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Study on the Optimal Crude Papaya Latex Content of Growing Rabbit Diet under Summer Conditions: Effects on Growth Performance and Immune Status

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Abstract: The study aimed to evaluate the effect of latex of *Carica papaya* as feed additive and enzymes complex on performance growing rabbits. A control diet was formulated with an estimated proportion of 18% crude protein and 14% crude fibre. Another three diets were formulated supplementing control diet with 0.1, 0.5 or 0.7% papaya latex. One hundred weaned New Zealand White (NZW) rabbits aged 35 days with an average initial weight of 570±8.30 g (mean±std. error) were used in the present work (25/diet). Daily weight gain and daily feed intake were recorded from weaning up to 91 d of age (fattening period). At this time seven rabbits from each group were slaughtered and immediately lymphoid organs were taken and weighted. Also, blood samples were collected of seven rabbits from each group. Besides, at the end of the experimental period cell mediated immunity was recorded. In the whole fattening period, final body weight at 91 d and feed efficiency values of growing NZW increased linearly by 296.3±49.5 g (P = 0.001) and 0.052±0.007 g/g (P = 0.001), respectively per each increment of 1 unit of papaya latex inclusion. Daily weight gain during the whole fattening period from 35-91 d increased linearly and quadratically (P < 0.031) as dietary concentrations of papaya latex increased obtaining the highest values by using up to 0.16 papaya latex. Spleen and thymus indexes increased linearly by 0.023±0.006 (P = 0.002) and 0.161±0.025 (P = 0.001), respectively per each increment of 1 unit of papaya latex inclusion. The values of WBCs; lymphocytes and total protein increased linearly by 0.95±0.324 (P = 0.007); 8.83±2.03 (P = 0.002) and 2.11±0.338 (P = 0.001), respectively per each increment of 1 unit of papaya latex inclusion. Cell mediated immunity increased linearly and quadratically (P < 0.002) as papaya latex inclusion, being optimized for 0.54% papaya latex inclusion. In conclusion, the addition of 0.7% latex of *Carica papaya* to the growing NZW rabbit diets, improved growth and immunity capabilities during summer heat stress.

Key words: Growing rabbits, *Carica papaya* latex, growth performance, immunity

Introduction

Exposing the growing rabbits to high ambient temperature (above 30°C) impairs the growth performance and immune system activity (El-Kholy, 2003; Ashour *et al.*, 2004). Numerous attempts have been made to improve the productive performance in rabbits by using growth promoters (Ashour *et al.*, 2004; El-Kholy *et al.*, 2008a) or enzyme supplementation (Sarhan, 2001; El-Mahdy *et al.*, 2002). Some consequences of heat stress affect digestive system functions, with impaired appetite, growth and feed conversion, but also with increased disease incidence (McNitt *et al.*, 2000; Ashour *et al.*, 2004; Bani *et al.*, 2005). The exogenous enzyme in feed additive can complement to endogenous enzymes in the digestive system during heat stress. The use of exogenous enzymes in animal nutrition has increased in the last years as alternative natural products that might improve animal productivities and reduce the morbidity and the mortality in intensive farms (Cachaldora *et al.*, 2004). The main objective of the enzyme supplementation is to complement the endogenous enzymatic capability of the animal, especially under heat stress conditions and to

increase the nutritive value of the feed. Papaya (*Carica papaya*) yields a milky sap, often called a latex, which is a complex mixture of chemicals. Chief among them is papain, a well-known proteolytic enzyme (Oliver-Bever, 1986). An alkaloid, carpaine, is also present, which has shown antibacterial activities (Martinian *et al.*, 1990; Starley *et al.*, 1999). Terpenoids are also present and may contribute to its antimicrobial properties (Thomson, 1978). Osato *et al.* (1993) found the latex to be bacteriostatic to *B. subtilis*, *Enterobacter cloacae*, *E. coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Proteus vulgaris*. The application of papaya latex that is probably of most interest to livestock producers is as an antimicrobial and antifungal (Suhaila *et al.*, 1994; Pandey *et al.*, 1996). The latex of *Carica papaya* is a rich source of the cysteine endopeptidases (digestive enzymes), including papain, glycy endopeptidase, chymopapain and caricain, which constitute more than 80% of the whole enzyme fraction (El Moussaoui *et al.*, 2001; Azarkan *et al.*, 2003). Other enzymes known from papaya latex include glycosyl hydrolases such as β -1,3-glucanases, chitinases and lysozymes, chitinase, protease inhibitors such as cystatin and glutaminyl

cyclotransferases and lipases (El Moussaoui *et al.*, 2001; Azarkan *et al.*, 2003). Two chitinolytic enzymes, chitotriosidase and chitosanase, were purified sequentially from a commercial dried papaya latex (Bei-Yu *et al.*, 2006). Papaya latex itself has been reported to exhibit antifungal activity against *Candida albicans* "cause immune deficiency" (Giordani *et al.*, 1996). Recently research found that the fresh papaya latex contained 40 ± 13 mg protein and 529 ± 162 units protease activity/g wet latex (Nitsawang *et al.*, 2006). The aim of this study was to investigate the efficacy of dietary inclusion of crude papaya latex on some productive and immunological parameters of New Zealand White rabbits under summer conditions. The study also aimed to establish the optimum level of papaya latex in rabbit diets.

Materials and Methods

Diets and animals: One hundred weaned New Zealand White rabbits aged 35 days and weighed $570 \text{ g} \pm 8.30$ (mean \pm std. error) were equally and randomly divided into four groups (25 in each one). The first group was fed *ad libitum* a commercial pelleted diet according to NRC (1977) recommendations and kept untreated and served as a control, while the other groups (second; third and fourth) were fed the same diet, but supplemented with 0.1, 0.5 and 0.7% dried crude papaya latex powder, respectively. Latex from *Carica papaya* was obtained by cutting the skin of the unripe fruit but almost mature papaya by steel razor blade and then collected into a test tube. The latex is simply spread on trays and left in the sun to dry. Then it was ground to powder in a ball mill. All the experimental animals were healthy and clinically free from internal and external parasites and were kept under the same management and hygienic conditions.

Experimental procedure: Daily weight gain and daily feed intake were recorded weekly for each rabbit during fattening period (from 5 wks up to marketing age: 13 wks of age).

At marketing age (91 d), seven growing rabbits from each experimental group were randomly chosen and slaughtered (by bleeding). Spleen and thymus weight was weighed after the rabbits were slaughtered and spleen index and thymus index were calculated according to the following formulae of Fu-Chang *et al.* (2004):

spleen index = spleen weight/body weight, thymus index = thymus weight/body weight.

Blood samples were taken at the time of slaughter from seven rabbits from each group. Plasma was separated by centrifugation at 3000 r.p.m. for 20 minutes and kept -20°C until blood analysis. Non-coagulated blood was tested shortly after collection for estimating blood pictures. Red blood cells (RBCs), white blood cells (WBC's) and different subclasses of WBC's

(lymphocytes, neutrophils, monocytes, eosinophils and basophils percentages) were counted according to Feldman *et al.* (2000). Total protein level was estimated according to Armstrong and Corri (1960). Albumin level was estimated according to Dumas *et al.* (1971). Globulin level values were obtained by subtracting the values of albumin from the corresponding values of total protein. All samples were run in duplicate and assayed by the same investigator, who was blind to the experimental situation.

At the end of the experiment (91 d), seven representative rabbits from each group were randomly selected to determine cell mediated immunity (CMI). Fifty μg of PHA (phytohemagglutinin-P; Difco, Detroit, MI) in 0.1 ml of sterile pyrogen-free physiologic saline or saline only was injected intradermally (ID) into the right and left ear, respectively. Ear thickness of each rabbit was measured with a constant-tension dial micrometer (Mitutoyo Co., Tokyo, Japan) just before the injection and again every 3 hrs later (from 0-24 hrs). The response was recorded in millimeters as the difference between PHA response (right ear) and the saline response (left ear) after injection (Heba El-Lethey *et al.*, 2003).

Housing: The present work was carried out in a rabbit farm, Egypt, from July till August, 2007. Rabbits were housed separately in individual cages ($35 \times 35 \times 60$ cm) of conventional universal galvanized wire batteries. All cages were equipped with feeding hoppers, which made of galvanized steel sheets and nipples for automatic drinking. The batteries were located in a well ventilated building. Averages of ambient temperature, relative humidity and temperature humidity index inside building were $32.1 \pm 0.9^\circ\text{C}$, $63.7 \pm 1.5\%$ and 30.1, respectively, which indicate severe heat stress. The temperature-humidity index (THI) was estimated according to the following formula of Marai *et al.* (2001):

$$\text{THI} = \text{db}^\circ\text{C} - [(0.31 - 0.31 \times \text{RH}) \times (\text{db}^\circ\text{C} - 14.4)]$$

where, db°C is the dry bulb temperature in centigrade and RH is the relative humidity. A cycle of 16 h of light and 8 h of dark was used throughout the experiment.

Statistical analysis: Data were subjected to analysis of variance by using the General Linear Procedure Program of SAS (SAS Inst. Inc., Cary, NC). Polynomial contrasts were used to detect linear and quadratic relationships for the effect of papaya latex dose on all parameters (PROC GLM). Initial weight was included as a covariate in statistical model for growth traits (PROC GLM). Repeated measures were used to analyze cell mediated immunity data over time (PROC MIXED) by using the compound symmetry (CS) model. Data presented as percentages were transformed to the corresponding arcsine values (Warren and Gregory,

2005) before being statistically analyzed. All data are presented least squares means. For all data analyses, each animal was considered as an experimental unit.

Results and Discussion

Growth performance: The effect of treatments on performance in the four weeks after weaning (35-63 days) and in the whole fattening period (35-91 days) is shown in Table 2. The values of final body weight at 63 d; daily weight gain and feed efficiency in the first four weeks after weaning increased linearly by 174.5±27.9 g (P = 0.001); 5.80±0.823 g (P = 0.001); and 0.088±0.011 g/g (P = 0.001), respectively per each increment of 1 unit of papaya latex inclusion. While daily feed intake values decreased linearly (P = 0.013) as proportion of papaya latex in diets increased. These results agree with the findings of Sarhan (2001), who found that daily feed intake of rabbits was significantly lower in the enzyme supplemented groups than control. In the whole fattening period, final body weight at 91 d and feed efficiency values of growing NZW increased linearly by 296.3±49.5 g (P = 0.001) and 0.052±0.007 g/g (P = 0.001), respectively per each increment of 1 unit of papaya latex inclusion. Daily weight gain during the whole fattening period from 35-91 d increased linearly and quadratically (P < 0.031) as dietary concentrations of papaya latex increased obtaining the highest values by using up to 0.16 papaya latex. There is a trend to increase quadratically daily feed intake values of growing NZW (P = 0.078) with increasing percentages of papaya latex in the diet. These results agree with the findings of Ibrahim *et al.* (2000) and Sarhan (2001), who found that feeding growing rabbit's diet supplemented with 500 mg Kemzyme[®] or Optizyme/kg[®] feed significantly improved live body weight at 9 and 13 weeks of age and daily weight gain from 5-9 and from 9-13 weeks of age. The improvement in body weight and daily weight gain may be due to the enhancing effect of Optizyme[®] on microflora growth in gut and cecum as well as the increase in the volatile fatty acids production and organic matter digestibility (Makled *et al.*, 2005). On the other hand, these results are different than what has been observed by García-Ruiz *et al.* (2006), who showed that enzyme supplementation (protease + xylanase) did not affect daily gain, daily feed intake and feed conversion during fattening period of rabbits. The trend of final live weight result could be a reflection of feed efficiency which was better for the rabbits on treated groups than those on control groups. Accordingly, increases in growth performance parameters for treated groups might be due to the better absorption of amino acids because of cysteine proteinases constitute as much as 80% of the enzyme fraction in papaya latex (El Moussaoui *et al.*, 2001; Azarkan *et al.*, 2003) or/and due to antibacterial properties of papaya latex.

Table 1: Ingredient and chemical composition of experimental diets

Items	% as fed
Ingredients	
Clover hay	40.5
Wheat bran	25.0
Yellow corn	14.0
Soybean meal (44%)	11.0
Molasses	3.00
Vinasse	3.00
Bone meal	1.75
Calcium carbonate	0.70
Sodium chloride	0.55
Vitamins and Mineral Premix ¹	0.35
DL-Methionine	0.15
Calculated chemical composition²	
Ash	7.8
Crude protein	18.0
Ether extract	3.00
Crude fiber	14.0
Digestible energy, kcal/kg ³	2720

¹Vitamins and minerals premix per kilogram diet contains: Vit. A = 6000.0 IU; Vit. D = 900.0 IU; Vit. E = 40.0 mg; Vit. K₃ = 2.0 mg; Vit. B₁ = 2.0 mg; Vit. B₂ = 4.0 mg; Vit. B₆ = 2.0 mg; Vit. B₁₂ = 10.0 µg; Nicotinic acid = 50.0 mg; Biotin = 50.0 µg; Folic acid = 10.0 mg; Choline chloride = 250.0 mg; Zinc = 50.0 mg; Manganese = 85.0 mg; Iron = 50.0 mg; Copper = 5.0 mg; Iodine = 0.2 mg; Selenium = 0.1 mg; Cobalt = 0.1 mg.

²According to NRC (1977) for rabbits. ³Digestible energy (kcal/kg DM) = 4253 - 32.6 CF (% DM) - 114.4 Ash (% DM). According to Fekete and Gippert (1986).

Effect of papaya latex on lymphatic organs system:

Results in Table 3 showed that spleen and thymus indexes increased linearly by 0.023±0.006 (P = 0.002) and 0.161±0.025 (P = 0.001), respectively per each increment of 1 unit of papaya latex inclusion. The increased in spleen weight observed in our trial is similar to that seen in previous studies, with enzymes fed to rabbits (Abdel-Malak *et al.*, 1994; El-Mahdy *et al.*, 2002). 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). Thymus is main immunity organ. It also is the main place of differencing, maturing T lymphocyte and excreting thymus hormone (Fu-Chang *et al.*, 2004). The size of thymus and spleen decreased in heat stressed broiler chicks (Anwar *et al.*, 2004). The bigger the immunity index is, the stronger the immunity capability is (Fu-Chang *et al.*, 2004). They added that when nutrient intake increase, organ is becoming mature and function is becoming perfect, and it is consistent with the growth rule of animal.

Immune function: The values of WBCs; lymphocytes; total protein; albumin and globulin increased linearly by 0.95±0.324 (P = 0.007); 8.83±2.03 (P = 0.002); 2.11±0.338 (P = 0.001); 1.22±0.229 (P = 0.001) and 0.886 ± 0.155 (P = 0.001), respectively per each

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Table 2: Effect of latex of papaya (*Carica papaya*) inclusion on growth performance of growing NZW rabbits at different periods during fattening period

Items	% papaya latex				P-value	
	0 (control)	0.1	0.5	0.7	Linear	Quadratic
Initial body weight at 35 d, g	656±8.78	676±8.78	667±8.78	678±8.78	0.27	0.88
Period 35-63 days						
Daily weight gain, g	23.9±0.43	26.6±0.41	26.0±0.40	29.5±0.40	0.001	0.095
Final body weight at 63 d, g	1333±11.9	1413±11.6	1399±11.1	1495±11.3	0.001	0.23
Daily feed intake, g	85.7±1.16	85.4±1.12	83.3±1.07	82.2±1.10	0.013	0.90
Feed efficiency, g/g	0.276±0.006	0.312±0.005	0.313±0.005	0.360±0.005	0.001	0.079
Period 35-91 days						
Daily weight gain, g	23.0±0.45	25.3±0.42	24.7±0.41	28.1±0.41	0.001	0.031
Final body weight at 91 d, g	1964±25.4	2092±23.7	2056±23.1	2246±23.1	0.001	0.056
Daily feed intake, g	98.6±1.31	99.1±1.22	96.0±1.19	99.4±1.20	0.67	0.078
Feed efficiency, g/g	0.234±0.004	0.256±0.004	0.257±0.003	0.283±0.003	0.001	0.24

¹n = 25 animals per treatment

Table 3: Effect of papaya latex inclusion on immunity index of growing NZW rabbits at 91 d of age

Items	% papaya latex					P-value	
	0 (control)	0.1	0.5	0.7	SEM ¹	Linear	Quadratic
Live body weight, g (BW)	1940	2105	2074	2238	42.6	0.002	0.67
Spleen index (weight % BW)	0.040	0.041	0.054	0.058	0.004	0.002	0.84
Thymus index (weight % BW)	0.101	0.129	0.221	0.240	0.012	0.001	0.18

¹n = 7 per treatment.

Table 4: Effect of papaya latex inclusion on white blood cells and some blood serum constitute of growing NZW rabbits at 91 d of age

Items	% papaya latex				SEM ¹	P-value	
	0 (control)	0.1	0.5	0.7		Linear	Quadratic
White blood cells (N×10 ³ /mm ³)	5.21	6.06	5.54	6.40	0.12	0.007	0.36
Lymphocytes (%)	54.9	59.3	57.8	63.9	0.88	0.002	0.18
Monocytes (%)	37.3	32.9	34.5	29.8	0.76	0.005	0.38
Neutrophils (%)	3.77	3.37	3.44	2.67	0.16	0.007	0.16
Eosinophils (%)	2.04	2.27	2.16	1.93	0.066	0.064	0.006
Basophils (%)	1.93	2.03	2.04	1.73	0.068	0.054	0.004
TP, g/dl	5.58	5.57	6.45	7.00	0.20	0.001	0.42
Alb, g/dl	2.90	3.00	3.37	3.81	0.13	0.001	0.32
Glb, g/dl	2.68	2.57	3.08	3.20	0.088	0.001	0.76

¹n = 7 per treatment.

increment of 1 unit of papaya latex inclusion. While, monocytes and neutrophils decreased linearly by -7.16±1.77 (P = 0.001) and -1.16±0.306 (P = 0.007), respectively per each increment of 1 unit of papaya latex inclusion. Basophils percentage of growing NZW rabbits increased (P < 0.054) linear and quadratically with papaya latex inclusion being maximized with 0.3% papaya latex. Similar results were obtained by El-Kholy *et al.* (2008b) using Arak inclusion as antibacterial and antifungal. These results might be related to the maximum spleen and thymus indexes obtained with papaya latex inclusion. However, Stephen, (2007) demonstrated that lymphocytes cells are made in the bone marrow and the lymphatic tissues (lymph nodes, thymus, spleen and gut-associated lymphatic tissue. The lymphocyte considered the main type of white blood corpuscles and a good indicator of increasing the immunity efficiency (Wieslaw *et al.*, 2006). This result is in harmony with finding of Wu and Tsai (2006) who showed that mice fed chitosan (isolated from papaya latex), at the dose of 2.5 g/kg body weight, increased phagocytic activity of macrophages,

lymphoproliferation, serum immunoglobulin amounts and cytokine production by splenocytes. The increase in WBCs in treated groups can be attributed mainly to the antibacterial functions of papaya latex. The increase of total protein in blood rabbits fed papaya latex may be associated with improvement of crude protein digestibility. On the other hand, these results are different than what has been observed by El-Mahdy *et al.* (2002), who showed that total protein, albumin and globulin improved not significant with enzyme supplementation in growing rabbits. Increased globulin concentration with papaya latex inclusion in the heat stressed rabbit's diet as observed in the present study may be an indication of increased immunity in the rabbits since the liver will be able to synthesize enough globulins for immunologic action as mentioned by Sunmonu and Oloyede (2007).

Cell-mediated immunity (cellular immunity): Figure 1 indicates linear and quadratic effects (P < 0.002) of cellular immunity as papaya latex inclusion, being optimized for 0.54% papaya latex inclusion. This result

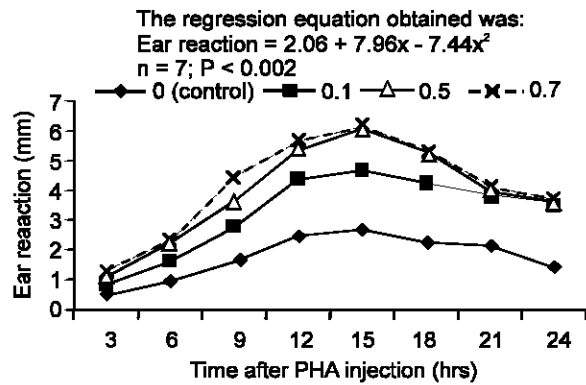


Fig. 1: Change of ear thickness response (mm) to phytohemagglutinin (PHA) at different times (hrs) post injection of NZW growing rabbits as affected by dietary papaya latex level

may be due to the effect of papaya latex improving lymphocytes percentage and thymus index which lead to produced more T-cells. T-cells undergo maturation in the thymus gland and play a major role in cell-mediated immunity (Stephen, 2007). There were good indications that cell-mediated immunity plays an important role in controlling and cleaning intracellular bacterium (Kougt *et al.* 1995). Many studies have demonstrated the antibacterial and anti-fungal properties of papaya latex (Suhaila *et al.*, 1994; Pandey *et al.*, 1996; Giordani *et al.*, 1996). Presence of these properties in papaya latex may explain the increase (enhancement) of immune system activity. This result is in a good agreement with results found by El-Kholy *et al.* (2008b) by using Arak which has antibacterial and anti-fungal properties. Papaya latex fed (at a rate of 2, 4, 6, or 8g/kg BW) to mice with experimental infections of *Heligmosomoides polygyrus* decreased infections rates by 55.5-84.5% compared to non-treated control mice (Satrija *et al.*, 1995). The changes in the cell mediated immunity and lymphocyte cells percentage are due to papaya latex inclusion, provided evidence of improved growing rabbit's adaptation to high environmental temperatures. Thus, protective immune responses require a supply of nutrients at the appropriate times and amounts (Humphrey *et al.*, 2002).

We concluded that the inclusion of 0.7 % of papaya latex in rabbit diets throughout fattening period improved their productive performance and immunity status during summer heat stress. Actually, further studies are needed to determine the effect of this additive on reproductive performance especially in the subsequent parities.

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