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INFLUENCE OF PAPAYA LEAVES AND THEIR EXTRACT SUPPLEMENTATION ON GROWING RABBIT PERFORMANCE, PHYSIOLOGICAL TRAITS, IMMUNE RESPONSES AND OXIDATIVE ENZYME STATUS.

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ABSTRACT: *The present study aimed to determine the effects of using different levels of papaya leaves (PL) and papaya leaves extract (PLE) on productive performance, some blood constituents, oxidative enzyme status, carcass traits, immune response, digestibility coefficients and economic efficiency of weaned New Zealand White rabbits (NZW). Eighty four weaned New Zealand White rabbits (NZW) aged 35 days and weighed 564g ± 3.40 were distributed randomly into seven groups (12 each). The experimental diets contained different levels of papaya leaves and papaya leaves extract (0.0 controls (C), 1, 2 and 3% papaya leaves or papaya leaves extract, respectively. The experiment was lasted for 8 weeks.*

The obtained results indicated that: The final live body weight, total weight gain and performance index were significantly (P<0.05) improved in the treated groups. Digestibility coefficient and nutritive values were significantly improved for rabbits fed diet contained PL and PLE compared to those fed the control diet.

There were significant increases detected for plasma total protein, albumin and globulin while blood plasma cholesterol, LDL and total lipids were significantly decreased by increasing PL and PLE inclusion level compared to control groups. Liver function as AST and ALT activity were not affected by dietary supplementation. Moreover the concentrations of IgG and IgM of rabbits were higher for rabbit supplemented with PL and PLE than the control group. GPx, SOD and CAT were significantly improved by dietary PL and PLE. The best economic efficiency had been recorded with rabbits fed 3% PLE inclusion followed by rabbits received 2% and 1% treatment of PLE, respectively. It is concluded that supplemental papaya leaves extract at the rate of 3 % of the diet improved feed conversion ratio, nutrients digestibility, immunity, and economic efficiency of the NZW rabbits.

Keywords*: Papaya leaves, growth performance, digestibility, rabbits and diet.*

INTRODUCTION

Papaya, a juicy and tasty fruit, belonging to family Caricaceae is scientifically known as Carica papaya Linn. It is grown in various parts of the world, including Egypt, India, America and Europe. It is commonly known as Papaya melon tree or Pawpaw, the leaves are large, 50-70 cm in diameter, deeply palmately lobed, with seven lobes. Papaya leaves are rich source of the proteolytic enzymes papain and chymopapain which have protein digesting properties and are useful in controlling digestive problems and intestinal worms (Burkill, 1985). Also, papaya leaves and their extract contain carotene, provitamin A, which serves as many as 18-50 IU and can be used as a source of natural Xanthophyll. Papaya leaves contain vitamin C, vitamin E, calcium, phosphorous and iron. Beside that the leaves contain 20.88% crude protein, 0.99% calcium, 0.47% phosphorous and 2912 kcal / kg gross energy (Mahendra and Nikhil, 2016).

Recent numerous research aimed to use of natural growth promoters to enhance feed utilization, growth performance and immune responses of poultry (El-Kholy *et al.,* 2008; Zeedan *et al.,* 2009 and Sorwar *et al*., ,2016). Moreover antioxidant supplements in animal diets present the advantage that living animals may efficiently distribute the compounds throughout the tissues, and the resulting enriched animal production ensures tolerable amounts for humans (Bou *et al*., 2009; Laudadio *et al*., 2015). Previous studies have indicated that a long-term dietary supplementation with a natural Papaya leaves extract leads to an antioxidant effect in pork, enhancing its oxidative status, (Rossi *et al.,* 2013).

Thus, the aim of the study was to investigate the effect of papaya leaves and their extract as feed additive on nutrients digestibility, immunity, performance, some blood constituents and economic efficiency of growing rabbits.

MATERIALS AND METHODS

Animals, diet, and experimental design:-

The experiment aimed to evaluate the growth promoting efficacy supplementation growing rabbit diets with papaya leaves and their extract by1, 2, 3%. Eighty Four weaned New Zealand White rabbits (NZW) aged 35 days and weighed 564g ± 3.40 were equally and randomly divided into seven groups (12 in each one). Rabbits were housed in galvanized metal rabbit battery cages supplied with separated feeders. Diets were offered in pellets form *ad libtum* and fresh clean water was available all times from automatic nipple drinkers. Feed intake and live weight were recorded weekly, feed conversion ratio and relative growth rate were calculated. All animals were kept under the same managements and hygienic conditions.

The experimental diets supplemented with different levels of papaya leaves or papaya leaves extract (0.0 control (C), 1, 2 and 3%), respectively. All diets were iso-protein and iso-digestible energy, and to satisfy the nutrient requirements according to Agriculture Ministry Decree (1996) recommendations. The experiment was lasted for 8 weeks. Composition and calculated analysis of the experimental main diet is presented in Table (1).

Preparation of plant extract

Mature and disease free papaya leaves were obtained from pawpaw trees. For the preparation of extract, 1,2, and 3 gram Leaves soaked in 100 ml distilled water for 1,2, and 3% (PLE) respectively , and macerated for 6 hours. At the end of 6 hours, the extract was filtered through Whatman No.1 filter paper (Mrinal and Bhushan, 2017).

Nutrients digestibility and carcass characteristics

At the end of the experimental period, digestibility trail was carried out using three rabbits from each treatment. Feces were collected daily, weighed and dried at 60-70 ^{o}C for 24 hours, finely ground and stored for chemical analysis. Data of quantities and chemical analysis of feed and feces were used to calculate the nutrients digestion coefficients and the nutritive values of the dietary treatments, as described by Cheeke *et al.* (1982). Carcass traits were studied; relative weight of giblets percentages (heart, liver and kidney) and dressing percentage was calculated according to Steven *et al.*, (1981). Boneless meat of breast and thigh muscle was determined according to A.O.A.C (2000).

Biochemical parameters

Blood samples were collected during slaughtering in heparinized tubes. Blood plasma were separated by centrifugation at 4000 rpm for 10 minutes, and then frozen at -20 C until analysis. Commercial kits were used to determine plasma total protein, albumin, Aspartate transaminase (AST), alanine transaminase (ALT), cholesterol, LDL, HDL and total lipids. Plasma immunoglobulin, IgG and IgM were detected.

Histology examination

The liver specimens were taken for histological study, fixed in 10% neutral-buffered formalin, dehydrated in a graded alcohol series (70%, 90%, absolute ethanol), cleared with methyl benzoate and embedded in paraffin wax. Sections of 5 μm were cut, fixed on glass slides and stained with hematoxylin and eosin for light microscopic examination (Bancroft *et al*., 1996; Oloruntola *et al.*, 2017). Stained sections were examined by light microscope and photographed using a digital camera.

Table 1. Composition and calculated analysis of the experimental diets for growing rabbits.

Ingredients	**%**
Clover hay (12%CP)	30.00
Barely	29.00
Yellow Corn corn	10.00
Soybean meal (44%CP)	18.00
Wheat bran	8.00
Molasses	3.00
DL-Methionine	0.10
Vit. & Min. mix.*	0.40
Salt	0.50
Limestone	1.00
Total	**100**
Ration Calculated analysis1	
Crude protein %	17.02
Digestible energy(Kcal/Kg)	2500
C/P ratio	147
Extract Ether %	2.72
Crude fiber %	13.25
NDF% m	37.63
ADF% n	21.52
Hemicellulose %o	16.11
Calcium %	1.10
Total Phosphorus %	0.80
Methionine %	0.36
TSAA	0.61
Lysine %	0.75

*Each 1.5Kg. of Vit. mix contained: 50,000,000 IU Vit. A; 1,000,000 IU D3; 10,000 mg Vit. E; 1170 mg Vit. K3;735 mg Vit.B1; mg Vit.B2; 15000 mg Vit B6;15 mg Vit;B12 ; 500 mg Vit.B5 Panathonic acid; 30,000 g Nicotinic acid; 84 mg Biotin; 500 g Folic acid; 300g choline chloride. Each 1.5 Kg Min. mix contained 25 g Zn (oxide); 33.4 g Mn; 26.7 g Fe; 2.67 g Cu; 67 mg cobalt; 1mg Se and.0.334 gI. l According to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001) ,except values of DDGS, which were determined (Table 2). [Mno] Calculated according to Cheeke (1987).

[m]% NDF = 28.924 + 0.657 (%CF)

[n]% ADF = 9.432 + 0.912 (%CF)

[o]Hemicellulose = %NDF - % ADF

Cecum activities

Samples of cecum contents were taken from slaughtered rabbits and used immediately for estimation of cecum pH, cecum microflora (bacteria) Aerobic total count, Fecal coliforms, Escherichia coli count, Bacillus cereus, Enterobacter, Clostridium sp., Enterococcus, yeasts, Salmonella and Shigella. Another sample of cecum content was strained through four folds of gauze and divided into two portions. The first portion was used immediately for the estimation of ammonia nitrogen concentration. The second portion was preserved by addition of 1 ml N/10 HCL and 2 ml orthophosphoric acid to each 2 ml of cecum contents juice for determination total volatile fatty acids. The pH of the cecum contents was measured immediately by using a digital pH meter. The microbial contents were studied in their selective media, as described by Postage (1969) for Aerobic total bacterial counts and Difco (1989) for Fecal coliforms and E.coli, while, the methods described by Baired Parker (1962) and Kim and Goepfert (1971) were used for Enterococcus and Bacillus cereus, respectively and Difco (1989) for Enterobacter and Clostridium sp.; while the method described by Lodder (1952) was used for yeasts determination. Salmonella and Shigella were enumerated according to the methods described by AOAC (1998). Technique of colony forming unit (CFU) was adopted. Incubation took place at 30 oC for 2-7 days. The ammonia nitrogen concentration was determined by applying method of Conway (1958). The total volatile fatty acids were determined by steam distillation of the distillate as mentioned by Eadie *et al*. (1967).

Economic efficiency

The economic efficiency of the experimental diets was calculated as the ratio between income (price of weight gain) and cost of feed consumed, calculated according to the price of the Egyptian market.

Statistical analysis

The data were analyzed using General Linear Models (GLM) procedure of SAS (2001). The statistical model was:

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

Where: Y_{ij}= Any observation, μ= Overall mean, Ti = Effect of treatment, and e_{ij} = Experimental error.

Variables having a significant F-test were compared using Duncan's multiple rang test (Duncan, 1955).

RESULTS AND DISCUSSIN

Growth performance traits

Results presented in Table 2 showed the effect of adding papaya leaves extract (PLE) and papaya leaves (PL) in diets for growing rabbits on growth

Table 2. Rabbit's performance values as affected by the experimental diets.

Items	Control Diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets			SEM	Sig
		1%	2%	3%	1%	2%	3%		
Initial body weight(g)	530.17	575.25	570.25	576.33	572.41	574.00	567.50	22.64	NS
Final body weight (g)	1836.50^{ab}	2026.33^{a}	2076.33^{a}	2047.42^{a}	1644.42^{b}	1945.00^{a}	1861.83^{ab}	76.28	**
Daily weight gain (g)									
5-13 weeks of age	17.85^{c}	21.01^{ab}	21.84^{a}	20.48^{ab}	17.02^{c}	18.91^{bc}	17.85^{c}	0.78	**
Daily feed intake(g)									
5-13 weeks of age	71.08^{cd}	76.57^{b}	82.18^{a}	76.32^{bc}	68. 14^{d}	74.12^{bc}	71.06^{cd}	1.90	*
Feed conversion ratio									
5-13 weeks of age	3.98^{a}	3.34^{b}	3.76^{b}	3.73^{b}	4.00^{a}	3.92^{a}	3.98^{a}	0.26	**
Relative growth rate (%)	99.37^{b}	101.52^{ab}	105.71^{a}	104.86^{a}	97.32^{b}	100.29^{b}	98.84^{b}	4.20	**
Performance index (%)	50.83^{b}	52.66^{ab}	55.75^{a}	55.23^{a}	47.00^{b}	52.55^{ab}	48.13^{b}	3.85	**

a, b, c, Means are bearing different superscripts in the same row , differed significantly ($P \leq 0.05$)

. SE = Standard error. * $P \leq 0.05$, ** P<0.01, NS: Not significant.

performance traits. It showed that final body weight and daily weight gain were significantly (P<0.01) higher in rabbits fed on PLE diets as compared to rabbits fed on control and PL diets. While, during 5-13 week of age period, there was no significant difference on daily weight gain between PL and control animals

The daily weight gain between 5-13 weeks of age was recorded to be 21.01, 21.84 and 20.48 (g) for rabbits fed on diets supplemented by 1, 2, 3 %, PLE respectively, when compared with rabbits fed on diets supplemented by PL ,17.02,18.91and 17.85 , respectively. Total Feed conversion ratio was significantly (P<0.01) improved with rabbits fed PLE diets compared to other treatments. During the experimental period (5-13 weeks of age) 1% PLE was the best value with 3.34 kg diet/kg growth, comparing with control diet which recorded 3.98 kg diet/kg growth. Although, feed intake did not significantly affected by dietary treatments throughout 5-13 weeks of age rabbit fed PLE diets recorded 76.57, 82.18 and 76.32, respectively for 1, 2 and 3% supplemented level comparing with control diet which recorded 71.08. Performance index and relative growth rate (%) increased significantly (P<0.0.01) in rabbits fed on PLE diets compared to that fed on other diets. Rabbit fed on 2% PLE supplemented diet had the highest relative growth rate with value 105.71 compared to other treatments without holding significant difference with other PLE diet groups. However, 2,and 3% PLE groups showed the highest performance index values 55.75 and 55.23 comparing with other treatments with non-significant difference with 1% PLE group and 2% PL group with values 52.66 and 52.55 respectively, but with significantly (P<0.0.01) difference with other groups. This result is confirmed by Nusrat *et al,* (2015) who reported that Addition of papaya leaves extract improved the weight gain of broilers. These results are agreement with the findings of Ahmad (2005), Wanker *et al.* (2009) , Onyimonyi, *et al.* (2009) and Sorwar *et al*,(2016) who reported that the body weight gains and feed efficiency increased in treated groups with PL compared to non-treated control group. This trend could be ascribed to the high nutritional value in terms of protein and minerals/vitamins supplied by PL particularly when increased. It could also be attributed to the papain in the PL which aid protein digestion thus enhancing the release of free amino acids necessary to enhance growth. This improvement may be attributed to proteolytic enzyme of exogenous origin plays an important role in feed digestibility.

The improvement of digestibility coefficient values may be attributed to the potential beneficial effect of these additives PL and PLE 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 enabling rabbits 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, 2015). Unigwe et al. (2014) reported that papain is an effective natural digestive aid which breaks down protein and cleanses the digestive tract.

Digestibility coefficients and nutritive value of the diets

Results in Table 3 indicated that apparent digestibility of DM and EE were not significantly affected by different treatments. On the other hand, digestibility of CP and CF were significantly (P<0.0.01) affected. The best upon feeding rabbits on 3% PLE supplemented diets which recorded (77.43 and 37.43) respectively compared to 3% PL supplemented diet which recorded (74.89 and 33. 23) respectively and control diet which recorded (74.9 and 30.67) respectively. Also, digestibility coefficients of NFE significantly (P < 0.001) increased with 2 and 3% PLE supplemented diet which recorded (77.48 and 78.28) respectively compared to 3% PL diets which recorded 75.75 and control diet which recorded 75.89. That, digestibility of NFE did not differ significantly with PL diets compared to control diet. Nutritive values in terms of DCP and TDN were significantly (P <0.01) improved compared to the control. That, DCP was significantly improved with PLE diets which recorded 13.31 in 3% diet compared to the control diet which recorded 12.94 and PL diets which recorded 12.87 in 3%diet. Also, there were significantly improved in DE (Kcal/kg diet) with PLE diets compared to the control group.

Carcass characteristics

Effect of different treatments on some carcass traits is showed in Table 4. Results indicated that carcass (weight and percentage), Liver%, Heart %, Kidneys %, Lungs% and dressing percentages were not significant affected with feeding on PLE and PL supplemented diets as compared to control diet. Also, there were insignificantly affected by different treatments in edible giblets (liver, heart and kidneys). Abdalla *et al.* (2012) and Navid, *et al*. (2011) concluded that dietary supplementation of 2% PL meal and papain in off-layer hens for a few days before slaughter improved meat quality in terms of meat tenderness and juiciness. Moreover Abdalla (2012) indicated that hens fed on supplemented diets with papaya leaves had significantly higher carcass and dressing percentage. Sorwar *et al,* (2016) also, agree with no significant effect papaya leaf in carcass traits in broiler.

Effect of different treatments on meat quality is showed in Table 5. Results indicated that Moisture, Ash % and EE % were not significantly affected by feeding rabbit on PLM and PL diets as compared to control diet.

Table 3. Digestibility coefficients of nutrients as affected by experimental treatments.

Items	Control Diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets			SEM	Sig.
		1%	2%	3%	1%	3%	2%		
DM	69.40	69.40	69.22	69.37	69.20	69.45	69.37	0.25	NS
OM	65.37^{d}	67.48^{b}	68.68^{a}	68.18^{a}	66.41^{c}	65.57^{d}	65.75^{d}	0.17	**
CP	74.9^{d}	75.80^{c}	76.60^{b}	77.43^{a}	75.45^{c}	75.41^{c}	74.89^{d}	0.16	**
CF	30.67^{d}	33.28^{c}	35.40^{b}	37.43^{a}	31.11^{d}	31.22^{d}	33.23^{c}	0.48	**
EE	73.65	73.63	73.67	73.45	73..80	73.66	73.33	0.55	NS
NFE	75.89^{cd}	76.43^{c}	77.48^{b}	78.28^{a}	76.31^{cd}	75.90^{cd}	75.75^{d}	0.19	**
DCP	12.94^{cd}	13.03^{c}	13.17^{b}	13.31^{a}	12.97^{cd}	12.96^{cd}	12.87^{d}	0.04	**
TDN	63.56^{d}	64.81^{c}	65.52^{b}	66.39^{a}	63.60^{d}	63.53^{d}	63.82^{d}	0.17	**
*DE kcal/kg	2815.86^{d}	2871.08^{c}	2902.54^{b}	2940.93^{a}	2817.63^{d}	2814.23^{d}	2827.37^{d}	7.68	**

a, b, c, Means are bearing different superscripts in the same row, differed significantly ($P < 0.05$)

** $P<0.01$ NS: Not significant. *DE = TDN X 44.3 (Schneider and Flatt, 1975).

Table 4. Some carcass traits of growing rabbits fed on experimental diets.

Items	Control Diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets			SEM	Sig.
		1%	2%	3%	1%	2%	3%		
Dressing, %	58.47	57.70	58.59	58.37	57.82	59.0	58.98	1.29	NS
Liver, %	5.32	5.42	5.20	4.82	5.02	5.01	5.62	0.45	NS
Heart, %	0.56	0.70	0.66	0.65	0.51	0.53	0.57	0.07	NS
Kidneys ,%	1.13	1.13	1.28	1.12	1.06	1.11	1.07	0.08	NS
Lungs,%	1.21	1.45	1.09	1.35	0.97	1.25	1.25	0.23	NS
Caecum,%	5.23	5.21	5.30	5.12	5.16	5.77	5.38	0.163	NS

Means on the same row with different superscripts a and b, are significantly different ($P<0.01$)
NS: Not significant.

Table 5 Meat quality (chemical composition) of growing rabbits fed on experimental diets.

Items	Control Diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets			SEM	Sig.
		1%	2%	3%	1%	2%	3%		
Moisture	73.09	72.99	72.73	72.21	72.41	72.05	72.05	0.35	NS
Ash%	1.41	1.22	1.28	1.22	1.43	1.59	1.60	0.13	NS
CP%	23.7^{c}	23.92^{bc}	24.41^{bc}	24.76^{a}	24.44^{abc}	24.59^{ab}	24.55^{ab}	0.22	**
EE%	1.73	1.87	1.58	1.81	1.72	1.78	1.80	0.17	NS

a, b, c, Means are bearing different superscripts in the same row , differed significantly ($P<0.05$)
** $P<0.01$, NS: Not significant.

However, carcass protein was significantly (P<0.0.01) affected, the highest value (24.76) was recorded for rabbit fed 3% PL diet followed by 24.59 which recorded for2% PLM diet comparing with rabbit fed control diet which recorded the lowest value (23.7). These results might be attributed to the effect of proteolytic enzyme found in the papaya leaves (papain enzyme) which may cause more degradation and availability of dietary protein. The increase in protein content of meat improves the nutritive value and quality of rabbit's meat. Also, this increase in meat protein in rabbits 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.

Blood plasma constituents:

Total protein, albumin and globulin were significantly increased by adding PLM or PL in diets as shown in Table 6. This increase may be due to the increase in protein synthesis, and digestion of protein. Increased globulin concentration with increased papaya inclusion which was observed in the present study may be an indication of increased immunity in rabbits since the liver will be able to synthesize enough globulins for immunologic action as mentioned by Sunmonu and Oloyede (2007). This result is agreement with El-Kholy *et al.* (2008), Zeedan *et al.* (2009) and El-Neney *et al.* (2013 & 2015). Lien and Wu (2012) found that papain supplementation markedly increased total serum globulin, total protein for laying hens.

All feed addition decreased cholesterol, LDL and total lipids compared to control diet. This result is agreement with Lien and Wu (2012) who indicated that papain supplementation reduced liver cholesterol. 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. Oloruntola and Ayodele (2017) reported that reduction of cholesterol level recorded may be as a result of activities of some secondary metabolites present in PL meal which exert reducing effect on cholesterol uptake in the intestine. Capability of saponin, one of the anti-nutrients present in PL in reducing absorption of cholesterol through intra-lumenal physiochemical interaction was reported by Yilkal (2015).

Low density lipoprotein, which is otherwise known as bad cholesterol collects in the blood vessels walls, narrows or blocks the blood vessel lumen and increased risk of atherosclerosis and heart disease. Therefore, reduced low density lipoprotein in rabbits due to dietary papaya leaves found in this study is

Table 6. Blood parameters of growing rabbits fed on experimental diets.

Items	Control Diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets			SEM	Sig.
		1%	2%	3%	1%	2%	3%		
Total protein (g/dl)	4.72 ^{c}	6.11 ^{ab}	6.72 ^{a}	6.83 ^{a}	5.45 ^{b}	5.71 ^{ab}	5.82 ^{ab}	0.13	**
Albumin(g/dl)	2.01 ^{c}	2.99 ^{a}	2.79 ^{a}	2.91 ^{a}	2.51 ^{b}	2.73 ^{a}	2.39 ^{b}	0.11	**
Globulin (g/dl)	2.71 ^{c}	3.12 ^{ab}	3.93 ^{a}	3.92 ^{a}	2.94 ^{b}	2.98 ^{b}	3.43 ^{a}	0.02	**
AST(u/l)	35.54	35.52	33.25	34.54	32.62	33.55	33.65	0.38	NS
ALT (u/l)	27.65	24.75	24.32	23.65	25.35	26.43	27.21	0.45	NS
CHOL (mmol/l)	89.65 ^{a}	67.45 ^{c}	68.25 ^{c}	66.52 ^{c}	78.36 ^{b}	77.65 ^{b}	76.25 ^{b}	1.32	**
HDL (mmol/l)	25.36 ^{b}	41.63 ^{a}	43.65 ^{a}	44.09 ^{a}	49.54 ^{a}	45.36 ^{a}	47.75 ^{a}	0.65	**
LDL (mmol/l)	64.29 ^{a}	25.82 ^{b}	24.60 ^{b}	22.43 ^{b}	28.82 ^{b}	32.29 ^{b}	28.50 ^{b}	0.23	**
T. lipids (mg/dl)	435.52 ^{a}	369.54 ^{b}	368.36 ^{b}	362.35 ^{b}	375.46 ^{b}	373.65 ^{b}	372.34 ^{b}	8.65	**
IgG (mg/ml)	3.15 ^{b}	6.52 ^{a}	6.83 ^{a}	6.82 ^{a}	5.85 ^{a}	6.45 ^{a}	6.12 ^{a}	0.06	**
IgM (mg/ml)	2.45 ^{b}	3.74 ^{a}	3.65 ^{a}	3.85 ^{a}	3.21 ^{a}	3.56 ^{a}	3.46 ^{a}	0.04	**

a, b, c, Means are bearing different superscripts in the same row , differed significantly ($P < 0.05$)
** $P<0.01$, NS: Not significant.

a health benefit and supports the wholesomeness of this leaf meal in rabbits' nutrition.

No significant differences ($P<0.05$) were observed in AST and ALT among treatments (Table 6). Liver function as AST and ALT activity were not affected by dietary treatments with supplementing papaya. 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 papaya leaves or its extract to 3% in rabbit diets 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 papaya to the diet of growing rabbits or laying hens decreased significantly ($P\leq0.05$) of AST and ALT

Data presented in Table 6 showed that Concentrations of IgG and IgM in plasma were high in PLM supplemented diets, while they low in control. These results are in agreement with those obtained by El-Neney *et al.* (2015) 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 Papaya leaves on the improvement of the immune response may be due to the increased availability amino acids, which are nutrients needed for an effective and vigorous immune response, and to the beneficial effects on the gastrointestinal microflora. 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 papaya leaves 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.

Higher glutathione peroxidase (GPx) ($P < 0.05$) , superoxide dismutase (SOD) and catalase (CAT) activities were recorded in rabbits fed diets supplemented with papaya (Table 7) these results are agreement with Oloruntola *et al*., 2018, who reported that GPx and SOD were significantly improved by dietary PL. The improved GPx and SOD activities in this study further supports the earlier claim that pawpaw is rich in antioxidant nutrients (Bolu, Sola-Ojo, Olorunsanya, and Idris, 2009) and that active ingredients of plants have strong antioxidant effects such as hydrogen peroxide, nitric oxide, and superoxide neutralization by increasing the production of SOD, GPx, and CAT (Ali, Marrif, Noureldayem, Bakheit, and Blunden, 2006; Dhama et al., 2015). In addition, since oxidative stress was identified as a cause for reduce growth rate in animals (Vara Prasad Reddy, Thangavel, Leela, and Narayana Raju, 2009); the increased antioxidant enzymes in rabbits fed diet contain PLE and PL in this study may also explain the enhanced performance recorded in the rabbits.

Caecum contents and Microbiological assay

Caecum content pH, total volatile fatty acids and ammonia concentration at the end of the study are presented in Table 8. Analysis of variance revealed that feeding growing rabbit on PLE diets which recorded 6.43 for 3% diet and PL diets which recorded 5.80 for 3% diet comparing with control diet which recorded 6.00 significantly ($P<0.01$) increased the value of PH. Total volatile fatty acid was increased and unfavorable ammonia was decreased significantly with feeding on PLE diets and which recorded 5.49 and 8.17 respectively for 3% diet and PL diets which recorded 4.26 and 8.42 respectively for 3% diet comparing with control diet which recorded 3.72 and 9.84 respectively these results may related to antimicrobial activity to papaya leaves and its extract. Microbial counts (x 10 81 CFU/ml) in caecum as affected by the experimental diets at the end of the study are presented in Table 9.

Results revealed that PLE diets and PL diets significantly ($P<0.01$) decreased the values of all studied microbes comparing with control diet except yeasts values were increased significantly ($P<0.01$) while Salmonella and Shigella were not detected in all diets. These results agree with Doughari *et al*., (2007), Emeruwa (1982) , Leite *et al*.,(2005) and Mahendra and Nikhil (2016) Who reported that seeds of Carica papaya were found to possess bacteriostatic activity against several enteropathogens such as bacillus subtilis, Enterobacter cloacae, Escherichia coli, salmonella typhi, staphylococcus, protease vulgaris, pseudomonas aeruginosa and klebsiella pneumonia. Among the grampositive and gram-negative bacteria tested the gram negative bacteria were more susceptible to the extract. The latex of papaya and fluconazole has synergistic action on the inhibition of Candida albicans growth (Giordiani *et al.,* 1997).

Table 7 Plasma antioxidant enzyme statuses of growing rabbits fed on experimental diets.

Items	Control Diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets			SEM	Sig.
		1%	2%	3%	1%	2%	3%		
GPx (µg/g)	49.51 ^{c}	71.24 ^{a}	73.62 ^{a}	75.43 ^{a}	63.33 ^{b}	67.54 ^{ab}	68.45 ^{ab}	0.21	*
SOD (%)	51.21 ^{c}	74.36 ^{a}	78.54 ^{a}	79.24 ^{a}	65.51 ^{b}	66.52 ^{b}	69.35 ^{b}	0.52	**
CAT (mM/ml/min)	5.38 ^{c}	10.62 ^{a}	11.81 ^{a}	12.32 ^{a}	8.5 ^{b}	8.72 ^{b}	9.54 ^{b}	0.06	*

a, b, c, Means are bearing different superscripts in the same row , differed significantly (P <0.05)
* P ≤ 0.05, ** *P*<0.01,

Table 8. Caecum contents of growing rabbits fed on experimental diets.

Items	Control Diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets			SEM	Sig.
		1%	2%	3%	1%	2%	3%		
Caecum pH	6.00^{b}	6.10^{ab}	6.07^{ab}	6.43^{ab}	6.77^{a}	6.13^{ab}	5.80^{b}	0.22	**
***TVFA (mg/100ml)**	3.72^{e}	5.11^{b}	5.25^{ab}	5.49^{a}	4.56^{c}	4.23^{d}	4.26^{cd}	0.10	**
Ammonia (mg/100ml)	9.84^{a}	8.48^{b}	8.49^{b}	8.17^{d}	8.56^{b}	8.26^{cd}	8.42^{bc}	0.06	**

a, b, c, d Means are bearing different superscripts in the same row , differed significantly (P <0.05)
** *P*<0.01, NS: Not significant. TVFA: Total Volatile Fatty Acids.

Table 9. Microbial Cecum (x 10^{8} 1 CFU/ml) as affected by the experimental diets.

Cecum microbes (CFU/ml)l	Control Diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets			SEM	Sig.
		1%	2%	3%	1%	2%	3%		
Aerobic total count	7.40^{a}	5.59^{b}	5.15^{b}	5.30^{b}	5.44^{b}	5.57^{b}	5.22^{b}	0.15	**
Fecal coliforms	6.19^{a}	4.27^{cd}	4.21^{d}	4.23^{d}	4.24^{d}	4.55^{bc}	4.71^{b}	0.10	**
E. coli	5.26^{a}	3.05^{b}	2.99^{b}	2.93^{b}	3.11^{b}	3.13^{b}	3.03^{b}	0.08	**
Bacillus cereus	4.32^{a}	3.66^{b}	3.38^{b}	3.29^{b}	3.47^{b}	3.62^{b}	3.52^{b}	0.18	**
Enterobacter	5.76^{a}	3.26^{c}	3.78^{b}	3.37^{bc}	3.30^{bc}	3.68^{bc}	3.55^{bc}	0.14	**
Clostridium sp	2.26^{a}	1.48^{bc}	1.21^{c}	1.24^{c}	1.49^{bc}	1.47^{bc}	1.76^{b}	0.12	**
Enterococcus	3.18^{a}	2.67^{b}	2.48^{bc}	2.26^{c}	2.47^{bc}	2.47^{bc}	2.51^{bc}	0.12	**
Yeasts	5.19^{b}	6.21^{a}	6.25^{a}	6.39^{a}	6.34^{a}	6.31^{a}	6.33^{a}	0.07	**
Salmonella & Shigella	ND	ND	ND	ND	ND	ND	ND	=	=

Each value is an average of 3 observations. LSD between treatments d.f (0.05).
ND =Not detected, Number of bacterial cells per gram of cecum content (log10-1 CFU/ml)
[1]CFU (Colony forming unite).

This synergistic effect results in partial cell wall degradation due to lack of polysaccharides constituents in the outermost layers of fungal cell wall and release of cell debris into the cell culture. Latex proteins appear to be responsible for antifungal action and minimum protein concentration for producing a complete inhibition was reported as about 138 mg/dl (Giordiani *et al.*, 1991).

Economic efficiency

Data presented in Table 10. showed that rabbits fed on PLE supplemented diets achieved the highest economic efficiency with values 1.92, 1.84 and 1.86 respectively followed by that fed on PL diets with values 1.86, 1.73 and 1.70 respectively, for relative economic efficiency the highest was for 1,3% PLE diets with values 115 and 111.38 followed by 1% PL diet with value 111.38 followed by 2% PLE diet with value110.18 followed 1,3% PL diets with values 103.59 and 101.02, and the least values for economic efficiency and relative economic efficiency were shown with group fed on control diet with values (1.67, 100) respectively. These results were agree with Sorwar *et al*.,(2016) and Rahman *et al*., (2014) who indicated that supplementation with PL was more profitable with a higher net profit/kg than control group in broiler.

Liver histological examinations:

Figures 1, 2, 3, 4, 5, 6 and 7 show the histological sections of the liver. The histological hepatic cells appearances in rabbits fed control diet showing normal histological structure of hepatic tissues composed of hepatocytes disposed of in the sheet which are separated by sinusoids free of collections and inflammatory cells, moderate congestion of the portal vessels of polymorph nuclear cells (Fig. 1). No Histological change observed in the liver of rabbits treated with 1, 2,and 3 % PL supplementation and 1, 2,and 3 % as PLE, Fig 2, 3, 4, 5,6,7 compared with control group Fig1 normal hepatocytes (H) and central vein (c).

Liver from 1%PLE group showing normal histology; normal hepatocytes (H) and central vein (C), Fig 2. Liver from 2% PLE group showing normal hepatocytes with prominent nucleus (H) and normal central vein (C), Fig 3. Liver from 3% PLE group showing normal hepatocytes with prominent nucleus (H), dilated blood sinusoids (arrows), and normal central vein (C), Fig 4. Liver from 1% PL group showing normal hepatocytes with distinct nucleus and prominent nucleolus (arrows), dilated blood sinusoids, and normal central vein (C), Fig 5. Liver from 2% PL group showing normal histology; normal hepatocytes (H) and central vein Fig 6. Liver from 3% PL group showing normal hepatocytes with distinct nucleus and prominent nucleolus (arrows), dilated blood sinusoids, and normal central vein (C), Figure7.

Table 10. Economic efficiency of using the experimental diets.

Items	Control diet	Level of Papaya Leaf extract in diets			Level of Papaya Leaf in diets		
		1%	2%	3%	1%	2%	3%
Total weight gain (kg)	1.070	1.260	1.310	1.228	1.096	1.134	1.070
Price of 1kg body weight	45	45	45	45	45	45	45
Selling price/rabbit (LE) (A)	48.15	56.70	58.95	55.26	49.32	51.03	48.15
Total feed intake	4.33	4.63	4.93	4.58	4.12	4.46	4.26
Price/kg diets (LE)	4.17	4.20	4.21	4.22	4.18	4.19	4.19
Total feed cost/rabbit (LE)(B)	18.06	19.45	20.76	19.33	17.22	18.69	17.85
Net revenue(LE)[1]	30.09	37.25	38.19	35.93	32.10	32.34	30.3
Economic efficiency[2]	1.67	1.92	1.84	1.86	1.86	1.73	1.70
Relative Econ. Eff.[3]	100	115	110.18	111.38	111.38	103.59	101.02

(1) Net revenue = A – B.
(2) Economic efficiency = (A-B/B x 100).
(3) Relative Economic Efficiency= Economic efficiency of treatments other than the control/ Economic efficiency of the control group.

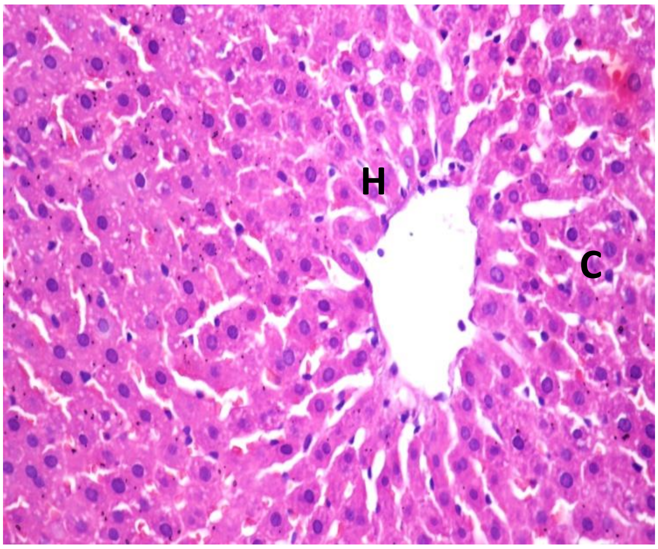

Fig. 1. .Liver from control group showing normal histology; note the normal hepatocytes (H) and central vein (c), (HE X400).

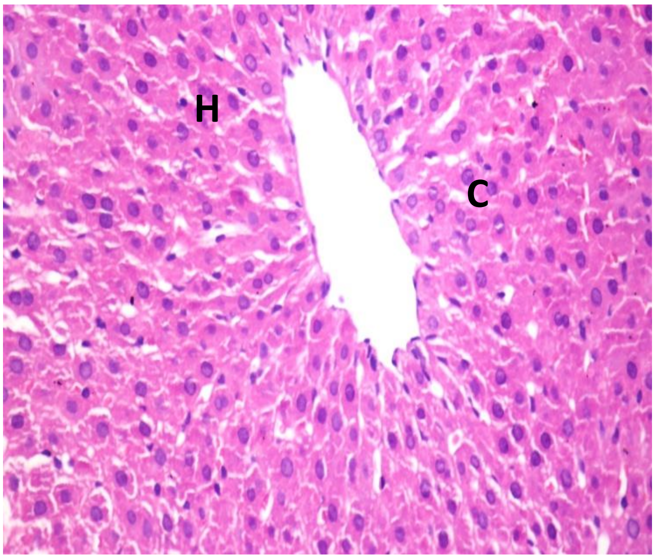

Fig. 2. Liver from 1%PLE group showing normal histology; note the normal hepatocytes (H) and central vein (C), (HE X400).

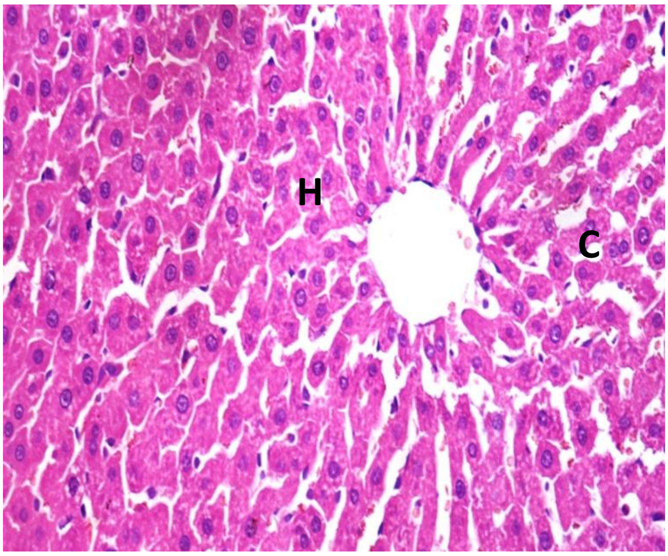

Fig. 3. Liver from 2% PLE group showing normal hepatocytes with prominent nucleus (H) and normal central vein (C), (HE X400).

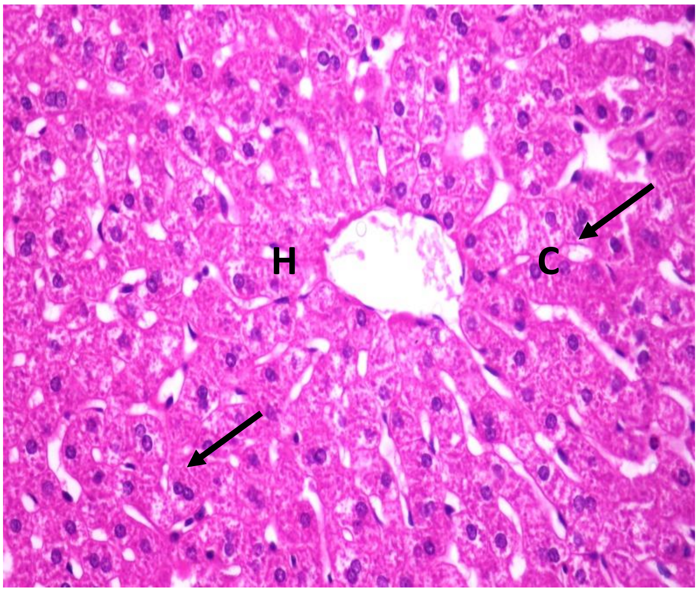

Fig. 4. Liver from 3% PLE group showing normal hepatocytes with prominent nucleus (H), dilated blood sinusoids (arrows), and normal central vein (C), (HE X400).

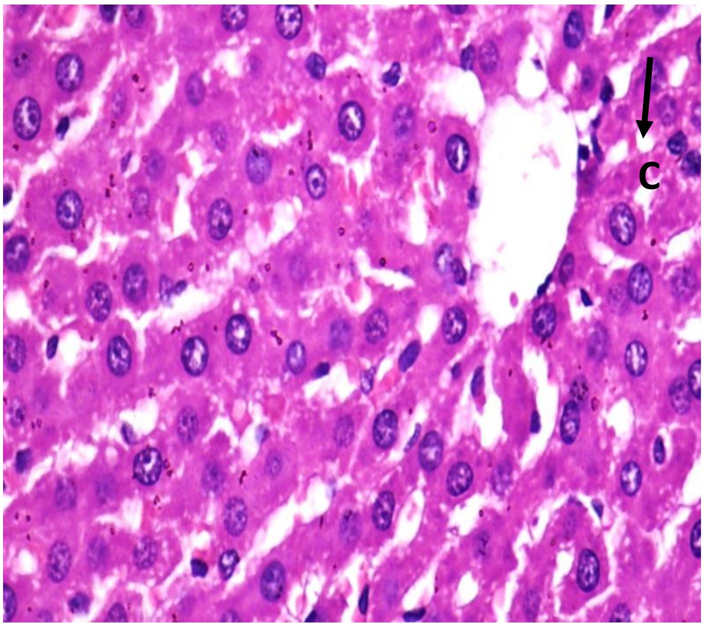

Fig. 5. Liver from 1% PL group showing normal hepatocytes with distinct nucleus and prominent nucleolus (arrows), dilated blood sinusoids, and normal central vein (C), (HE X400).

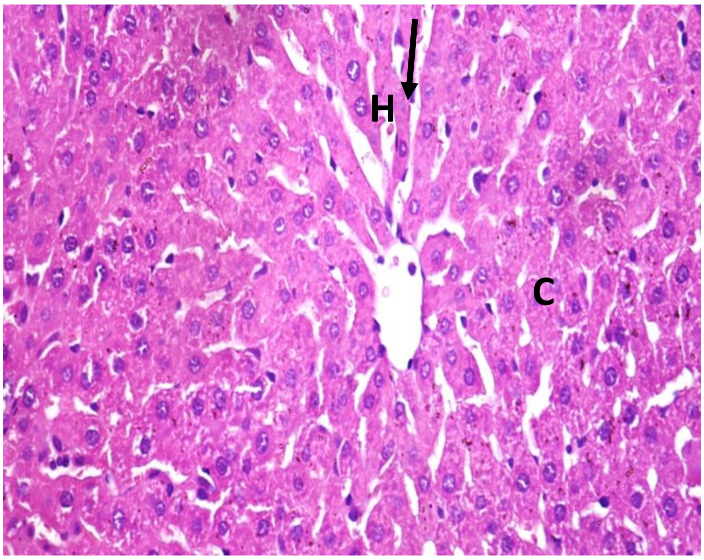

Fig. 6. Liver from 2% PL group showing normal histology; note the normal hepatocytes (H) and central vein (C), (HE X400).

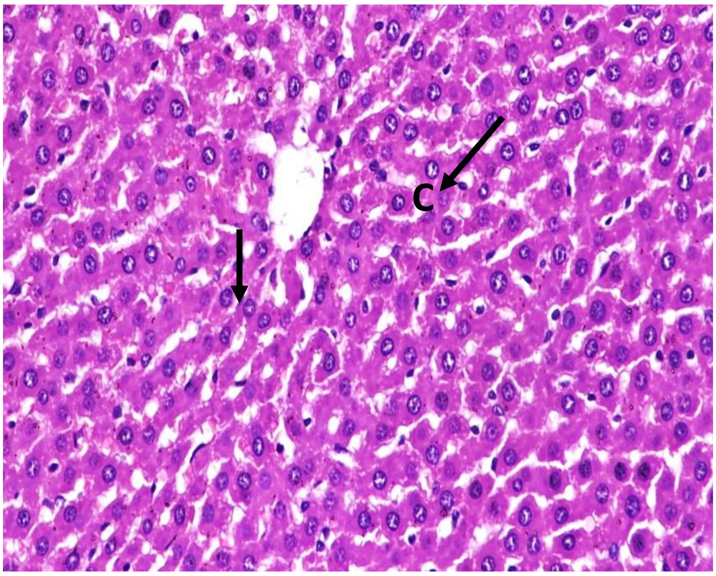

Fig. 7. Liver from 3%PL group showing normal hepatocytes with distinct nucleus and prominent nucleolus (arrows), dilated blood sinusoids, and normal central vein (C), (HE X400).

Conclusively, supplementation growing rabbit diet by 3% PLE improved the performance, crude protein, digestibility, immunity and the antioxidant enzyme status.

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تاثير اضافة اوراق الباباظ و مستخلص اوراق الباباظ على الاداء الانتاجى والصفات الفسيولوجية و الاستجابة المناعية و نشاط الانزيمات المضادة للاكسدة فى الارانب النامية

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2 قسم تربية الدواجن – معهد بحوث الانتاج الحيوانى – مركز البحوث الزراعية – الدقى - جيزة- مصر.

3 قسم انتاج الدواجن – كلية الزراعة – جامعة عين شمس - مصر.

أجريت هذه الدراسة لبحث تأثيرإستخدام مستويات مختلفة من اوراق الباباظ و مستخلص الاوراق على الأداء الإنتاجي وصفات الذبيحة ومكونات الدم ونشاط مضادات الأكسدة و معامل الهضم و الاستجابة المناعية للارانب النامية. تم استخدام عدد اربعة و ثمانون ارنب نيوزيلاندى عمر35 يوم وزن حوالى 564 جرام . تم تقسيمهم عشوائى إلى سبع مجموعات 12 فى كل مجموعة.تم أستخدام سبع علائق تجريبية كالتالي : المجموعة الأولى غذيت على عليقة كنترول بدون اى اضافات ، المجموعة الثانية و الثالثة و الرابعة غذيت على اوراق الباباظ بنسبة 1-2-3 % على التوالى و المجموعة الخامسة و السادسة و السابعة غذيت على مستخلص اوراق الباباظ فى صورة بودر بنسبة <u>1-2-3 %</u> على التوالى استمرت التجربة ثمانية اسابيع. **أظهرت النتائج مايلى :**

- سجلت المجموعات المضاف اليها الباباظ زيادة فى وزن الجسم النهائي ، معدلات النمو و تحسن فى كفاءة التحويل الغذائي عن الكنترول.
- تحسنت معنويا قيم معاملات هضم المواد الغذائيه للعلائق المضاف اليهاالباباظ بالمقارنة بالكنترول.
- اضافة الباباظ ادى الى انخفاض كل من الكوليسترول – الليبدات الكلية LDL- فى البلازما بالمقارنة بالكنترول.
- اضافة الباباظ ادى الى زيادة معنوية للبروتين و الالبيومين و الجلوبيولين فى البلازما
- لم تتاثر معنويا وظيفة الكبد من خلال نشاط انزيمات AST – ALT باضافة الباباظ .
- ادت اضافة الباباظ الى تحسين الاستجابة المناعية و زيادة كلا من IgM - IgG، وكذلك زيادة فى نشاط الانزيمات المضادة للاكسدة GPx , SOD و CAT
- سجلت افضل كفاءة اقتصادية للارانب المغذاة على 3% من مستخلص الباباظ يليها 2% و1% من المستخلص على التوالى.

التوصية: عموما ،يمكن القول أن اضافة مستخلص اوراق الباباظ الى علائق الارانب النامية ادى الى تحسين وزن الجسم وكفاءة التحويل الغذائى و معاملات هضم الغذاء والمناعة وتحسين الكفاءة الاقتصادية .