Egyptian Poultry Science Journal

http://www.epsaegypt.com

ISSN: 1110-5623 (Print) – 2090-0570 (On line)

THE PRODUCTIVE PERFORMANCE AND IMMUNOLOGICAL TRAITS OF LOCAL CHICKEN STRAIN BY USING NATURAL ENZYMES (PLANT PAPAIN) AND REMNANTS OF PLANT PAPAYA. 1- EFFECT OF PAPAYA LATEX ON LAYING PERIOD.

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Received: 26/11/2014 Accepted: 26/12/2014

ABSTRACT: The objective of this study was to evaluate the influence addition of papaya latex on productive performance, digestibility, egg qualities, semen quality, carcass traits, immunity, some blood constituents and economic of efficiency of Dokki4 strain. A total number of 132 Dokki4 hens and 12 Dokki4 cocks, 32 weeks of age were randomly distributed into 4 groups with 3 replicates each (11 hens+1cock). Chicks were randomly divided into control (without any addition of PL_0) and 3 treatment groups (basal diet containing papaya latex ground powder at levels 0.01(PL_1), 0.03 (PL_2) and 0.05% (PL_3).
The obtained results could be summarized as follows:

1) Final body weight, body weight gain, egg production, egg weight and egg mass for the layers fed diet supplemented with PL were increased significantly than those fed control diet. A feed conversion ratio was significantly improved with PL, whereas, daily feed intake significantly ($P \leq 0.05$) decreased with PL inclusion.
2) Digestibility coefficient values were significantly improved for hens fed diet supplemented with PL compared to those fed control diet.

Key Words: Laying Hens, Papaya, Blood, Egg, Digestibility, Immunity, Semen Quality

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3) Diets supplemented with PL had significantly increased shell thickness, egg shape index and HU. However, no effect on shell weight, yolk percentages and albumen were found.
4) Semen quality, fertility and hatchability were significantly (P≤ 0.05) improved by PL supplementation.
5) Feeding PL at different levels led to significant (P<0.05) decrease of total lipids and cholesterol on plasma and yolk. Also, add PL led to improved immune response.
6) Good economic efficiencies were observed with hens fed supplemented PL.

Generally, it could be concluded that supplementation of papaya latex diets of laying hens improved egg production, feed conversion ratio, nutrients digestibility, immunity, semen quality, fertility, hatchability and economical efficiency especially with the addition of 0.05% papaya latex.

INTRODUCTION

Enzyme "papain" is a proteolytic enzyme from the cysteine proteinase family. It is derived from papaya (Carica papaya) leaf, unripe fruit and papaya latex, a milky fluid that oozes out of green papaya. Carica papaya contains many biologically active compounds. Two important compounds are chymopapain and papain, which are supposed to aid in digestion. Vitamins and traces of an alkaloid called Carpaine have also been found in the latex. Papaya latex is a thixotropic fluid with a milky appearance that contains about 85% water. An insoluble particulate fraction whose composition is still practically unknown makes up 25% of the dry matter. The soluble fraction, however, contains both the usual ingredients such as carbohydrates (~10%), salts (~10%) and lipids (~5%), and representative biomolecules such as glutathione, cysteine proteinases (~30%) and several other proteins (Macalood et al., 2013). The latex of Carica papaya is rich source of cysteine endopeptidases (digestive enzymes), including proteolytic enzymes- papain, lipase, proteinases, peptidases isoenzymes like carboxy-exo-peptidase, glycyl endopeptidase, chymopapains, A, B, and C, peptidase A and B and caricain, which constitute more than 80% of the whole enzyme fraction, also, the application of papaya latex is probably of most interest to livestock producers is as an antimicrobial, antibiotic and antifungal (Krishna, et al., 2008, Nwinyi et al. 2010 and Aravind et al., 2013).

Papaya latex proteases are composed of four cysteine proteases which contribute 69- 89% of total protein: less than 10% papain, 26-30% chymopapain, 23-28% glycyl endopeptidase, and 14-26% caricain (Barrett, et al., 1998). Lien and Wu (2012) demonstrated that papain not only contains protease, but also phospholipase. Saha and Paul (2013) reported that papain helps the body to digest proteins, fats and sugars while amylase digests carbohydrates and sugars. Papain enzyme is a protein with papain profeinase, chymopapain and lysozyme. Enzymes accelerate reaction within body cells. Also, papain is a digestive proteolytic enzyme, used in several digestive formulations.

Carica papaya is an excellent aid to digestion, which helps to digest the protein in food at acid, alkaline or neutral medium. Many countries tend to prevent the application of some feed additives for their side effects on both animal and human body. It is interesting to replace these feed additives with other products which have good effects on both animal and human welfare.

The use of exogenous enzymes in animal nutrition has increased in the last years as an alternative natural product that might improve animal productivities and reduce the morbidity and the mortality in intensive farms (Cachaldora et al., 2004). Unigwe et al. (2014) reported that papain is an effective natural digestive aid which breaks down protein and cleanses the digestive tract. Miyamoto et al. (2004) showed that papain can hydrolyze lipids and carbohydrates as well as proteins, and it has been shown to have activity in a wide range of pH and temperature. Simply breaks down the proteins into amino acids. Amino acids in the form of micronutrients become easily digested. They added that papaya enzyme help us get the most nutrition from the food and may ease the bloating ,gas and digestive disturbances and/or may increase immune system function. Lien and Wu (2012) found that papain not only contains protease, but also phospholipase. Papain could enhance animal immunity, also showed reduced serum cholesterol. They added that papain could enhance protein metabolism, ameliorate inflammation and regulate immunity. Gayosso-Garcia et al. (2010) reported that the papain super family and it's an antioxidant properties can be useful in preventing certain types of illnesses. Nowadays, El-Kholy et al. (2008) and Zeedan et al. (2009) showed that papaya latex can be used as an alternative growth promoter with favorite growth performance and/or enhanced immune system in feeding growing rabbits. They added that reported that rabbits received the papaya latex had the highest litter size and weight at birth or weaning were significantly ($P \leq 0.05$) higher, while, number of still birth or number of pre-weaning mortality were significantly ($P \leq 0.05$) lower than that in the control group. Also, they added that rabbits received the level of 0.09% papaya latex had the increasing in WBCs in treated groups can be attributed mainly to the antibacterial functions of papaya latex.

The aim of the study was to investigate the effect of papaya latex (PL) as feed additive on nutrients digestibility, immunity, performance of laying hens, egg quality traits, some blood constituents and economical efficiency.

MATERIALS AND METHODS

Animals, diet, and experimental design:-

This study was carried out at the Poultry Farm, Department of Animal and Poultry Production, Faculty of Agriculture, Kafrelsheikh University, Egypt. Dokki4 laying hens provided by Sakha Animal Research Station, Animal Production Research Institute, Ministry of Agriculture, Egypt. The chemical analyses were carried out at Laboratories of the Animal Production Research Institute, Ministry of Agriculture, Egypt. A total number of 132 Dokki4 hens and 12 Dokki4 cocks, 32 weeks of age were randomly distributed into 4 groups with 3 replicates each (11 hens+1cock). One group served as a control without any addition (PL_0) and the other three groups were fed control diets with three levels of papaya latex (PL) 0.01 (PL_1), 0.03 (PL_2) and 0.05% (PL_3) as ground powder PL. The experimental diets (Table 1) were formulated to be isonitrogenous (16% CP) and isocaloric (2744 Kcal ME/Kg diet) according to Feed composition Tables for animal & poultry feedstuffs used in Egypt (2001). The birds were reared under the same managerial conditions on floor in open-sided house. Feed and water were offered ad libitum during the experimental period for 12 wks.

Collection of latex:

Papaya latex (PL) is the powder obtained from pawpaw latex which is the milky juice obtained from unripe pawpaw fruits by making 3- 4 incisions (vertically) of about 2mm using a sharp pointed knife/object on each of the selected fruits still intact on the mother plants. On incision of the sharp object, the latex was collected in a crucible and immediately began to coagulate and it can be processed either by solar or oven drying at 40°C for 14 hrs or 55°C for 1 hrs with the aid of laboratory mortar and pestle, it was then ground thus forming a greenish or grey powder known as papain (Macalood et al., 2013, Zeedan and Abdel-Latif, 2013 and El-Neney et al., 2013). In this study latex from Carica papaya was obtained by cutting the skin of the unripe fruit papaya by steel razor blade and then collected. The latex is simply spread on trays and left in the sun to dry. Then it was ground to powder in a ball mill.

Productive and reproductive traits:-

Birds were weighted at 32 wks of age (the beginning of the experiment, IBW) and then every 4 wks till the end of the experiment (FBW) after 12 wks of beginning. Egg weight (EW), egg mass (EM= TEP x EW), total egg production (TEP), egg production (EP), total feed intake (TFI) and feed conversion ratio (FCR= TFI / EM) were recorded and calculated throughout the whole experimental period (12 wks). Crude protein conversion (CPC) and caloric conversion ratio (CCR) were also calculated. Three eggs from each hen were randomly chosen to determine egg shell weight, egg shell thickness (mm), egg shell index (ESI), albumin and yolk percentage. Haugh units (HU) were evaluated by (Haugh, 1937). At the end of 44 weeks of age, the all cocks were used to determine semen volume, mass motility, sperm abnormality and dead sperms were measured according to Kamar (1960). For evaluating egg fertility and hatchability, three hatches of eggs were made every 4 weeks of the experimental period. Weights of healthy chicks were also recorded.

Nutrients digestibility:-

Digestion coefficients of dry matter (DM), organic matter (OM), crude protein (CP), crude fibre (CF), ether extract (EE) and nitrogen free extract (NFE) were determined at the end of the study using 3 cockerels from each group. Faecal nitrogen content was determined according to the method outlined by Jakobsen et al. (1960), while the urinary organic matter fraction was calculated according to Abou-Raya and Galal (1971). Proximate analyses of feed and excreta were carried out following A.O.A.C. (1995).

Carcass characteristics:-

At the end of the experimental period (44 wks of age), six hens were chosen randomly from each treatment for slaughter test and carcass weights were determined and presented as a percentage of live body weight. Meat of birds was analyzed for crude protein (CP) and ether extract (EE). Intestinal content samples were collected by pressing the outer wall of cut ileum to push its content into sterile glass bottle. The pH value was determined in intestinal samples at once then frozen until used for microbiological examination.

Biochemical parameters:-

At the end of the experimental period, individual blood samples were taken from six hens from each treatment.

The biochemical characteristics of blood were determined colorimetrically, using commercial kits (Produced by Bio-Diagnostics Company, Egypt). Blood samples were centrifuged at 3000 rpm for 20 minutes. Plasma was decanted and stored frozen at -20°C until the time of analysis. After measuring the egg quality, three yolk samples from each treatment were separated from the broken eggs and extracted to determine yolk cholesterol and total lipids. Total lipids, total cholesterol, Low Density Lipoprotein (LDL~cholesterol), High Density Lipoprotein (HDL~ cholesterol) were determined in both of blood plasma and egg yolk. Total protein, albumin, transamiase (AST), alanine transamiase (ALT) and total antioxidant capacity (mmol/L) were determined in blood plasma. Plasma immunoglobulin, IgG and IgM in plasma were employed using the method of Leslie and Frank (1989). Non-coagulated blood was tested shortly after collection for determination blood pictures including, red blood cells count (RBCs, 10^6/mm^3), white blood cells count (WBCs, 10^3/mm^3), different subclasses of WBC's (lymphocyte, monocytes, eosinophils and basophil percentages), hemoglobin concentration (Hb) and hematocrite value (Ht) according to Drew et al. (2004).

Economic efficiency:-

The total feed cost (L.E/hen) at the end of the experiment for each treatment was calculated depending on the local market prices of the ingredients used for formulating the experimental diet. Economic efficiency (EE) and relative economic efficiency (REE) were calculated.

Statistical analysis:-

Data were statistically analyzed according to SPSS (2012) computer program using the following fixed model: $Yij=\mu + T_i + e_{ij}$

Where: Yij = the observation; μ = overall mean; T_i = effect of treatments; e_{ij}= random error component assumed to be normally distributed.

Duncan's multiple range tests was performed (Duncan, 1955) to detect significant differences among means.

RESULTS AND DISCUSSION

1- Chemical composition of papaya latex (PL):

The results in Table 2 showed that the content of organic matter (OM), crude protein (CP), ether extract (EE), nitrogen-free extract (NFE), crude fiber (CF) and ash were 93.15, 62, 4.50, 25.99, 0.66 and 6.85 %, respectively for PL. This result was in agreement with those of Macalood et al. (2013) who reported that complete proximate analysis of Carica papaya latex dried latex showed that it contained higher amount of crude protein at approximately (57.24 %) over other components such as moisture (17.76 %), ash (7.00 %), crude fat (5.21 %) and crude fiber (0.67 %).

2- Productive traits:

2-1. Body weight and body weight gain:-

Data of Table 3 indicated that there were a significant ($P<0.05$) difference in values of final body weight and body weight gain for Dokki4 laying hens with different levels of papaya latex (PL). Hens fed diet supplemented with PL_3 had the highest final body weight and body weight gain compared to other groups. These findings were confirmed upon examination of papaya latex on rabbits (El-Kholy et al., 2008, Zeedan et al., 2009 and El-Neney et al., 2013). Papain is a protease enzyme that hydrolyzes proteins to short peptides in diet, which is the key factor to increase protein digestibility and fast absorption, and helps to increase growth factors (Wong et al., 1996).

The improvement in growth performance resulted from the addition of papaya latex could be due to the better absorption of amino acids because of cysteine proteinases, papain, proteolytic and chymopapain enzyme (digestive enzymes) constitute as much as 80% of the enzyme fraction in papaya latex (El-Moussaoui et al., 2001, Azarkan et al., 2003) and due to antibacterial properties of PL.

2-2. Egg Production:

Total egg production (TEP) and egg production percentage (EP) were significantly ($P<0.05$) increased by PL treatments (Table 3). The highest numerically value was for PL_3 (77.03%). These findings were confirmed upon examination of PL on laying hens (Lien and Wu, 2012). In the present experiment, Increase in the EP in the treated groups with the PL could have been due to its antibacterial, antibiotic, antioxidant and antifungal effects which can lead to decrease in the harmful microbes of digestive system, improve their immunity and performance. This is possible that it is result of synergetic influence of effective substances in increasing antimicrobial activity. Also, increased egg production with PL may be due to improve their nutrient digestibility and improving nutrient utilization (Table 4).

2-3. Egg weight (EW) and egg mass (EM):

The analysis of variance of obtained data from Table 3 showed that there were significant increase in EW and EM of fed diet supplemented with PL compared with control group. The result was supported with Lien and Wu (2012) who found that papain supplementation to diet of laying hens were 64.84, 64.47 and 65.48 g egg weight for 0, 0.14 and 0.28 g papain/kg feed, respectively. The increase in EW and EM throughout the trial may have been due to limited amino acid (Papaya latex proteases are composed of four cysteine proteases which contribute 69- 89% of total protein). Increase egg mass with PL supplementation can be due to the increase of laying rate rather than the individual egg weight. Likewise, the tendency for improvement in feed conversion was mainly due to the increased egg mass rather than the effect on feed intake. Also, increased egg production with PL may be due to improve their nutrient digestibility and improving nutrient utilization (Table 4).

2-4. Feed intake and feed conversion:

The data showed that there are significant effect of PL on TFI, FCR, crude protein conversion (CPC) and caloric conversion ratio (CCR) (Table 3). The decrease in daily feed intake by adding papaya latex may be due to an increase in the efficiency of nutrition absorption and utilization. This improvement in FCR, CPC and CCR may due to that intestinal pH may alter both microbial populations (Table 8) and nutrient absorption and this may improve efficiency of feed digestion and utilization (Table 4). These findings were confirmed upon examination of papaya latex on rabbits El-Kholy et al. (2008), Zeedan et al. (2009), El-Neney et al. (2013) and Singh et al. (2011) on fish. The reason may be attributed to fast metabolism in laying hens fed on papain supplemented feed which in turn resulted in better FCR. Papain is a protease enzyme that hydrolyzes proteins to short peptides in diet, which is the key factor to increase protein digestibility and fast absorption, and helps to increase growth factors (Singh et al., 2011 and Wong et al., 1996). On the other hand, Lien and Wu (2012) indicated that papain supplementation increased FI than those fed control diet.

In the current study, improved FCR with PL may be due to improve their crude protein digestibility and attributed to the antibacterial, antioxidant and improving nutrient utilization. Also, the improvement of FCR of PL levels may be due to a beneficial microbial environment in the gut, which in might have enhanced digestion, absorption and utilization of nutrients. Protein conversion is a measure to show how the protein sources in the diet could provide essential amino acids requirement of the laying hens. During the whole experiment there was an improvement in crude protein conversion in all treatments than the control (Table 3) which could be due to the exogenous application of vegetable pepsin.

3- Nutrient digestibility:

The effect of treatments on the nutrients digestibility coefficients of CP, CF, EE and NFE are summarized in Table 4. Nutrient digestibility coefficients of CP, CF, EE and NFE were significantly ($P<0.05$) increased by PL supplementation. Protease activity in the digestive tract is a key determinant of the digestibility and assimilation efficiency of ingested proteins. This improvement may be attributed to proteolytic enzyme of exogenous origin plays an important role in feed digestibility in hens. The improvement of digestibility coefficient values may be attributed to the potential beneficial effect of these additives PL on gastrointestinal tract micro-organisms and metabolites which be reflected on improving the digestibility of feed nutrients and conversions. Also, this improvement may be attributed to reduction in digesta viscosity, increases the diffusion rates of nutrients and enzymes (PL) enabling the bird to digest and absorb more nutrients. Similar results were confirmed by Krishna et al. (2008), Zeedan et al. (2009) and El-Neney et al. (2013). Unigwe et al. (2014) reported that papain is an effective natural digestive aid which breaks down protein and cleanses the digestive tract. Papain is a protease enzyme that hydrolyzes proteins to short peptides in diet, which is the key factor to increase protein digestibility and fast absorption, and helps to increase growth factors (Wong et al., 1996). This may be due to the more extensive hydrolysis of protein which was caused by papain with results shown by Singh et al. (2011). They added that, the digestibility values recorded in the common carp fed on papain supplemented diet (2%) that exogenous enzymes play a considerable role in fish digestion process and added enzymes advantageously influence fish growth and food utilization.

The increase of CP digestibility in treated groups can be discussed from the point that papain enzyme as proteolytic enzyme may be associated with improvement in crude protein digestibility. A portion of the increase in digestibility may have resulted from the reduction in intake that occurred. So, increased feed digestibility corresponded to improved production efficiency of hens received PL. Papaya latex may cause the better absorption of amino acids because of cysteine and proteinases (digestive enzymes) constitute as much as 80% of the enzyme fraction in papaya latex (El Moussaoui et al., 2001 and Azarkan et al., 2003) and due to antibacterial properties of papaya latex.

4- Egg Quality:

Data presented in Table 5, in general, showed that there were significant ($P \leq 0.05$) increased in shell thickness values (Sh. T mm), egg shape index (ESI) and Haugh unit. While, treatments had no significant effect on shell weight, yolk and albumen percentages. These positive effects were evidenced by increases of growth performances and digestibility together with improvement of egg shell thickness and egg weight in comparison to non-supplemented birds. The decrease in yolk percentage was probably associated with the increase in albumen percentage. On the other hand, Lien and Wu (2012) reported that egg shell thickness was not significantly different among the groups (0.14g/kg feed and 0.28g/kg feed papain groups).

5- Reproductive traits:

Table 6 shows the effect of the treatments on semen characteristics (semen volume, live, dead and abnormal sperms and motility), fertility, hatchability and hatching chick weight. As shown, semen volume, live sperms, motility, fertility, hatchability and hatching chick weight were significantly higher ($P \leq 0.05$) in PL supplementation groups than the control treatment. While, dead sperms and abnormal sperms were decreased in cocks fed PL supplementation. Any negative impact on motility would seriously affect fertilizing ability. Sperm motility may be affected by altered enzymatic activities of oxidative phosphorolytic process. The improvement of fertility, hatchability and hatching chick weight can be due to increased metabolism for the digestion protein. Also, papaya latex consists of proteolytic enzyme this component increased from digestibility and may be due to an increase in the efficiency of nutrition absorption and nutrients utilization thus may be reflected on better performance production and reproductive animal. The improvement in fertility traits of treated rabbit does may be discussed from the view which demonstrated by El-Kholy et al. (2008) who showed that papaya latex inclusion have a great effect on immune system, and this may be responsible to increase immune system activity of offspring, where there was a positive correlation between the immune system of rabbit does and their offspring (Fortun-Lamothe and Drouet-Viard, 2002). The marked improvement in fertility and hatchability of hens supplemented with PL compared with PL_0 is in agreement with the findings of Zeedan et al. (2009) on rabbits reported that NZW rabbit does fed diets supplemented with PL is very useful for pregnant rabbit does. Also, they reported that NZW rabbit does fed diets supplemented with PL increased of conception rate and enhancing the reproductive performance due to presence of certain digestive enzymes.

6- Carcass characteristics and chemical composition of meat:

Results tabulated in Table 7 were clearly that, laying hens received the level of PL had the best values ($P<0.05$) of carcass, spleen and total giblets percentages as compared to control group. However, there were insignificantly differences in liver, gizzard and heart % of the laying hens received the level of PL compared to control group. Spleen is peripheral immunity organ and is the biggest immunity organ in animal body, which larges when the body weight increase. Spleen is identified as the secondary lymphoid tissue (Stephen, 2007, El-Kholy et al, 2008 and El-Neney et al. 2013). Hens fed PL had significantly (P<0.05) low abdominal fat%. Low abdominal fat percent obtained with supplemented PL in hens might be due to the increased efficiency of energy utilization as shown in Table 3. Also, decrease the fat content can be due to because the decrease plasma lipid levels or yolk and attenuate obesity. These results are in agreement with Zeedan et al. (2009) and El-Neney et al. (2013).

It seems that the literature is still sparse on the effect of PL on laying hens carcass traits. The increase in carcass traits for treated groups may be mainly related to the increase in growth performance and digestibility. Also, the improvement in growth performance resulted from the addition of PL could be due to the better absorption of amino acids and due to antibacterial properties of PL. The increase of spleen weight % may be due to immunostimulate activity of PL compound. Abdalla et al. (2012) and Navid, et al. (2011) concluded that dietary supplementation of 2% papaya leaf meal and papain in off-layer hens for a few days before slaughter improved meat quality in terms of meat tenderness and juiciness.

Data in Table 7 showed that, carcass protein of hens received diets with PL increased significantly (P<0.05) as compared with the control group. Whereas, EE was significantly decreased (P<0.05) by PL compared to the control. The increase in protein content of meat improves the value and quality of meat hens. This increase may be due to the presence of proteolytic enzymes (10% papain, 50% chymopapain, 24% peptidases A and B, and 16% lysozyme) in the milky juice (latex) of papaya fruits (Pendzhiev, 2002). Also, this increase in protein in hen meat can be attributed mainly to the presence of amino acids, vitamins, increase of protein digestibility as mentioned and improving intestinal absorption and digestion of all nutrients due the stimulus to the development, proliferation and differentiation of intestinal cells. These enzymes increased of all nutrient digestibilities in Table 4.

7- Microbial determination:

Data presented in Table 8 showed that the total microflora count (10^4 cfu/g) significantly decreased (P<0.05) with increasing PL in intestinal of laying hens compared with the control group. These results are in agreement with those obtained by El-Neney et al. (2013). This was due to the decreasing of the ceca pH with increasing papaya latex level when compared to the control diet. In the present experiment, decreased pH and total microflora count with PL may be due to the antimicrobial, antibiotic, antifungal, antioxidant and antibacterial. Also, the low pH with PL levels may be due to a beneficial microbial environment in the gut, which might have enhanced digestion, absorption and utilization of nutrients. Concerning the antimicrobial effect of PL, it was attributed to increase the immune status of the host through activation of the macrophages, increase phagocytic activity. It could be declared that decreasing pH may increase the beneficial bacteria and decrease the harmful one. Okunola et al. (2012) and Abdeldaiem (2014) concluded that papaya leaves had antimicrobial activity against both gram-negative and gram-positive bacteria. Osato et al. (1993)

found that latex has been found to be bacteriostatic to B. subtilis, Enterobacter cloacae, E. coli, Salmonella typhi, Staphylococcus aureus, and Proteus vulgaris. The application of papaya latex is probably the most interest to livestock producers as an antimicrobial and antifungal (Nwinyi, et al., 2010 and Aravind et al., 2013). Presence of these properties in papaya latex may explain the enhancement of immune system activity. Papaya latex itself has been reported to exhibit antifungal activity against Candida albicans "which cause immune deficiency" (Krishna et al., 2008).

8- Biochemical parameters:

8-1. Some constituents of egg yolk extract:

Results of yolk total lipids and cholesterol of laying hens are presented in Table 9. Yolk total lipids and cholesterol were significantly reduced ($P<0.05$) of laying hens fed PL compared to control. Yolk LDL start to decrease significantly by PL as compared with the control, while the useful HDL values were numerically higher than the control. These results are in agreement with those obtained by Lien and Wu (2012) who showed that supplemental papain reduced the level of yolk cholesterol (12.17 and 11.30 mg/g for 0.14 and 0.28 papain (g/kg feed, respectively) compared with the control group (13.05 mg/g). They added that this implies that papain affect cholesterol absorption or metabolism, indicating that papain supplementation was good for coronary health. This may lead to produce enriched eggs that are healthier for human consumption and useful for those suffering from heart diseases.

8-2. Blood plasma constituents:

Significant ($P<0.05$) differences were observed in total protein, albumin and globulin as shown in Table 10. The increase in total protein, albumin and globulin with PL addition may be due to the increase in protein synthesis, and digestion of protein as show in Table 4. Increased globulin concentration with increased PL inclusion which was observed in the present study may be an indication of increased immunity in the laying hens since the liver will be able to synthesize enough globulins for immunologic action as mentioned by Sunmonu and Oloyede (2007). This result is in harmony with finding of El-Kholy et al. (2008), Zeedan et al. (2009) and El-Neney et al. (2013). Lien and Wu (2012) found that papain supplementation markedly increased total serum globulin, total protein for laying hens.

The data in Table 10 showed that there was a significant effect due to dietary treatment on cholesterol, LDL, HDL and total lipids. All feed addition decreased cholesterol, LDL and total lipids compared to control diet. This result is in harmony with finding of Lien and Wu (2012) who indicated that papain supplementation reduced liver cholesterol and the high level group showed a decreased serum cholesterol concentration. They added that this implies that papain affect cholesterol absorption or metabolism, indicating that papain supplementation was good for coronary health. Also they added that papain not only contains protease, but also phospholipase.

No significant differences ($P<0.05$) were observed in AST and ALT among the four groups (Table 10). Liver function as AST and ALT activity were not affected by dietary treatments with supplementing PL. Values AST and ALT were within the normal range and indicated that the animals were generally in a good nutritional status and their livers were in a normal health condition. These results may explain that increasing PL is safe with liver functions and so it did not have any harmful effects on liver tissues. This result is in harmony with finding of El-Neney et al. (2013). On the other hand, Zeedan et al. (2009) and Lien and Wu (2012) reported that supplementing PL to the diet of growing rabbits or laying hens decreased significantly ($P \leq 0.05$) of AST and ALT.

Data presented in Table 10 showed that, the analysis of variance indicated that there were significantly ($P<0.05$) differences between total antioxidants capacity values, IgG and IgM. Concentrations of total antioxidants capacity values, IgG and IgM in plasma were high in PL_3 followed by PL_2 and PL_1, while they low in control. These results are in agreed with those obtained by Lien and Wu (2012) showed that papain supplementation markedly increased IgG levels. Total globulin was mainly synthesized into antibodies, implying that papain could enhance animal immunity. Rose et al. (2006) reported that papain enhanced IL-6 production dose-dependently. The additive effects of PL on the improvement of the immune response may be due to the effects of PL on the increased availability amino acids, which are nutrients needed for an effective and vigorous immune response, and to the beneficial effects of PL on the gastrointestinal microflora.

Data in Table 10 illustrated that Hb, Ht, RBCs and WBCs were significantly ($P<0.05$) increased with addition of PL. The immunity responsiveness represented in leukocytes counts and mainly on lymphocytes increased with increasing PL. The increase in WBCs in treated groups can be attributed mainly to the antibacterial functions, antibiotic, antifungal and antioxidant of papaya latex. These results are in accordance with El-Kholy et al. (2008), Zeedan et al. (2009) and El-Neney et al. (2013) they noted that rabbits fed diet containing papaya latex had the best value of WBC and lymphocytes when compared to control. The lymphocyte is considered the main type of white blood corpuscles and a good indicator of increasing the immunity efficiency (Wieslaw et al., 2006). Many studies have demonstrated the antibacterial and anti-fungal properties of papaya latex (Nwinyi et al., 2010 and Aravind et al., 2013). Essence of these properties in papaya latex may explain the enhancement of immune system activity. Miyamoto et al. (2004) showed that papaya enzyme increase immune system function. Lien and Wu (2012) found that papain could enhance animal immunity, enhance protein metabolism, ameliorate inflammation and regulate immunity.

Generally the increase in blood constituents may be due to the role of PL in improving all nutrient digestibility especially CP (Table 4). Papaya latex consists of proteolytic enzyme and may possess antimicrobial, antifungal, antioxidant and against many different parasites. This component increased from digestibility and efficiency of nutrition absorption and utilization thus may be reflected on better performance production and reproductive. Also, it may be probably led to an increase in the absorption rate from the digestive tract, thus blood constituents of the supplemented animals reflected a corresponding increase of these values

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9- Economical efficiency:

Data illustrated in Table 11 showed that laying hens supplemented with PL gave more EP than the control group. Moreover, PL supplementation tended to get more economic efficiency (0.97, 1.13 and 1.28) and increased net revenue. These results were in agreement with those obtained by El-Neney et al. (2013) who found that the buffalo and rabbits treated with PL showed a higher value of economical efficiency than in the control group.

In conclusion:

Papaya latex is able to improve productive and reproductive performance of Dokki4. Standing on our results, the best concentration of papaya latex in Dokki4 diets seems to be 0.05 %. At this level, both laying hens and cockers improved egg production, egg and semen quality, immunity some blood biochemical parameters and economic efficiency. Also, produce eggs with lower yolk cholesterol that are healthier for human.

Table (1): Composition and calculated chemical analyses of the experimental basal diet.

Ingredients	%
Yellow corn	65.14
Soybean meal (44%)	25.10
Limestone	7.60
NaCl	0.30
Di-Calcium Phosphate	1.50
DL-Methionine	0.06
Premix*	0.30
Total	100
Calculated analyses**	
Crude protein %	16.09
ME, Kcal / kg	2744
C / P ratio	170.54
Crude fiber %	3.33
Ca %	3.29
P (Available) %	0.40
Lysine %	0.90
Methionine %	0.35
Met. + Cyct	0.62
Tryptophan	0.19
Price/ ton	2388

*Each 3 Kg of vitamins and minerals premix contained: 10000000 IU Vit. A, 10000 mg Vit. E, 1000 mg Vit. K3, 2000000 IU Vit. D, 1000 mg Vit. B, 10000 mg Pantothenic acid, 10 mg Vit. B12, 1500 mg Vit. B, 5000 mg Vit. B2 , 30000mg Niacin, 300000 mg Choline chloride, 1000 mg Folic acid, 50 mg Biotin , 300 mg I, 60000 mg Mn, 50000 mg Zn, 30000 mg Fe, 4000 mg Cu, 100 mg Se and 100 mg Co.

**According to Feed Composition Tables for animal & poultry feedstuffs used in Egypt (2001).

Table (2): The chemical composition analysis of dried crude papaya latex (PL) of Carica papaya.

Item	OM	CP	EE	CF	NFE	Ash
Dried papaya latex	93.15	62	4.50	0.66	25.99	6.85

Table(3): Effect of papaya latex inclusion on the productive performance of Dokki4 laying hens during the experimental period.

Parameter	PL_0	PL_1	PL_2	PL_3	SEM	Sig.
Initial body weight, g (IBW)	1531.3	1537.0	1532.7	1536.8	8.71	NS
Final body weight, g (FBW)	1600.0^{d}	1624.0^{c}	1660.7^{b}	1685.3^{a}	10.01	*
Body weight gain, g (BWG)	68.7^{d}	87.0^{c}	128.0^{b}	148.5^{a}	2.14	*
Total egg production (TEP)	54.20^{d}	58.67^{c}	61.26^{b}	64.71^{a}	0.32	*
Egg production, % (EP)	64.52^{d}	69.84^{c}	72.93^{b}	77.03^{a}	0.24	*
Egg weight, g (EW)	45.12^{c}	49.08^{b}	50.65^{b}	55.04^{a}	0.24	*
Egg mass, kg (EM)	2.44^{c}	2.88^{b}	3.10^{b}	3.56^{a}	0.01	*
Total feed intake, kg (TFI)	9.74^{a}	9.13^{b}	8.50^{c}	8.06^{d}	0.20	*
Feed conversion ratio (FCR)	3.99^{a}	3.17^{b}	2.74^{c}	2.26^{d}	0.01	*
Crude protein conversion (CPC)	0.64^{a}	0.51^{b}	0.44^{c}	0.36^{d}	0.01	*
Caloric conversion ratio (CCR)	10.95^{a}	8.70^{b}	7.52^{c}	6.21^{d}	0.07	*

Means in the same row having different letters are significantly different ($p \leq 0.05$). NS, not significant. PL_0 = control, PL_1= 0.01, PL_2= 0.03 and PL_3= 0.05%.

Table(4): Effects of feeding papaya latex on nutrients digestibility coefficient values of Dokki4 laying hens.

Item	DM%	CP%	CF%	EE%	NFE
PL_0	78.65	73.27^d	19.87^c	75.98^c	75.20^c
PL_1	79.54	75.79^c	22.62^b	79.44^b	79.22^b
PL_2	80.53	79.75^b	25.23^a	80.60^b	81.01^b
PL_3	81.71	81.81^a	25.75^a	84.30^a	85.28^a
SEM	2.23	0.46	0.15	0.95	1.00
Sig	NS	*	*	*	*

Means in the same column having different letters are significantly different ($p \leq 0.05$). NS, not significant. PL_0 = control, PL_1= 0.01, PL_2= 0.03 and PL_3= 0.05%.

Table(5): Effects of feeding papaya latex on egg quality parameters for Dokki4 laying hens during the experimental period.

Item	Shell weight	(Sh. Tmm)	ESI	Yolk, %	albumen,%	H U
PL_0	12.55	0.42^b	74.60^c	35.86	54.57	77.50^c
PL_1	12.54	0.40^b	76.32^b	33.71	54.76	79.18^b
PL_2	14.40	0.49^a	76.92^{ab}	32.18	55.18	76.92^c
PL_3	13.37	0.49^a	77.22^a	31.58	55.92	82.38^a
SEM	0.95	0.01	0.10	0.05	1.01	0.62
Sig	NS	*	*	NS	NS	*

Means in the same column having different letters are significantly different ($p \leq 0.05$). NS, not significant.
PL_0 = control, PL_1= 0.01, PL_2= 0.03 and PL_3= 0.05%. Sh. T= Shell thickness and ESI= Egg shape index.

Table(6): Effects of feeding papaya latex on the semen quality, fertility, hatchability and hatched chick weights of Dokki4.

Parameter	PL_0	PL_1	PL_2	PL_3	SEM	Sig.
Semen volume, (ml)	0.34^c	0.35^c	0.44^b	0.56^a	0.001	*
Live sperm, (%)	66.00^d	77.21^c	80.33^b	87.00^a	0.04	*
Dead sperm, %	34.00^a	18.00^b	13.00^c	12.00^d	0.12	*
Abnormal sperm, (%)	4.53^a	3.00^b	2.50^c	1.20^d	0.01	*
Motility, (%)	75.00^d	80.02^c	85.01^b	89.10^a	0.33	*
Fertility, (%)	85.53^c	88.83^{bc}	90.85^{ab}	94.15^a	0.55	*
Hatchability, (%)	68.12^c	73.40^b	74.87^b	80.98^a	0.50	*
Average chick weight,(g)	28.85^c	29.97^c	33.09^b	36.10^a	0.15	*

Means in the same row having different letters are significantly different ($p \leq 0.05$).
PL_0 = control, PL_1= 0.01, PL_2= 0.03 and PL_3= 0.05%.

Table (7):. Effects of feeding papaya latex on the carcass yield and some slaughter traits for Dokki 4 laying hens.

Item	PL_0	PL_1	PL_2	PL_3	SEM	Sig
Carcass, %	66.79[c]	70.30[b]	75.61[a]	78.09[a]	1.20	*
Liver, %	2.38	2.39	2.39	2.41	0.05	NS
Gizzard, (%)	1.75	1.77	1.78	1.80	0.20	NS
Heart, %	0.47	0.48	0.50	0.49	0.02	NS
Abdominal fat (%)	4.55[a]	3.12 [b]	2.54 [c]	2.50[c]	0.01	*
Spleen, %	0.13[b]	0.14[b]	0.18[a]	0.20[a]	0.002	*
Total giblets (%)	4.60[b]	4.64[b]	4.67[ab]	4.70 [a]	0.02	*
Chemical analysis of carcass meat on DM basis						
CP, %	13.61[c]	15.40[b]	17.98[a]	18.32[a]	0.01	*
EE, %	4.88 [a]	3.01[b]	2.18 [c]	2.15[c]	0.01	*

Means in the same column having different letters are significantly different ($p \leq 0.05$). NS, not significant.
PL_0 = control, PL_1= 0.01, PL_2= 0.03 and PL_3= 0.05%.

Table (8): Effects of feeding papaya latex on the intestinal pH, total microflora count (10^4 cfu/g) for Dokki4 laying hens.

Item	Ceca pH	Total microflora count (10^4 cfu/g)
PL_0	7.12[a]	5.75[a]
PL_1	6.80[b]	3.15[b]
PL_2	5.86[c]	1.50[c]
PL_3	5.75[c]	0.75[d]
SEM	0.01	0.01
Sig.	*	*

Means in the same column having different letters are significantly different ($p \leq 0.05$).

Table (9): Effect of feeding papaya latex on cholesterol, LDL, HDL and total lipids of eggs yolk.

Item	Cholesterol mg/g	LDL mg/g	HDL mg/g	Total lipids mg/g
PL_0	16.98[a]	12.67[a]	4.31[d]	331.10[a]
PL_1	15.49[b]	11.04[b]	4.45[c]	293.53[b]
PL_2	14.43[c]	9.32[c]	5.11[b]	273.00[c]
PL_3	13.69[c]	7.67[d]	6.02[a]	260.25[d]
SEM	0.11	0.12	0.02	7.10
Sig	*	*	*	*

Means in the same column having different letters are significantly different ($p \leq 0.05$).

PL_0 = control, PL_1= 0.01, PL_2= 0.03 and PL_3= 0.05%.

Table (10): Effect of feeding papaya latex on blood picture and some blood constituents.

Items	PL_0	PL_1	PL_2	PL_3	SEM	Sig.
Total protein (g/dl)	4.51^d	5.45^c	6.32^b	6.69^a	0.12	*
Albumin (g/dl)	2.23^c	2.55^b	2.57^a	2.71^a	0.01	*
Globulin (g/dl)	2.28^c	2.90^b	3.75^a	3.98^a	0.01	*
Cholesterol (mg/dl)	123.22^a	102.30^b	85.02^c	90.30^d	4.52	*
HDL - Cholesterol (mg/dl)	27.72^c	45.01^b	45.10^b	50.01^a	1.05	*
LDL - Cholesterol (mg/dl)	95.50^a	57.29^b	39.92^c	40.29^c	0.85	*
T.lipids (mg/dl)	386^a	355^b	339^c	333^c	3.30	*
AST (U/ L)	63.33	64.52	65.65	66.01	2.14	NS
ALT (U/ L)	25.53	26.10	26.22	26.33	1.75	NS
Total antioxidants capacity (mmol/L)	0.330^d	0.380^c	0.490^b	0.695^a	0.01	*
IgG (mg/dL)	55.45^d	81.22^c	100.21^b	138.85^a	10.04	*
IgM (mg/dL)	191.21^d	218.66^c	240.80^b	275.12^a	8.50	*
Hemoglobin (Hb, g/dl)	6.50^c	9.90^b	10.21^b	11.32^a	0.10	*
Hematocrite(Ht, %)	28.10^c	30.66^b	30.75^b	34.60^a	0.50	*
Red blood cells (RBC, $N \times 10^6$ /mm^3)	2.30^c	3.33^b	3.61^b	4.21^a	0.01	*
White blood cells (WCR, $N \times 10^3$/mm^3)	4.45^d	5.10^c	6.15^b	6.75^a	0.05	*
Lymphocytes (%)	57.08^d	59.85^c	62.56^b	66.67^a	0.75	*
Monocytes (%)	2.50^a	2.00^b	1.52^c	1.00^d	0.01	*
Eosinophils (%)	2.20^a	1.65^b	1.05^c	0.75^d	0.01	*
Basophils (%)	1.71^a	1.35^b	1.12^c	0.87^d	0.01	*

Means in the same row having different letters are significantly different ($p \leq 0.05$). NS, not significant.

PL_0 = control, PL_1= 0.01, PL_2= 0.03 and PL_3= 0.05%.

Table (11):. Effect of feeding papaya latex on economic efficiency of Dokki4 laying hens.

Items	PL_0	PL_1	PL_2	PL_3
Total feed intake / hen (kg)	9.74	9.13	8.50	8.06
Price/kg diet (LE)	2.39	2.44	2.54	2.64
Total feed cost/ hen (L.E)	23.28	22.28	21.59	21.28
Total EN/ hen	54.20	58.67	61.26	64.71
Price/ egg (L.E)	0.75	0.75	0.75	0.75
Total price of egg (L.E)	40.65	44.00	45.95	48.53
Net revenue per hen	17.37	21.72	24.36	27.25
Economic efficiency* (EE)	0.75	0.97	1.13	1.28
Relative (REE)	100	129	151	171

*Economic Efficiency (EE) = (net return / Feed cost) x100. Price of kg papaya latex=500 LE

PL_0 = control, PL_1= 0.01, PL_2= 0.03 and PL_3= 0.05%.

Relative economical efficiency (REE), assuming control treatment = 100.

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الملخص العربى

تحسين الأداء الانتاجى و المناعي للدجاج المحلى باستخدام الانزيمات الطبيعية (انزيم البابين النباتى) ومخلفات نبات الباباظ. ١- تأثير صمغ الباباظ على انتاج البيض.

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*****قسم انتاج الدواجن. كلية الزراعة ــجامعة كفر الشيخ- مصر.***

الهدف من هذه الدراسة هو تقييم تأثير إضافة الإنزيمات الطبيعية (البابين النباتى) فى دجاجات دقي 4 على الأداء الإنتاجي، الهضم، وجودة البيض والسائل المنوي، وصفات الذبيحة و المناعة وبعض مكونات الدم والكفاءة الاقتصادية . استخدم فى هذة الدراسة 132 دجاجة دقي٤ و ١٢ ديك ، فى عمر 32 أسبوعا، تم تقسيم الدجاج إلى أربع مجموعات كل مجموعة بها ثلاث مكررات كل مكررة (١١ دجاجه + ١ ديوك) .تم تقسيم الدجاجات عشوائيا إلى المجموعة الاولى كنترول بدون اضافة و ثلاث مجموعات معاملة (تحتوي على (PL1)٠,٠١ و (PL2)٠,٠٣ و (PL3) ٠,٠٥ % من البابای لاتكس).

النتائج المتحصل عليها يمكن تلخيصها على النحو التالي :

١- سجلت المجموعات المضاف اليها البابای لاتكس زيادة فى وزن الجسم النهائي ، معدلات النمو ، إنتاج البيض ، وزن البيض و كتلة البيض بالمقارنة بالكنترول .واستهلاك الغذاء كان اقل فى المجموعات المضاف اليهاPL عن الكنترول ، وحدث تحسن فى نسب التحويل الغذائي .

٢- تحسنت معنويا قيم معاملات هضم المواد الغذائيه للعلائق المضاف اليها PL بالمقارنة بالكنترول.

٣- اضافة PL كان لة تأثير على كل من سمك قشرة البيضة ، دليل شكل البيضة بينما لم يكن هناك تأثير على نسبة كل من الصفار والالبيومين.

٤- وجد ان اضافة PL حسن كل من صفات السائل المنوى والخصوبة ونسبة الفقس بالمقارنة بالكنترول.

٥- ادى اضافة PL الى انخفاض كل من الكوليسترول والدهن فى الدم والصفار بالمقارنة بالكنترول. ايضا اضافة PL ادت الى تحسين الاستجابة المناعية.

٦- اضافة PL حسنت الكفاءة الاقتصادية بالمقارنة بالكنترول.

عموما، يمكن القول أن اضافة البابای لاتكس الى علائق الدجاج البياض ادى الى تحسين انتاج البيض وكفاءة التحويل الغذائى و معاملات هضم الغذاء والمناعة وجودة السائل المنوي ونسبة الخصوبة والفقس وتحسين الكفاءة الاقتصادية، و كانت افضل النتائج مع إضافة 0.05٪ البابای اللاتكس.