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Enhancing Performance of Growing Rabbits Rearing Under Salinity Water by Adding Different Levels of Vitex Extracts

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Abstract

For improving the performance of growing rabbits given salinity water and two levels of aqueous and ethanolic vitex extract, 75 New Zealand White growing rabbits of five weeks old and body weight of 553.00±0.091g were divided equally into 5 treatments. Control treatment was fed control diet. 5 and 7.5g/kg diet of the vitex leaves aqueous extract were supplemented to treatments two and three and 5.0 and 7.5g / kg diet of the vitex leaves ethanolic extract were supplemented to groups four and five for 15 weeks. Blood samples were collected at the end of the experiment for the clinical analysis. Body weight and feed intake were recorded weekly. Carcass characteristics and gut microbiome were determined. Rabbits given saline water and supplemented aqueous and alcoholic vitex extracts had higher ($P<0.0001$) final body weight. The ethanolic extract reduced ($P<0.0001$) feed intake and feed conversion. The control had the lowest digestibility coefficient of all nutrient's digestion coefficients compared to treated groups. Carcass weight ($P<0.022$), carcass percent ($P<0.017$), and protein in the meat increased in all supplemented groups with no difference in the internal organs weight, dressing percent, and edible giblets. Vitex extracts ($P\leq 0.05$) increased total protein ($P<0.003$), triglycerides ($P<0.001$), and LDL ($P<0.042$) but declined ALT ($P<0.028$). Control group had the lowest cecum pH ($P<0.011$) and total volatile fatty acids (($P<0.017$), high ammonia ($P<0.009$) and cecum microbial counts ($P<0.001$). Rabbits supplemented Vitex ethanolic extracts consumed the lowest feed intake and cost associated increased body weight gain with increasing the concentration. In conclusion, growing rabbits bred for meat production can be supplemented either the aqueous or the ethanolic extracts for longer intervals for improving their health, meat composition and reduce the costs.

KEYWORDS
Carcass traits, Digestibility, Growth performance, Rabbits, Saline water, Vitex extracts

INTRODUCTION

Water means life for all living organisms on Earth. Oxygen and water are essential for life. Water contains impurities and several dissolved solids. Underground water and sea water contain high percentages of these impurities which varies in their contents of the dissolved solids. Livestock bred in coastal areas with salinity drinking water affected their health and growth (Sandford, 1996). Water and feed consumption, health, and the production state of animals can be affected by using poor quality water (NRC, 2007). Saline water increases the thirst feeling, water consumption, and urination to allow kidneys to excrete harmful minerals (Marai *et al*., 1995; Masters *et al*., 2007). Drinking salinity water in growing rabbits' stopped drinking and feed consumption to avoid toxicity. The decrease in feed intake consequently decreased the body weight gain (Abdel-Samee and El-Masry, 1992; Marai *et al*., 2005). Carcass traits of rabbits given saline water showed the lowest dressing percentage and the weight (Ayyat *et al*., 1991). The increase of salts to 9.11g TDS l-1 in the drinking water of Barki sheep improved their digestibility coefficients (Shawki *et al*., 1985).

Medicinal plants were used to reduce the negative impact of salinity and improve the productive performance of rabbits. Also, Herbal mixture and their interactions with feed restriction systems for growing rabbits have a beneficial role in growth performance, carcass traits, and microbial aspects (Abou-Kassem *et al*., 2021). The improvement in digestibility coefficients of Braki sheep offered saline water while grazing on vitex extract was referred to the phenolic compounds in vitex extract (Shawki *et al*., 1985).

Vitex plant belongs to Verbenaceae family and is native to the middle Asian, southern European, and Mediterranean countries. This plant is considered within phytogenic sources which can use different parts of it or their extracts to improve the performance due to their impressive range of phytochemicals (iridoids and flavonoids). Vitex improved appetite, intestinal microflora, immune functions, oxidative status, growth, and carcass traits when included in growing rabbits' diet (El-Speiy *et al*., 2020), rabbit does' diet (Abd- El Ghany *et al*., 2017; Basyony and Abdel-Khalek, 2021), and broilers' diet (Güclü *et al*., 2016). Vitex extract has relaxing effects (Dalle Zotte *et al*., 2016; Thaçi *et al*., 2022).

Neither the aqueous nor the ethanolic plant extracts contained cardiac glycosides. The absence of tannins in the ethanolic extract refer to their destruction by Ethanol. Medicinal rates

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of the most medical plants' leaves refer to their content of some phytochemicals which vary during extraction according to the used solvent (Varadarajan *et al*., 2008). The aqueous extract of vitex demonstrated the presence of Isovitexin and Casticin (El-Speiy *et al*., 2020). The solvent used for the phytochemical extraction plays important role in determining the composition of any plant extract. The identification of flavones, crisimartin, genkwanin, 3α-friedeliniol, and 3β-friedeliniol in the different parts of the vitex plant were isolated from the leaves of *vitex peduncularis* (Rudrapaul *et al*., 2015). The petroleum ether extracted steroids and terpenoids from *Vitex trifolia* and the ethanolic extract extracted steroids, trepenoids, and flavonoids (Hossain *et al*., 2001). In contrast, the methanolic extract of *Vitex agnus-castus* showed no important antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Candida albicans* (Kuruüzüm-Uz *et al*., 2003).

Vitex agnus castus extracts can be used safely in nontoxic concentration that were previously determined (Sahib *et al*., 2014; Gholampour *et al*., 2020). Therefore, this study was conducted to consider the effects of vitex aqueous extract (VAQE) or alcoholic extract (VALE) with two levels (5.0 and 7.5g / kg of diet) on productive performance, health status and economics of growing rabbits reared under salinity water.

MATERIALS AND METHODS

Vitex extracts preparation

The vitex leaves were rinsed with tap water, dried in the dark for 5 days, and then ground to a powder using an electric blender. One hundred grams were placed in 900 ml of 80% ethanol for four to five days with daily stirring. The mixture was filtered through filter paper (Whatman No. 1). The filtrate was evaporated using a rotary evaporator at 40°C and the percentage yield of vitex extract powder was obtained for alcoholic extract. For aqueous extract the same method was followed using bi-distillated water. Part of aqueous and alcoholic before evaporating kept in a refrigerator at 4°C for the phytochemical screening to identify alkaloids, saponins, tannins, anthraquinones, flavonoids, terpenoids and cardiac glycosides in both extracts (Harborne, 1973; Trease and Evans,1983).

Animals Experimental protocol

This study was approved by the animal care and use committee of the National Research Centre (NRC-2019-143). New Zealand white rabbits (NZW) belonged to the production branch of a private rabbit farm (Ismailia Governorate, Egypt). Seventy-five growing NZW rabbits aged five weeks of body weight 553.00±0.091g were used in this study. Rabbits were divided into five equal groups (15 in each). Two groups were fed diets treated with aqueous extract of vitex powder leaves (5 or 7.5 g/ kg of diet), other two groups were fed diets treated with ethanolic extract of vitex leaves (5 or 7.5 g/ kg of diet) as powder and one group received none of the two leaves extracts, and served as a control group. All experimental animals were kept under the same managerial, hygienic, and environmental conditions throughout the experimental period. Growing rabbits were housed in galvanized wire batteries provided with feeders and automatic stainless-steel nipples for supplying each cage with water all the time. Drinking water contained 2000 ppm total dissolved salts (Dacisamenz / m; Muller, 1995). All batteries were in an open rabbitry and exposed to natural environmental temperature and photoperiod and ventilated by windows and exhausted fans. Ceiling electric fans were also used when needed. Growing rabbits were fed experimental diet to cover their requirements according to Agriculture Ministry Decree (1996) recommendations. The basal experimental diet composition and chemical composition are presented in Table 1.

Growth performance

The live weight and feed intake were recorded weekly. The average daily feed intake, average daily gain, and feed conversion ratio were calculated. Data of live rabbits were used for the estimation of the performance production.

Digestibility trail

Digestibility trial was carried out to determine the digestion coefficients and nutritive value using five rabbits from each treatment. Feces were collected daily, weighed, and dried at 60oC for 48 h, finely ground and stored for chemical analysis. Data of quantities and chemical analysis of feed and feces were used to

Table 1. Ingredients and chemical composition of the basal experimental diet.

Ingredients%	Content	Chemical analysis%[1]	
Barley	30.28	OM%	90.34
Clover hay (12%)	27.05	CP%	17.76
Wheat bran	18.4	CF%	12.97
Soybean meal (44% CP)	18.1	EE%	2.18
Molasses	3	NFE%	57.43
Di calcium phosphate	2.2	Ash%	9.66
NaCl	0.3	DE (kcal/kg)	2518
Premix (Vit. Min) *	0.3	Calcium	1.11
Limestone	0.22	Total phosphorus	0.85
DL-Methionine	0.1	Methionine+ cyct.	0.65
Anticoccidia (Diclazuril)	0.05	Lysine	0.91
Total	100		

Each 3 kg of vitamins and minerals mixture contains: Vit. A 10.000.000 IU, Vit.B1 1000mg, Vit.B2 5000 mg, Vit.D3 2.000.000 IU, Vit E 10.000 mg, Vit. K 31000 mg, Pantothenic acid 10.000mg; Nicotinic acid, 30.000g; Vit. B6 15000 mg; Vit. B12 10 mg, Folic acid 1.0g, Biotin 50 mg, Cu 4g, choline chloride 200 mg, Mn 60g, Fe 30g, , Co 0.1 g, Se 0.1 g, Zn 50 g, Iodine 0.3 g and Antioxidant 10.000 mg.

[1] according to Feed composition for animal and poultry feed stuff used in Egypt (2001).

calculate the nutrients digestion coefficients and the nutritive values of the dietary treatments (Cheeke, 1982). The samples of feed and feces were chemically analyzed (AOAC, 2005).

Slaughter procedures and blood sampling

At week 15 of the end of experimental period, five rabbits per group with a weight close to the average of the group were selected and slaughtered in a commercial slaughterhouse. The carcass was weighed and expressed as a percentage of slaughter weight (SW). The weight of kidneys, lungs, heart, spleen, liver, giblets, and dressing were recorded for each carcass. Chemical composition of boneless breast's meat and thigh muscles were determined (AOAC, 2005). Blood samples were taken from five animals in each group after slaughtering in lithium heparin test tubes then centrifugated at 4000 rpm for 10 minutes then plasma was harvested and kept at -20º C until the clinical analysis. Total proteins, albumin, creatinine, urea, cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured using commercial colorimetric kits (Bio-Diagnostics, Egypt).

Cecum activity and cecum microbial counts

Samples of cecum contents from the same slaughtered rabbits under each treatment were taken and used immediately for estimation of cecum pH, cecum microflora (bacteria) aerobic total count were detected in each sample and referred as Colony Forming Unit (CFU). Fecal coliforms were counted by the enumeration of anaerobic bacteria using Reinforced Clostridial agar (Difco, 1989). Anaerobic incubation was made in anaerobic jars (Oxoid) for a minimum of 7 days before initial examination. Anaerobic conditions were obtained with Anaerogen (Oxoid) and were controlled by methyl blue strips as oxidation reduction indicator. After incubation of 7 days at 37°C, primary anaerobic plates were examined and all types of colonies grown on selective agar were described and subcultured. For counting *Escherichia coli*, MacConkey agar was used for its isolation and counting (Difco, 1989). *Bacillus cereus*-selective-agar (Merck) was used for the counting *Bacillus cereus* (Kim and Goepfert, 1971). *Enterobacter* was counted using Brilliant-Green Phenol-red lactose agar (BPL; Merck) then DHL Agar (Baired-Parker, 1962). For counting *Clostridium perfringens*, *Clostridium perfiingens*-selective-Agar was used (Difco, 1989). After that, the isolated colonies were kept on nutrient agar slants and identified by standard biochemical tests (Difco, 1989), *Enterococcus* and yeasts were enumerated on Potato-Dextrose-Agar (PDA; Merck). The pH of the medium was adjusted to 3.7 after sterilization by means of a sterile 10% solution of tartaric acid. The plates were incubated at 25°C for 3-5 days (Lodder, 1952). The presence of *Salmonella* species and *Shigella* were determined qualitatively. The methods of (AOAC, 1998) were modified in order to detect any possible *Salmonella*. Enrichment was achieved by suspending 20g of sample into 180 ml each of selenite and tetra- thionate broth and after 6, 12 and 18 h enrichment cultures were streaked onto Bismuth-Sulphite Agar (BSA). The second portion was preserved by addition of 1ml N/10 HCL and 2 ml orthophosphoric acid to each 2 ml of cecum contents juice for determining the total volatile fatty acids by steam distillation of the distillate method (Eadie *et al.*, 1967). The pH of the cecum contents was measured immediately by using a digital pH meter.

Economic efficiency (EEF)

According to price marketing during 2020, the percentage of economic efficiency (EEF) was calculated.

Net revenue / rabbit (LE) equal (Total revenue / rabbit in Egyptian pounds) – (Total feed cost / rabbit in Egyptian pounds).

Economic efficiency equal (Net revenue/rabbit in Egyptian pounds) / (Total feed cost/rabbit in Egyptian pounds).

Feed cost / kg gain equal Total feed cost/rabbit in Egyptian pounds *1000 / Total weight gain/rabbit weight (g).

Statistical Analysis

Data of different parameters were statistically analyzed using SPSS 20.0. Duncan's Multiple Range test was performed to detect significant differences between means. The statistical model used was: $Y_{ijk} = \mu + T_i + E_{ijk}$ where: Y_{ijk} = the observation μ = Overall mean T_i = Treatments E_{ijk} = Experimental error, associated with i, j and k observations assumed to be randomly distributed

RESULTS

Phytochemical composition of aquatic and alcoholic extracts of Vitex agnus-castus

Phytochemical composition of aquatic extract contains flavonoids, saponins, terpenoids, anthraquinones, alkaloids, and tannins. The Alcoholic extract has the same components except Tannins.

Growth performance

The initial body weight was nearly similar for all treatments (Fig.1). The Body weight of all treated rabbits increased ($P<0.0001$) starting from week 1 till week 10. The VAQE 7.5g/kg and VALE 5 g/kg achieved the same body weight and the VALE 7.5g/kg achieved the highest body weight. The body weight gain (Fig. 2) varied ($P<0.0001$) from week 1 till week10 compared to controls. From week 11 to week 15, rabbits supplemented with VALE 7.5g/kg showed the highest ($P<0.0001$) body weight gain but rabbits supplemented with VAQE 5g/kg showed the lowest body weight gain. The control rabbits had lower ($P<0.0001$) final body weight gain with higher ($P<0.0001$) total feed intake (Table 2). Control rabbits average daily weight gain ($P<0.017$) and feed conversion rate ($P<0.011$) from 5-10 weeks is lower than those supplemented vitex aqueous and Ethanolic extracts. The highest value of average daily weight gain from 5-10 weeks 39.7±6.16 kg is recorded for the rabbits fed 7.5g Ethanolic extract compared with controls 29.05±1.30 kg. Throughout the experimental period (5-15) weeks, body weight improved in rabbits supplemented dried aqueous and ethanolic vitex extracts. Daily feed intake ($P<0.005$) and daily weight gain from 10-15 weeks of 7.5 vitex ethanolic extract supplemented are the lowest that associated with the same feed conversion of controls (Table 2).

Digestion coefficients of nutrients and nutritive values

Nutrients digestibility and nutritive values of rabbits offered saline water (Table 3) indicate that the dry matter ($P<0.01$), organic matter ($P<0.015$), crude protein ($P<0.01$), ether extract ($P<0.01$), crude fiber ($P<0.01$), total digestible nutrients ($P<0.05$), and digestible crude protein ($P<0.05$) ascended linearly with the increase of the concertation of vitex aqueous and ethanolic extracts in the diet. Control group recorded linearly ($P<0.05$) the lowest digestibility coefficient values of all nutrients' digestion coefficients.

Table 2. Body weight gain, total feed intake and feed conversion

Item	Control Diet	VAQE Diets		VALE Diets		P-Value
		5g/kg	7.5g/kg	5g/kg	7.5g /kg	
Total BW gain (g)	1935±8.46^{a}	1971±13.68^{b}	2020±7.25^{c}	2033±4.58^{c}	2066±6.92^{d}	0.000
Daily weight gain 5-10 wks (g)	29.05±1.30^{a}	30.00±1.35^{a}	34.00±5.64^{b}	36.80±1.07^{b}	39.70±6.16^{b}	0.017
Daily weight gain 10-15 wks (g)	24.05±1.28	26.10±1.10	23.90±6.16	20.65±1.17	19.28±1.13	0.687
Total feed intake (g)	6835±38.2^{c}	6666±88.8^{bc}	6617±121.8^{bc}	6284±76.1^{a}	6532±67.7^{b}	0.000
Daily feed intake 5-10 wks (g)	84.57±3.57^{a}	88.28±3.76^{b}	88.28±4.91^{b}	91.7±7.90^{b}	97.14±5.86^{b}	0.011
Daily feed intake 10-15 wks (g)	110.57±2.86^{b}	102.00±7.78^{b}	100.28±8.52^{b}	87.42±8.27^{a}	88.57±2.87^{a}	0.005
Total feed conversion rate	3.53±0.02^{d}	3.39±0.06^{c}	3.26±0.06^{b}	3.11±0.04^{a}	3.16±0.04^{ab}	0.000
Feed conversion rate 5-10 wks	3.11±003^{b}	2.94±006^{a}	2.59±0.043^{a}	2.47±0.047^{a}	2.46±0.04^{a}	0.019
Feed conversion rate 10-15 wks	4.61±0.07	3.90±0.10	4.19±0.10	4.24±0.085	4.61±0.087	0.597

BW: Body wight; wks: Weeks. Data are expressed as Mean±SEM

Table 3. Digestion coefficients of nutrients and nutritive values of growing rabbits as affected by tested diets.

Item	Control Diet	VAQE Diets		VALE Diets		P-value
		5g/kg	7.5g/kg	5g/kg	7.5g /kg	
Dry matter	72.99±0.57^{a}	74.29±0.23^{b}	75.42±0.14^{c}	76.65±0.10^{d}	76.82±0.057^{d}	0.011
Organic matter	61.70±0.78^{a}	63.81±0.19^{b}	65.31±0.57^{bc}	66.45±0.40^{cd}	67.51±0.19^{d}	0.015
Crude protein	74.21±0.06^{a}	76.50±0.34^{b}	77.56±023^{c}	80.72±0.26^{d}	81.59±0.09^{e}	0.013
Ether extract	70.73±0.35^{a}	72.42±0.36^{b}	72.56±0.17^{b}	74.36±0.08^{c}	75.01±0.02^{c}	0.011
Crude fiber	39.64±0.04^{a}	42.51±0.68^{b}	43.78±0.18^{c}	46.22±0.43^{d}	47.28±0.18^{ad}	0.011
Nitrogen free extract	75.93±0.045^{a}	76.91±0.049^{b}	77.07±0.26^{b}	77.48±0.27^{b}	77.39±0.30^{b}	0.014
Digestible crude protein	12.90±0.01^{a}	13.30±0.06^{b}	13.48±0.04^{c}	14.03±0.04^{d}	14.19±0.01^{d}	0.012
Total digestible nutrients	64.33±0.04^{a}	65.85±0.11^{b}	66.35±0.14^{c}	67.66±0.16^{d}	67.99±0.14^{d}	0.016

Data are expressed as Mean±SEM. Means with different superscripts (a,b,c,d,e) within row differ significantly at P<0.05.

Table 4. Carcass characteristics of growing rabbits as affected by tested diets.

Item	Control Diet	VAQE Diets		VALE Diets		P-value
		5g/kg	7.5g/kg	5g/kg	7.5g/kg	
Carcass weight (kg)	1.72±4.13^{a}	1.89±1.37^{b}	1.93±3.21^{b}	1.93±8.11^{b}	1.93±8.02^{b}	0.022
Carcass %	48.93±4.55^{s}	67.19±2.21^{b}	67.87±3.37^{b}	66.83±7.99^{b}	68.91±8.35^{b}	0.017
Liver (g)	80.70±1.08^{a}	70.33±1.08^{b}	63.66±4.36^{b}	89.26±1.42^{c}	91.33±1.28^{c}	0.085
Kidney (g)	18.43±0.76	19.33±1.74	17.50±2.95	14.83±2.13	17.16±2.20	0.687
Heart (g)	8.60±0.37	9.46±2.43	7.73±0.93	7.90±2.32	7.13±1.00	0.768
Lungs (g)	14.20±3.45	12.60±0.52	11.73±0.40	14.53±1.93	16.90±1.85	0.711
Spleen (g)	1.33±0.28	1.10±0.100	1.19±0.35	1.23±0.12	1.26±0.43	0.699
Dressing %	53.5±2.73	52.25±3.11	51.62±2.77	51.73±3.45	52.77±2.89	0.745
Edible giblets (g)	107.73±3.22	99.12±3.37	88.89±3.89	111.99±4.25	115.62±2.33	0.821
Edible giblets %	4.14±0.77	3.78±0.56	3.28±0.34	4.13±0.87	4.22±0.67	0.867

Data are expressed as Mean±SEM. Means with different superscripts (a,b,c,d,e) within row differ significantly at P<0.05.

Carcass characteristics

Carcass weight (P<0.05) and carcass percent (P<0.05) of treated rabbits are high compared to controls (Table 4). No significant differences in dressing percentage were observed between control group and the supplemented groups. The 7.5g/kg ethanolic vitex extracts got the highest carcass percentage and liver weight whereas the control group obtained the lowest values.

Meat quality

Supplementing 7.5 g vitex Ethanolic extract increased (P<0.05) the crude meat protein but 7.5 vitex aqueous extract increased fat (Table 5).

Blood parameters

Blood plasma metabolites of rabbits offered saline water in response to the dietary supplementation with vitex (Table 6) high total protein (P<0.003),, triglycerides (P<0.001), and LDL (P<0.05), ALT (P<0.05). The lowest cholesterol concentrations (P<0.003) can be noticed in rabbits supplemented 5g aqueous vitex extract. The lowest AST (P<0.007), ALT (P<0.028), urea (P<0.028), and creatinine (P<0.011) with the highest can be observed in rabbits drank saline water and 7.5 g aqueous vitex extract.

Cecum activities and cecum microbial counts

Regarding the effect of adding vitex levels on cecum activities

presented in Table 7. It could be noticed that the pH increased (P<0.003) from 5.96 to 6.33 with increasing the vitex concentration and with changing type and all treated groups showed higher pH compared to control (5.66±0.14). However, TVFAs ranged between 4.04-4.58 (ml eq/100ml) for treatments groups vs. 3.77 mg/100 ml for control. On the other hand, Ammonia concentration decreased (P<0.05) in rabbits drank saline water and fed vitex extract diets compared to control group. Cecum microbial counts (log^{-1} CFU/ml) (Table 7) descended linearly with increasing the concentration of the supplemented vitex extract groups. Control group recorded the highest count for all tested microbes and yeasts.

Economical evaluation

The economic efficiency Table 8, showed the least feed cost/ Kg body weight gain, economic efficiency and the best relative economic efficiency for the rabbit supplemented with alcoholic extracts. The worst values were recorded for control. The values of economic efficiency for 5 and 7.5 g/kg alcoholic extract were 1.24 and 1.27 compared 1.00 for the control diet. Moreover, the highest value relative of economical evaluation of 127 was recorded for 7.5 g/kg alcoholic extract comparing to control group value which was 100. The improvement of economical evaluation for vitex extract groups comparing to control group related to increasing of total weight gain/rabbit of aqueous extract and alcoholic extract groups with a low total feed cost comparing to control group.

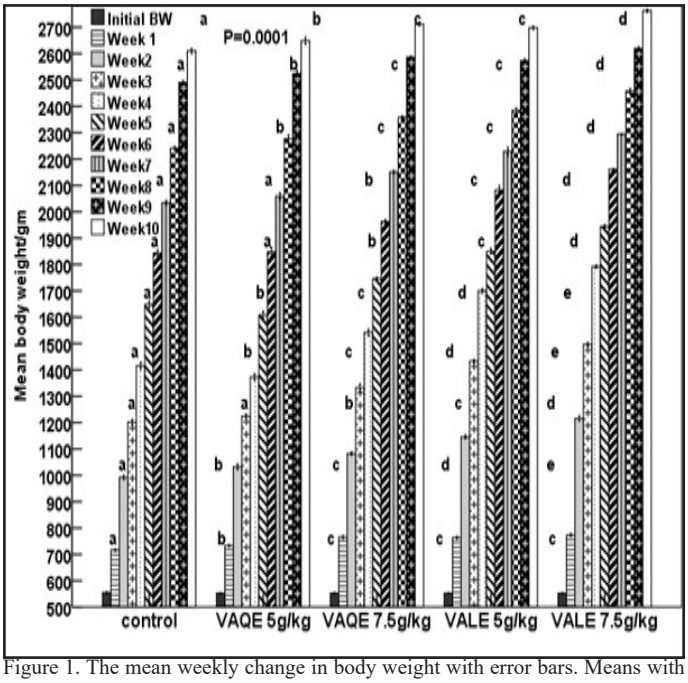

Figure 1. The mean weekly change in body weight with error bars. Means with different superscripts (a, b, c, d, e) are significantly different at P<0.05.

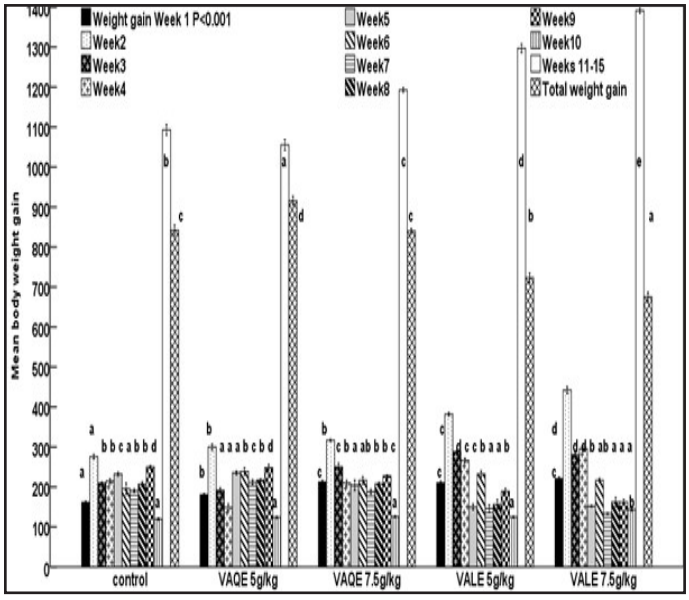

Figure 2. The mean weekly and total body weight gain with error bars. Means with different superscripts (a, b, c, d, e) are significantly different at P<0.05.

DISCUSSION

In agreement with the obtained results, saline water decreased feed intake in growing rabbits (Abdel-Samee and El-Masry, 1992). Vitex plant extracts compensated the decline in the growth performance due to the salinity of the drinking water that reduced feed consumption. The increase in the feed intake of vitex supplemented rabbits from 5-10 weeks was attributed to the presence of natural antioxidants which can protect the intestinal mucosa against oxidative damage and pathogens and limit peristaltic activity in digestive disorders thus preventing diarrhea and improved growth (Kermauner and Laurenčič, 2008), and its potential effects on improving digestion and appetite (Regiane *et al*., 2017). Vitex had a positive impact on feed conversion ratio (Güclü *et al*., 2016). The use of vitex in the broiler rations produced beneficial effects and it is recommended as an alternative growth promoter for boosting feed efficiency (Nath *et al*., 2012; Thaçi *et al*., 2022). Vitex also affected the growth indices (Gholampour *et al*., 2020). In heat stressed broilers, the final body weight did not vary between treatments supplemented with vitex essential oils with or without vitamin A, Vitamin E, and vitamin C but the feed consumption and feed conversion ratio varied significantly (Güclü, *et al*., 2016). Supplementing mature female new-Zealand white rabbits for four months before breeding with the same concentrations of either aqueous and ethanolic vitex extract increased the body weight and feed intake (Abd-El Ghany *et al*., 2017). In agreement with the improved body weight gain, daily weight gain, daily feed intake, and feed conversion rate from 5 to 10 weeks after supplementing 7.5g aqueous vitex extract to the growing rabbits of this study, El-Speiy *et al*. (2020) reported increase the final body weight and the average daily weight gain of 35-day old growing heat stressed male California rabbits supplemented orally 1ml (100mg) vitex leaf aqueous extract with and

Table 5. Meat composition of growing rabbits as affected by tested diets.

Item	Control	VAQE Diets		VALE Diets		P-value
		5g/kg	7.5g/kg	5g/kg	7.5g /kg	
Moisture (%)	72.89±0.12^{b}	72.77±0.05^{ab}	72.70±0.07^{ab}	72.71±0.13^{ab}	72.46±0.09^{a}	0.019
Protein CP (%)	24.39±0.03^{a}	24.77±0.10^{b}	24.79±0.05^{b}	24.82±0.09^{b}	25.18±0.09^{c}	0.032
Fat EE (%)	1.85+0.07^{ab}	1.78±0.017^{ab}	1.89±0.011^{b}	1.72±0.03^{a}	1.76±0.05^{ab}	0.051
Ash (%)	0.85±0.05	0.67±0.17	0.61±0.12	0.74±0.14	0.59±0.13	0.378

Data are expressed as Mean±SEM. Means with different superscripts (a,b,c,d,e) within row differ significantly at P<0.05.

Table 6. Blood plasma parameters of growing rabbits as affected by tested diets.

Item	Control	VAQE Diets		VALE Diets		P-value
	Diet	5g/kg	7.5g/kg	5g/kg	7.5g /kg	
Total Protein (g/dl)	6.37±0.22^{a}	7.37±0.20^{b}	7.30±0.06^{b}	7.37±0.09^{b}	7.36±0.09^{b}	0.003
Albumin (g/dl)	4.05±0.14^{b}	4.21±0.16^{b}	4.18±0.13^{b}	3.70±0.04^{a}	4.06±0.09^{b}	0.009
Urea (mg/ dl)	37.66±2.96^{ab}	39.00±1.15^{ab}	35.00±4.16^{a}	42.00±3.51^{b}	39.33±1.76^{ab}	0.028
Creatinine (mg/ dl)	1.10±0.11^{cd}	1.11±0.03^{d}	0.94±0.01^{a}	0.96±0.05^{ab}	1.02±0.02^{bc}	0.011
AST (U/l)	39.90±3.46^{b}	26.67±0.88^{a}	26.00±4.58^{a}	39.00±1.15^{b}	36.33±3.48^{b}	0.007
ALT (U/l)	55.67±3.84^{b}	40.67±12.33^{a}	39.00±6.66^{a}	41.33±7.22^{a}	46.67±6.23^{ab}	0.028
Cholesterol (mg/ dl)	29.00±1.15^{bc}	21.67±1.45^{a}	31.00±2.31^{c}	28.33±3.53^{bc}	26.00±1.53^{b}	0.003
Triglycerides (mg/dl)	47.33±2.19^{a}	55.00±16.52^{a}	75.00±12.50^{b}	78.00±4.04^{b}	78.00±6.66^{b}	0.001
LDL (mg/dl)	9.47±0.21^{a}	11.00±1.65^{a}	15.00±1.25^{b}	15.60±0.40^{b}	15.60±1.00^{b}	0.042
HDL (mg/dl)	5.67±0.43^{a}	5.66±0.46^{a}	7.66±0.45^{b}	6.33±0.44^{b}	8.00±0.28^{b}	0.051

Data are expressed as Mean±SEM. Means with different superscripts (a,b,c,d)within row differ significantly at P<0.05.
AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDL: Low density lipoprotein; HDL: High density lipoprotein.

Table 7. Cecum activity and cecum microbial counts (log-1 CFU/ml) of growing rabbits as affected by the tested diets.

Item	Control	VAQE Diets		VALE Diets		P-value
	Diet	5g/kg	7.5g/kg	5g/kg	7.5g /kg	
pH	5.66±0.14^{a}	5.96 ±0.12^{ab}	5.93±0.21^{ab}	6.26±.0.23^{b}	6.33±0.08^{b}	0.011
Total volatile Fatty acids (TVFAs mg/100ml)	3.77±0.07^{a}	4.04±0.04^{b}	4.26±0.074^{bc}	4.58±0.109^{c}	4.56±0.12^{c}	0.017
Ammonia (mg/100ml)	11.51±0.15^{b}	10.40± 0.06^{a}	10.43±0.16^{a}	10.43±0.15^{a}	10.42±0.07^{a}	0.009
Aerobic total count	9..20±0.04^{e}	8.24±0.05^{d}	6.59±0.12^{c}	6.08±0.008^{b}	5.74±0.047^{a}	0.001
Fecal coliform	6.65±0.16^{e}	5.14±0.03^{d}	4.06±0.01^{c}	3.80±0.017^{b}	2.89±0.06^{a}	0.001
E. coli	7.32±0.11^{e}	6.16±0.04^{d}	3.81±0.15^{c}	3.21±0.03^{b}	2.63±0.12^{a}	0.001
Bacillus	3.36±0.12^{d}	2.81±0.08^{c}	2.32±0.05^{b}	2.04±0.02^{a}	2.01±0.01^{a}	0.001
Enterobacter	4.44±0.06^{d}	4.09±0.02^{c}	3.71±0.08^{b}	3.12±0.01^{a}	3.06±0.03^{a}	0.001
Clostridium	1.72±0.09^{b}	1.13±0.008^{a}	1.10±0.04^{a}	1.06±0.01^{a}	1.04±0.017^{a}	0.001
Enterococcus	7.34±0.06^{d}	6.33±0.06^{a}	6.63±0.15^{bc}	6.62±0.12^{bc}	6.68±0.06^{c}	0.001
Yeasts	6.11±0.06^{b}	5.68±0.11^{a}	5.59±0.13^{a}	5.56±0.07^{a}	5.54±0.15^{a}	0.001

Data are expressed as Mean±SEM. Means with different superscripts (a,b,c,d,e)within row differ significantly at P<0.05.

Table 8. Mean economical evaluation of growing rabbits as affected by tested diets.

Item	Control	VAQE Diets		VALE Diets	
	Diet	5g/kg	7.5g/kg	5g/kg	7.5g /kg
Total feed cost/rabbit (LE)	29.04	28.46	28.58	27.02	27.33
Feed cost / kg gain	15.01	14.44	14.06	13.37	13.23
Total revenue/rabbit (LE)	58.05	59.14	60.98	60.61	61.98
Net revenue/rabbit (LE)	29.01	30.68	32.4	33.59	34.65
Economic efficiency (EE)	1	1.08	1.13	1.24	1.27
Relative EE%	100	108	113	124	127

Based on prices of the Egyptian market during the experimental period (2020). price of kg/rabbit 30 LE.
Net revenue / rabbit (LE) = (Total revenue / rabbit (LE)) – (Total feed cost / rabbit (LE)). Economic efficiency = (Net revenue/rabbit (LE)) / (Total feed cost/rabbit (LE). Feed cost / kg gain = Total feed cost/rabbit (LE) *1000 / Total weight gain/rabbit (g).

without peppermint oil for 7 weeks. The antioxidants of the vitex aqueous and ethanolic extracts improved both performance and the production quality (Ansari *et al*., 2012)

The highest digestion coefficient noticed in the rabbits supplemented with 7.5 g/kg ethanolic extract agree with improved digestibility coefficients of Barki sheep given saline drinking water containing 9.11g TDS l-1 (Shapasand, 2010). This improvement in digestibility coefficients was referred to the ability of the phenolic compounds in vitex extract to modulate gut microbiota (Mosele *et al*., 2015). The antioxidants activity in the vitex extract also plays a role in improving the digestibility and the health status of the supplemented animals (Hajdú *et al*., 2007). In addition, proanthocyanidin in vitex increased the growth of potentially beneficial gut bacteria, the activity of the endogen digestive enzymes in the pancreas and small intestine and the digestibility and absorption of nutrients especially protein in broiler chickens (Jang *et al*. 2007). It is known that plant feed additive extraction has positive effects on gastrointestinal enzymatic activity and enhanced nutrient absorption and digestibility in rabbits (Hassan *et al*., 2016). The improvement in digestion coefficients in the growing rabbits supplemented with two aqueous and ethanolic vitex extracts was also noticed in mature female new-Zealand rabbits fed the same extracts in the same concentrations for one month before starting breeding (Abd-El Ghany *et al*., 2017).

In agreement with findings from this study, saline water in sheep (7 g NaCl /liter) did not influence slaughter weight, carcass weight and liver weight (Yousfi *et al*., 2016). Different levels of salinity (640, 3188, 5740 and 8326 mg TDS/l) in the drinking water did not affect slaughter or carcass weights (Castro *et al*., 2017). Furthermore, the increased carcass weight and percent in the

growing rabbits supplemented with 5.0 and 7.5 g Vitex aqueous extract, is supported by findings from a study by El-Speiy *et al.* (2020) who recorded increased preslaughter weight in male California heat stressed rabbits of similar age supplemented orally vitex aqueous extract for 9 weeks with and without peppermint oil(. Contrary to the current study, the phenolic compound of vitex extract did not improve carcass characteristics except for the breast muscle and liver (Chen *et al*., 2017). When phenolic compound added in the diet of growing rabbits for 9 weeks, it did not induce positive effects on the carcass traits (Dalle Zotte *et al*., 2012). In heat stressed broilers, the carcass trait except the breast muscle percent did not vary between treatments supplemented with vitex essential oils with or without vitamin A, Vitamin E, and vitamin C (Güclü, *et al*., 2016).

The meat chemical composition of the investigated rabbits coincided with broiler fed diets supplemented with flavonoids and produced more CP content in breast meat ($P < 0.02$) than those in the non-supplemented group (Ahmed *et al*., 2015).

The supplementation of vitex extracts reversed the adverse effects of saline drinking water on the retention of body fluids, that dilute plasma proteins and decrease its concentration (Tietz, 1986 Ayyat *et al*., 1991; Qar and Abdel-Monem, 2014; Attia *et al*., 2015). The effect of vitex extracts in improving the total proteins and globulins in growing rabbits was observed in male California rabbits after 9 weeks of supplementation (El-Speiy *et al*., 2020) but was not observed in mature female rabbits after one month of supplementation (Abd-El Ghany *et al*., 2017). The decrease of AST and ALT activities in growing rabbits from this study supplemented with 5.0 and 7.5g vitex aqueous extracts is similar to that observed in male California rabbits (El-Speiy *et al*., 2020) and contrasts the absence of its effect on mature female rabbits (Abd-El Ghany *et al*., 2017). Contrary to the decrease of urea and creatinine in the investigated rabbits supplemented with 7.5g aqueous vitex extract, neither creatinine nor urea levels were affected by vitex aqueous or ethanolic extracts supplemented to mature female rabbits (Abd-El Ghany *et al*., 2017). The decrease of total cholesterol in the growing rabbits of this study fed 5.0 g aqueous vitex extract is similar to the decrease of total lipids when female rabbits were fed 5.0 and 7.5 g aqueous vitex extracts (Abd-El Ghany *et al*., 2017).The significant increase of triglycerides, LDL, and HDL in growing rabbits that were supplemented with 7.5 g aqueous and ethanolic vitex extracts and 5.0 g ethanolic extracts are in contrast to the slight decrease of triglycerides in mature female rabbits supplemented with the same treatments (Abd-El Ghany *et al*., 2017). The supplementation of 5 g vitex aqueous extract did not exert significant changes on the circulating the triglycerides, LDL and HDL levels in this experiment contrasting their significant increase after supplementing 7.5 g. In contrast, 9 weeks to male California rabbits supplemented orally with 100mg/kg BW of vitex aqueous extract with and without peppermint oil showed a decline in serum triglycerides and LDL level, and increased HDL level (El-Speiy *et al*., 2020).

The reduction in the aerobic, fecal coliform count, E coli, *Bacillus*, *Enterobacter* and *Clostridium*, *Enterococcus*, and yeast count with increasing the concentration of vitex in the diet is similar to the moderate inhibiting activity against both gram-positive and gram-negative bacteria observed in the *Vitex trifolia* leaves (Hossain *et al*., 2001) and its essential oils (Ekunday *et al*., 1990). The significantly increased cecum volatile fatty acids (TVFA) values of rabbits fed aqueous or alcoholic vitex extract is similar to the supplementation of mulberry leaf which modulated the intestinal micro-flora used in streptozotocin-induced diabetic rats (Sheng *et al*., 2017). The flavonoids in vitex extracts showed antimicrobial effect on harmful micro-organisms (Lansdown, 2006). Moreover, using flavonoids inhibited the microbial assemblies in the methanogenesis leading to improved digestion of the organic matter and reduced methane production (Ma *et al*., 2016). Adding vitex leaves extract in the diet can effectively optimize the cecum micro-flora as well as the intestinal micro-flora of rabbits, and this capacity of vitex leaves extract can be attributed to the constituent phytochemicals. The changes in the formation of the commensal and intestinal micro-flora greatly influences the levels of NH_3-N and TVFAs. Some of the cecum microbes arise from gastrointestinal tract.

The results of economic evaluation agree with Nath *et al*. (2012) who indicated that adding phenolic compound like vitex improved the economic efficiency of broiler and this formulation could be used as an alternative to commercial growth promoters. The decrease of total feed cost/rabbit and feed cost/kg BW gain, associated with the increased net revenue/rabbit (LE), the economic efficiency, and the relative economic efficiency (EE) in the growing rabbits supplemented the two ethanolic extracts is contrasting their increase in mature female rabbits (Abd-El Ghany *et al*., 2017).

CONCLUSION

Aqueous and ethanolic extracts modulated the adverse effects of the drinking salinity water. The two ethanolic extracts have better economic efficiency compared to the two aqueous extracts. Aqueous and alcoholic extracts of vitex can be used in growing rabbits' diets reared under salinity water within tested levels with positive effect on productive efficiency and physiological functions. 7.5 g vitex ethanolic extract for each kilogram of diet improves all the body gain and is recommended to be used as feed additive.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related.

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