed that (table2), G3 had a significant increase in FCR than G5 while had insignificant difference with other groups. There is no significant difference between G1, G2, G4 and G5. This result agreed with ***Özek et al. (2011)*** who said that 2 g/kg organic acids had not significant effect on feed conversion ratio of laying hens. And also agreed with ***Polat and Denli (2019)*** who said that laying hen which fed basal diet supplemented with 0.1% enzyme mixture that contain protease, had no effect on the feed conversion ratio. But our results (average FCR through the all experimental period) disagreed with ***Youssef et al. (2013)*** who told that, mixture of organic acids that contain fumaric acid, some organic acid salts and hydrogenate vegetable oil which were used in brown laying hen basal diet at dose 0.06% resulted in, feed conversion ratio was improved. Also disagreed with ***Agunbiade et***

al. (2016) who reported that, when use Lohmann Brown laying hens in a study to evaluate the effect of replacing maize with sorghum with or without enzyme supplementation on egg production, egg mass, feed intake and feed conversion ration. The results showed that diets supplemented with phytase, protease and other enzymes had significantly (p<0.05) better feed conversion ratio when compared with other groups (significant difference (p<0.05) was observed among dietary treatments).

Results in (table 3) showed the egg quality parameters.

There is no significant difference between G1,G2,G3and G5 towards mean egg mass, this mean protease had not obvious effect on egg mass this agrees with ***Alagawany and Attia (2015)*** who studied that, addition of sugar beet pulp in the diet of laying quail hens which supplemented with enzyme mixture contain xylanase, protease and amylase at levels (0, 1 and 2 g /kg diet) had not affect egg mass throughout the experimental period (12–20 weeks of age). And also agreed with ***Ludke et al. (2018)*** who found that, no effect of the enzyme (p>0.05) on the evaluated parameters (egg mass)for laying Japanese quail. But disagreed with ***Ali et al. (2006)*** who said that when use enzyme mixture contain protease to 50% wheat bran diet at level 0.1%, egg mass of laying hens will be decrease. And also disagreed with ***Khan et al. (2011)*** who said that, when added 2.0 g/kg of enzyme mixture (contains protease) to the basal diet of laying hen, there was a significantly enhancement of the egg mass.

There is no significant difference in the egg mass between low protein 17% group (G2) and control through the all experimental period (table 3), this result agreed with ***Pérez-Bonilla et al. (2012)*** who found that, crude protein content of the diet treatments (16.5%-17.5%-18.5%) with (1.8%-3.6% fat) and iso caloric diets, did not affect any of the productive performance traits studied (egg weight, egg mass) in brown egg-laying hens. And agreed also with ***Torki et al. (2015)*** who used five crude protein (CP) levels of laying hens diets: CP present on commercial diet (16.5 % CP) and low-CP diets containing 15.0, 13.5, 12.0, or 10.5 % CP. All experimental diets were supplemented with crystalline amino acids, they found that, by comparison, egg mass in the hens fed with control diet (16.5%) was similar to the values in the birds fed with 15.0, 13.5, and 12.0 % CP diets. However, disagreed with ***Roberts et al. (2007)*** who reported that a decrease in CP content (19.8 to 19.1% from 23to 31 wk., 18.1 to 17.1% from 32 to 44 wk., and 16.9 to15.8% from 45 to 58 wk. old of laying hen) of isoenergetic diets with similar indispensable amino acids profile, reduced egg production, egg mass, and feed efficiency. However, egg weight and body weight gain were not affected by a decrease in CP content.

G3 and G5 had no significant difference with each other and with G2 and control in the total average egg weight throughout the all experimental period (table 3). This agreed with ***Adrizal et al. (2011)*** who explored that, when use phytase and protease mixture at level 0.035% of (0, 15, 30) % of palm kernel meal diet, there is no significant effect on any of the parameters measured (egg weight) for laying hens by enzyme supplementation. Also agreed with ***Abousekken (2014)*** who concluded that, partial substitution (0, 10, 15 or 20%) of Distillers Dried Grains with Solubles for yellow corn and soybean meal in laying quail diets with enzyme supplementation at level 100 mg/kg feed or without supplementation, showed that there is no significant difference (P>0.05) in laying Japanese egg weights. But disagreed with ***Kim et al. (2009)*** who said that supplementation of Hy-Line Brown layers with spider-derived protease enzyme at level of (0.05 %) improved egg weight as compared with that of control. And also with ***Khan et al. (2011)*** who said that when added 2.0 g/kg of enzyme mixture to the basal diet of laying hen, there was a significantly improvement in egg weight than the negative control group.

G2 which is a low protein diet had a significant increase in the total average egg weight throughout the all experimental period when compared to control (table 3), this may be accordance with ***Keshavarz (1995)*** who said early egg weight can be increased through proper diets during the early stages of the egg production cycle, which in our study there was two weeks of adaption from 6 to 8 weeks old and all birds fed NRC recommended diet except for protein (17%) and 20% for control, this agreed with ***Thornton et al. (1957)*** who told, 17 percent of protein group had considerably more small eggs than the 15 or 13 percent groups (which supplemented with lysine and/ or methionine in isocaloric laying hens diet). And agreed also with ***Fernandez et al. (1973)*** who mentioned that there was no significant differences in egg weight of laying hens among the treatments (as diet containing 13% protein and supplemented with lysine and methionine was as effective as 15, 17 and 18% protein diet only). However, the results disagreed with that of ***Keshavarz and Nakajima (1995)*** who told**,** the results indicated that egg weight during the early stages of the egg production cycle of laying hens may be increased due to increasing the protein level from 17 to 21%.

Numerically G4 was the highest in egg shell percent between all groups (table 3), this agreed with ***Swiatkiewicz and Arczewska-***

Wlosek (2012) who said that inclusion of 5 g/kg of short chain fatty acids that contain formic, propionic and acetic acid enhanced eggshell characteristics (eggshell percent) of laying hens. Also agreed with ***Shalaei et al. (2014)*** who said that organic acid mixture at dose 3 kg/ ton increase egg shell percent numerically in laying hens. But disagreed ***Fouladi et al. (2018)*** who studied the effect of different levels of organic acids on some egg quality parameters of laying Japanese quail. The results showed that, the diets containing butyric acid + acetic acid and butyric acid + lactic acid significantly impacted some quality traits such as eggshell thickness, eggshell weight. And also disagreed with ***Manafi and Hedayati (2018)*** who told that when laying Japanese quails fed basal diet with 0.01% of organic acids combination, egg weight and egg shell percentage were enhanced significantly compared to the control group .

G2 had no significant difference with G1(control) in egg shell weight and percent throughout the all experimental period (table 3) as ***Penz Jr and Jensen (1991)*** who found that group fed on diet contain 16% protein and group fed on diet containing 13% protein had the same shell percent in laying hen 10.2%, also agreed with ***Novak et al. (2006)*** who found that, laying hens which consuming 14.6 and 16.3 g of protein/hen per day (protien16%-14.5% diet) had a similar effect on egg shell percent, but disagree with the same author in this point of hens consuming the 13.8 g of protein/hen per day (13% protein diet) produced eggs with more shell than those consuming the 14.6 and 16.3 g of protein/hen per day (protien16%-14.5% diet).

As protease increase albumin percentage numerically through the all experimental period (table 3), this agreed with ***Alagawany and Attia (2015)*** who studied that inclusion of sugar beet pulp (SBP) in the diet of laying quail hens which supplemented with enzyme mixture (xylanase, protease and amylase) at doses as (0, 1 and 2g/kg diet) made insignificantly effect on the external and internal egg quality parameters (albumen as a percentage of total egg weight) of the laying quails. But numerically there is an increase in albumin percent at dose 2g/kg diet. Also agreed with ***Ribeiro et al. (2015)*** who studied the supplementation effect of 300 ppm protease alone or with combination of other enzymes of laying Japanese quail diet which reduced in some nutrients as protein, digestible amino acids, energy, calcium and phosphorus, to evaluate egg quality parameters (proportion of egg constituents and specific egg weight) of Japanese quail. The results showed that there was no significant effect of the treatments on the variables analyzed (P>0.05). But the authors concluded that the enzymes supplementation had beneficial effects such as maintaining egg quality of Japanese quail. But this disagreed with ***Abousekken (2014)*** who studied enzyme mixture (contain protease) supplementation at level (100 mg/kg feed). The results showed that addition of enzymes to the diets containing 10% Distillers Dried Grains with Solubles significantly enhanced albumin weight as compared to control diets. And also disagreed with ***Rao et al. (2020)*** who studied the effects of protease supplementation (0 and 4000 u/kg) in diets containing three levels of CP (15, 16.5 and 18% CP with uniform metabolisable energy (10.67 MJ/kg) on egg production and egg quality in laying hens. They found that, supplementation of protease significantly ($P < 0.05$) improved egg albumen quality.

Protease may numerically increase egg albumin percent as Protease had a function as breaking down feed proteins into simpler parts (oligopeptides) so it will be more easily absorbed and rearranged into egg proteins ***(Dawson-Hughes, 2003)***.

G2 (low protein) had no significant difference with control in average of egg albumin weight and egg albumin percent through the all experimental period (table 3). Also agreed with ***Alagawany et al. (2014)*** who found that, there is no significant difference in egg albumin percent between control and low protein group16% in laying quails. Also disagreed with ***Penz Jr and Jensen (1991)*** who found that weight and percentage of egg albumen of laying hens was consistently less for eggs from hens fed the 13% CP diets than that of those fed the 16% CP diet.

G2 and control group had no significant difference between each other in average of egg yolk weight and egg yolk percent through the all experimental period (table 3), this agreed with ***Novak et al. (2006)*** who found that protein intake which as (during phase I, there were 3 levels of dietary protein calculated: 18, 16, and 14% with 0.97, 0.85, and 0.82 total sulfur amino acid: Lysine and during phase II, the dietary protein was reduced to 16, 14.5, and 13% with 0.92, 0.82, and 0.72 total sulfur amino acid: Lysine) there is no significant difference between all groups in egg yolk percent of laying hens during phase one but during phase two there is no significant difference between medium (14.5%) and low protein (13%) percent groups. But disagree with the same author in this point: high protein percent (16) in phase two had lower yolk percent than medium and lower protein percent.

Protease or formic acid had no effect on yolk% (table 3), this agreed with ***Koshy (2005)*** who studied the effect of addition of protease at dose 0.02 and 0.04 percent in low protein diet as (CP 18%) with same level of metabolisable energy for laying Japanese quails on their egg quality parameters. He found that, yolk parameters were not affected by enzyme treatment on laying quails. And also agreed with ***Gopinger et al. (2016)*** who said that organic acid treatment (2% acetic and propionic acid) maintained the quality of the whole rice bran up to 120 day of storage and could comprise 20% of the laying quails' diet without affecting egg quality parameters including yolk percentage. But disagreed with ***Jaroni et al. (1999)*** who conduct study to evaluate the effect of ration contain wheat middlings with or without enzyme mixture (xylanase and protease) at two levels (0.1% and 0.2%) of diet on egg composition (shell quality, specific gravity, albumen weight and yolk weight) in two strains of single combe white Leghorn hens (DeKalb Delta2 and Hisex White3). They found that percentage yolk was significantly higher ($P < 0.05$) for diets supplemented with enzyme when compared with control or WM diets without enzyme. Also disagreed with ***Attia et al. (2013)*** who stated that when used basal layer quail diet supplemented with 0, 1.5, 3 and 6% of acetic acid. The results revealed that there is an increase in yolk weight at 1.5 and 3% acetic acids doses. Also there is an increase in the egg weight may be explained by the increase in the yolk weight.

Towards triglyceride mg/whole yolk, there is a significant decrease in all groups (G2, G3,G4 and G5)when compare with control (table 3), this may be due to low protein diet with egg production as ***Morris (1992)*** who said that cholesterol level and triglycerides (lipid profile) of egg yolk differ according to genetic background and also due to composition of total diet which must be taken in consideration.

There is no significant difference between all groups in triglyceride mg/1g yolk (table 3). However numerically G3 was the lowest value, may be due to protease increases T_3 hormone in our results and according to ***Goldberg et al. (2012)*** who said that thyroid hormone reduces plasma cholesterol via a non-LDL receptor-mediated pathway. And according to ***Bondi and Drori (1987)*** who said that, in attempt to reduce cholesterol of egg we should use substance into the diet that impair cholesterol absorption. So the egg yolk cholesterol decreased in protease supplemented group as protease mainly enhance protein digestibility ***(Wang et al., 2006).*** There is no significant difference between all groups in cholesterol mg/1g yolk, but numerically G3 was the lowest value among all groups. Formic acid had no effect on egg cholesterol as compared with low protein group (table 3). This results agreed with ***El-Deek et al. (2008)*** who studied the effect of enzymes supplementation (which is a multienzyme preparation) at level of 1g/kg diet on egg yolk cholesterol. They found that, yolk total lipids of fresh and stored egg were reduced (P < 0.01) with increasing inclusion level of date waste meal which supplemented by enzyme. Also agreed with ***Elabbas (2012)*** who studied the effect of addition of 0.15 and 0.3% formic acid only or combinations of formic and propionic acids at doses of 0.15 + 0.2 , 0.15 + 0.4 , 0.3 + 0.2 and 0.3 + 0.4 % respectively. The results showed that, there was no significant difference between the treatments in the cholesterol level of egg yolk of Lohmann Brown laying hens. But our results disagreed with ***Khan et al. (2011)*** who said that, when added 2.0 g/kg of enzyme mixture to the basal diet of laying hen, the result showed that, addition of enzyme to the basal diet did not affect the cholesterol levels of chicken egg. And also disagreed with ***Yusuf et al. (2014)*** who found that when one weeks old Japanese quail chicks took diet with (2g benzoic acid + 5g citric acid / kg), there is a significant decrease of yolk cholesterol due to supplementation of organic acids mixture.

In table (4) and figure (1) the results showed that, there is considerable cost saving during experimental period (most groups had cost saving). In group two which fed low protein diet 17% without any supplementation which had the highest net revenue (48 LE) in compare with control (46 LE), and other groups which were 47.1, 43.3 and 46.2 LE in groups G3, G4 and G5 respectively. These values were parallel to economic efficiency (EE), that was 106.98, 114.3, 107.3, 101.4 and 105.5 % in G1, G2, G3, G4 and G5 respectively. G2 significantly has the heavier egg weight among groups (p<0.05) as the average egg weights were 11.77g and 12.48g in control and G2 respectively, this means about 6.03% more weight than control group. G2 showed also numerical improvement than the control group and other experimental groups regarding egg production, egg mass, FCR, egg shell weight, albumin weight, egg yolk weight and dressing percentage.

These results agree with ***Amer et al. (2021)*** who said, the economic efficiency indicators remained unaffected by the difference feeding regimens, protease supplementation (250mg/kg), or their interaction (there was no positive effect of these difference diets and protease supplementation on economic efficiency) in low protein broiler diet. On the contrary ***Costa et al. (2008)*** who found that supplementation of enzymes (including protease) resulted in reduction of feed cost due to reduction of the amount of corn needed into the diets of the chicks, and disagreed with ***Yadav and Jha (2019)*** who concluded that exogenous enzymes that contain protease in combination with non-conventional ingredients, are used to reduce the cost of feeding and to utilize the non-conventional feed ingredients efficiently. Groups supplemented with formic acid G4&G5 had lower net revenue than control group which in contrary with ***Soltan (2008)*** who said that there is a possibility of increasing economic efficiency by organic acid supplementation of laying hens diets, and also ***Kamel and Mohamed (2016)*** who mentioned that the value of total return was the highest for organic acid groups of both Cobb and Ross breed (L.E 27.72 and 27.41, respectively), while the lowest value was found for the enzyme group (0.2%) of Cobb breed (L.E 25.16). And they concluded that organic acids and synbiotic feed additives were better than the other used feed additives, and they had an important role in improving productive and economic efficiency of broiler chicks. The disagreement with our findings may be attributed to the differences in species, the mixture in which the enzyme or the organic acid incorporated, level of supplementation, duration of supplementation, feed composition, antinational factors and environmental factors.

Conclusion

It could be concluded that, feeding laying Japanese quail hens on diet containing 17% protein without any feed additives not differ significantly than those on control diet containing 20% protein. Moreover, it showed numerical improvement regarding laying performance, egg quality parameters and net revenue.

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الملخص العربى

كفاءة الأداء فى إناث السمان اليابانى البياض المغذى على عليقة منخفضة البروتين ومضاف إليها مستويات مختلفة من مخلوط البروتييزوالحمض العضوى.

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صممت الدراسة الحالية لمعرفة تأثير إضافة بعض إضافات الأعلاف (البروتييز وحمض الفورميك) على الأداء ، وبعض معايير جودة البيض ، والتقييم الاقتصادى.
تم تقسيم إجمالي 120 أنثى من السمان اليابانى عمر (من 5-6 أسابيع) بشكل عشوائي إلى خمس مجموعات تجريبية متساوية بعد أسبوعين من التكيف و النضج الكامل ، تم تقسيم كل مجموعة إلى ثلاث مكررات (كل منها 8 طيور) لمدة 12 أسبوعًا. تم تقسيم المجموعات التجريبية على النحو التالي:
غذيت المجموعة الأولى(الضابطة) على النظام الغذائي الأساسي (يتكون من الذرة وفول الصويا طاقة تمثيل غذائى 2900 كيلو كالورى لكل كيلو جرام عليقة والبروتين 20 % طبقا الى الاحتياج المقرر للسمان البياض). اما باقى المجاميع من الثانية اللى الخامسة غذيت على نظام غذائي منخفض البروتين 17% بنفس السعرات الحرارية للمجموعة الضابطة . كانت المجموعة الثانية بدون اضافة إنزيم البروتييزاو حمض الفورميك. المجموعة الثالثة اضيف اليها إنزيم البروتييز بنسبة 0.05٪ من العلف . المجموعة الرابعة اضيف اليها حمض الفورميك 85٪ بمستوى 0.5٪ من العلف. المجموعة الخامسة اضيف اليها مزيج من حمض الفورميك بنسبة 0.5٪ من العلف وإنزيم البروتييز بنسبة 0.05٪ من العلف. تم تسجيل عدد البيض اليومي ووزن البيض وكتلة البيض لكل مكرر وكذلك كمية العلف الأسبوعية. في نهاية كل أسبوع ، تم جمع البيض من كل مكرر لتقييم صفات جودة البيض [النسبة المئوية لقشرة البيض ، لصفار البيض والألبومين] . تم تقييم الكوليستيرول فى صفار البيض في نهاية الفترة. وخلصت النتائج الى أن : تغذية السمان اليابانى البياض على عليقة منخفضة البروتين وليس مضاف اليها انزيم البروتيزوحمض الفورميك لم تختلف معنويا عن المجموعة الضابطة بل حدث تحسن عدديا فى انتاج البيض ووزن البيض وكذلك وزن القشرة وبعض صفات البيضة الداخلية مثل وزن صفار البيض وكذلك الالبومين بالمقارنة بالمجموعة الضابطة وايضا الاعلى كفاءة اقتصادية عن باقى المجموعات والمجموعة الضابطة.