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EVALUATION OF *ALOE VERA*, OLIVE POMACE, VITAMIN C AND VITAMIN E AS ANTIOXIDANT ON FUNCTIONALITY AND INTEGRITY OF GASTROINTESTINAL TRACT OF GROWING RABBITS

By

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ABSTRACT

The aim of the present study was to investigate the antioxidant function of *aloe vera* gel, olive pomace, vitamin C, and vitamin E as a feed additive on gastro-intestinal functionality and integrity of growing rabbits. The present study was carried on 48 New Zealand White rabbits (NZW) of 6 weeks old. The experiment lasted for 10 weeks. Rabbits were equally divided into eight groups, control group which was fed on a basal diet, group (A) was fed on a basal diet and water supplemented with *aloe vera* gel at a dose (500mg/L) and group (C) was fed on a basal diet and water supplemented with vitamin C(250mg/L), group (E) was fed on a basal diet and water supplemented with vitamin E (1ml/L), (O) group was fed on a basal diet to which 10% olive pomace was added, (CE) group fed a basal diet and water supplemented with both vitamins (C&E) by the previous doses, (ACE) group, of which its water supplemented with *aloe vera* gel ,vitamin C and vitamin E by the previous doses, (OCE) group fed the diet to which olive pomace and both vitamins in water was added. Performance parameters were measured (Live body weight (LBW), feed intake (FI) and feed conversion ratio (FCR)) at the end of the experiment. Serum samples were collected to measure biochemical and antioxidant parameters. Tissue samples of duodenum were collected and prepared for histomorphology and molecular studies. The results revealed that: [1] most supplements had no significant effect on body weight, while (FI) was decreased in (C), (O) and (OCE) groups. Moreover, the (FCR) was only decreased in group (C). [2] A hypoglycemic and a hypocholesterolemia were detected in group (A), (E), (CE), (ACE) and (OCE). [3] There were no significant variations in liver enzymes (ALT and AST) and kidney function tests

(Creatinine and Urea) between all groups. [4] Regarding the antioxidant parameters of all supplemented groups, a significant increase of most of antioxidant parameters was determined with a significant decrease of serum Malonaldehyde (MDA).[5] DNA concentration in duodenum and pancreatic tissues increased significantly in group supplemented with (ACE), while protein concentration of pancreatic tissue recorded a significant higher value in group (CE). DNA to Protein ratio revealed a significant increase in groups supplemented with (A), (C) and (ACE) which indicate increase of cell size. [6].The duodenum morphometry showed best villous height/crypt depth ratio of group supplemented with (A) than other supplemented groups. Conclusively it was obvious that the rabbits supplemented with *aloe vera* gel mixed with vitamin C and E in supplied water had the best antioxidant activity and the used supplements have no harmful effect on liver and kidney measured parameters. Furthermore the olive pomace supplemented diet can be considered as a cheapest diet.

Keywords:

New Zealand White rabbits, *Aloe vera* gel, Olive pomace, Vitamin C and Vitamin E.

INTRODUCTION

Production of rabbits has a potential in developing countries to supply cheap and high quality animal protein within the shortest possible time **(Mehrez and Mousa, 2011).** Optimal gastrointestinal functionality is essential for sustainable animal production, effective functionality of the gastrointestinal tract (GIT) and its health are important factors in maintaining animal performance **(Celi *et al*., 2017).**

Antioxidants are routinely supplemented in livestock's diet as alternative prevention of excessive production of reactive oxygen species (ROS) and to improve health and productivity of animals, they have acquired more reliability and acceptability among consumers as safe and natural additives as *aloe vera*, olive pomace, vitamins C and E **(Loh *et al*., 2010, Salim *et al*., 2013 and Pourhossein *et al*., 2015).**

Aloe barbadensis miller, also known as *aloe vera*, is the most widely used and commercially available. The *aloe vera* leaf has two major liquids including a yellow exudate, and mucilaginous gel produced by parenchyma cells **(Femenia *et al*., 2003)**. *Aloe vera* gel is commonly used in herbal medicines as an agonist for various properties as oxidant, diabetic, cancer, inflammatory, microbial, coughs, headaches and arthritis. Moreover, it may be used for wound, burns and incision healing, as well as in curing some immune deficiencies

(Rodriguez *et al.*, 2010).Vitamin C is an essential micronutrient that acts as non-enzymatic, water soluble antioxidant**,** it exert its antioxidant effect in both direct and indirect ways, in the direct way vitamin C scavenges free radicals formed **(Dawson *et al*., 1990)** or interact with reduced glutathione **(Dudek *et al*., 2005).**As an indirect way vitamin C can recycle the lipid soluble vitamin E by reducing alpha -tochopheroxyle radicals in membranes **(Gramlich *et al*., 2002).**The effect of vitamin C is due to its action as an electron donor, after vitamin C donates electrons, they turned into a free radicals, ascorbyle radical or semi dehydro-ascorbic acid which is relatively stable and is fairly unreactive. Ascorbate is therefore a good free radical scavenger **(Padayatty *et al*., 2003).**Vitamin E as one of the most abundant lipid-soluble antioxidants exists in two forms, as tocopherols and tocotrienols. That α-tocopherol is the most potent antioxidant and plays an important role in peroxyl radical scaveng **(Fino and valberg, 2012).** Vitamin E terminates the chain reaction of lipid peroxidation in membranes and lipoproteins, therefore, vitamin E can alleviate the toxic effects of ROS, limits the effects of oxidant molecules on tissues and is active in the defense against oxidative cell injury by means of its being free radical scavenger **(EL-Demerdash *et al*., 2013)**.Olive pomace (OP) is one of the agro-industrial by-products that represent as important feed resources for animal and play important economic and social roles in Mediterranean Sea Countries **(Molina and Yanez, 2008).** Moreover, OP is considered a natural of a highest antioxidant activity, which has been widely accepted due to the presence of some phenolic compounds (oleuropein, verbascoside, ligstroside, lutein, a pigenine, tyrosol, and hydroxytyrosol) of biological activities as antioxidants **(Visioli *et al*., 2002 and Jemai *et al*., 2009).**The rabbit as an economic animal especially for meat production, the development of duodenum is more important specially the mucosa (villous for absorption) and sub-mucosa (glands) for enzymatic digestion **(Elnasharty *et al*., 2013),** therefore intestinal morphology is very important to study. The aim of the present study was performed to evaluate and compare the effects of *aloe vera*, olive pomace, vitamin C and vitamin E on performance, gastro intestinal functionality and integrity of growing rabbits.

MATERIAL AND METHODS

2.1. Animals and Experimental design:

The present study was carried out in the rabbitry of Physiology Department, Faculty of Veterinary Medicine Cairo University during the period from October to December 2016 in

accordance with the Chinese guidelines for animal welfare and approved by the animal welfare committee of Animal Science College, Zhejiang University. All rabbits were apparently active and healthy. The experiment lasted for 10 weeks one week for adaptation and 9 weeks after supplementation of the experimental feed and water; and duration was from (6-15 week old).The rabbits were housed in cages (55×60×34 cm) equipped with automatic drinkers and J feeder. Food and water were available (*ad-libitum*) all over the experiment period, the whole rabbitry was well ventilated through both natural windows and electric fans, illuminated to 14:10 light dark cycle through natural and fluorescent lighting, all cages are kept under the same management and hygiene. Basal and experimental diets were formulated to cover the nutrient requirements of rabbits as recommended in NRC (1994).

The composition and chemical analysis of formulated diets are shown in (Table 1). Diets were subjected to chemical analysis according to AOAC (1999). The forty-eight rabbits were equally distributed into 8 groups (6 rabbits each) and were treated as follows:

I -Control group (control): Rabbits fed on a basal diet without any additives.

II-*Aloe Vera* group (A): was supplied by *aloe vera* gel in water (500mg/L drinking water**) Channa *et al*. (2014).**

III-Vitamin C group (C): Vitamin C (100% concentration) was added to water at a dose of vitamin C (250mg/L drinking water) as recommended by the providing company (Misr feed additive company).

IV-Vitamin E group (E): Vitamin E (20% concentration) was added to water at a dose of (1ml/L drinking water) it was manufactured by Miavit company and provided as a gift for this research by the importer company (United Bio Med-Egypt).

V- Olive Pomace group (O): Rabbits supplied with basal ration to which olive pomace was mixed (10% of diet) as recorded by **Dorbane *et al*. (2016)**, It was provided as a gift for this research from EL-wadi company for poultry industries**.**

VI-Vitamin C + Vitamin E group (CE): Rabbits supplied with vitamin C and vitamin E in drinking water by the previous recommended doses.

VII-*Aloe Vera* gel, Vitamin C and Vitamin E group (ACE): Rabbits drinking water was supplemented by *aloe vera* gel plus vitamin C and Vitamin E with previous recommended doses.

VIII- Olive Pomace, Vitamin C and Vitamin E group (OCE): were fed on basal ration to which olive pomace was mixed by 10% of diet plus both vitamin C and Vitamin E were added with previous recommended doses in drinking water.

***Aloe vera* gel preparation:**

Mature, healthy and fresh leaves of *aloe vera* having a length of approximately 25-50 cm were obtained from El-Hossary garden plantations and identified by Botanist as *aloe barbadensis* Miller belongs to the Liliaceal family which is the most effective species as recorded by **Gong *et al.* (2002).**The *aloe vera* leaf was harvested and washed with clean water. A clean, dry cloth was then used to mop up the water from the *aloe vera* leaf, then with sharp knife remove the lower 25 mm of the leaf base (the white part attached to the large rosette stem of the plant), the tapering point (50-100mm) of the leaf top and the short sharp spines located along the leaf margins were also removed, the leaf was splitted into two parts; white transparent pulp was scraped out and weighed, the solid gel in the center of the leaf was homogenized with mixer, the gel was prepared freshly each time as described by **Oyewopo *et al*. (2011).**

Table (1): Composition percentage and analysis nutrients profile of the basal and experimental diet.

Ingredients (%)	Basal diet	Experimental diet
Berseem hay	30.0	29.1
Olive cake meal	---	10.0
Barley grain	21.0	19.0
Yellow corn	5.0	3.0
Wheat bran	21.1	20.0
Soybean meal	17.5	13.5
Molasses	3.0	3.0
$CaCl_2$	1.5	1.5
NaCl	0.4	0.4
Vit. &Min. premix	0.3	0.3
DL-Methionine	0.2	0.2
Chemical analysis (%)		
Moisture	9.4	9.5
Crude protein	17.5	16.5
Crude fiber	14.0	23.3
Ether extract	2.70	6.1
Total Ash	7.10	7.5
Nitrogen free extract	49.30	47.6
Calculated digestible energy (kcal /kg)	2600	2500

The Rabbits vitamin and mineral premix /kg contained the following IU/g for vitamins or minerals : A-4,000,000, D3-5000,000, E-16,7 g, K-0.67 g, B1-0.67g , B2-2g ,B6-0.67 g, B12-0.004g, B5-16.7g , Pantothinc acid -6.67 g, Biotein-0.07 g, Folic acid-1.67 g , Choline chloride -400 g ,Zn-23.3 g, Mn -10 g, Fe-25 g , Cu -1.67 g , I -0.25 G , Se-0.033 g , and Mg-133.4 g (Rabbit premix).

2.2. Sampling:

2.2.1- Blood samples

Blood samples were collected at the end of experiment from ear veins for glucose level measurement and during slaughter of rabbits another blood samples for serum separation were collected. Glucose level was measured immediately after collection. The coagulated blood centrifuged at 860_{xg} for 20 minutes, sera were separated and kept at -20° C for determination of serum total proteins, alanine aminotransferase & aspartate aminotransferase activities, urea, creatinine, triglyceride, cholesterol and antioxidant parameters.

2.2.2-Tissue samples:

At slaughter time, the rabbits were immediately eviscerated for collection of pancreas tissue segment and an intestinal segment of 5 cm was taken from the duodenum (one piece of this tissue was kept in liquid nitrogen tank at – 196 C° for estimation of antioxidant and molecular parameters, while the other piece was fixed in 10% formol saline for histomorphology examination.

2.3. Measured Parameters:

2.3.1-Performance Parameters: The live body weight (LBW) for rabbits were recorded at the beginning of the experiment (6 weeks old) weekly till the end of the experiment. The feed intake was recorded daily in order to calculate feed intake and feed conversion ratio.

It was determined as follow:

a. Feed intake per group = feed consumption by all rabbits in each group per day.

b. Daily feed intake per individual rabbit = Daily feed intake per group **/** number of rabbits in the same group.

c. Weekly feed intake per individual rabbit = Average weekly feed intake per group **/** number of rabbits in the same group.

d. Body weight Gain (BWG kg/week):

It is the difference between two successive weekly body weights, for each rabbit.

e. Feed conversion ratio (FCR):

Feed conversion ratio was calculated by dividing average weekly feed intake by average weekly body gain **(Fromageot *et al*., 1985).**

Feed conversion ratio = Average weekly Feed intake / Weekly body gain.

2.3.2- Serum Biochemical Parameters:

The serum total proteins was determined as described by **Kaplan and Szalbo (1983)** and albumin was measured as described by **Grant *et al*. (1987).**Serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities were measured using kits purchased from biodiagnostic company , Dokki , Egypt, for determination of AST as described by**(Reitman and Frankel, 1957).** Serum Creatinine were measured spectrophotometrically according to the method described by **Houot (1985),** Urea concentration was measured spectrophotometrically according to the method described by **(Fawcettandsoctt,1960)** Serum Triglycerides and Cholesterol were measured spectrophotometrically according to the methods described by **(Allain *et al*., 1974 and Fossati *et al*., 1980 respectively).** Serum Glucose was determined as described by **Trinder (1969).**

2.3.3- Antioxidant Parameters:

A-Serum total antioxidant capacity (TAC) and lipid peroxide Malonaldhyde (MDA) were measured using kits purchased from biodiagnostic company, Dokki, Egypt, according to method described by **koracevic *et al*. (2001) and Ohkawa *et al*. (1979)** respectively. **Catalase** (CAT) enzymes activity was measured according to the method described by **Aebi (1984) and Fossati *et al*. (1980).** Glutathione-s-transferase (GST) activity was determined by the method described by **Habig *et al*. (1974).**

B-Duodenal tissue Antioxidants:

Tissue superoxide dismutase (SOD) and glutathione peroxidase (GPx**)** were performed using kits purchased from biodiagnostic company, Dokki, Egypt as described by **Nishikimi *et al*. (1972) and Paglia and Valentine (1967)** respectively.

2.3.4. Intestinal and Pancreatic DNA and Protein Concentrations:

Determination of DNA and protein in duodenal and pancreas tissues homogenate. DNA Purification from tissues (QIAamp DNA, RNA Mini Kit) This protocol is for purification of total (genomic, mitochondrial, and viral) DNA concentration of tissues were determined using the QIAamp Mini Kit **(Fisher, 2011).**And protein concentration was determined according to

Bradford (1976).

2.3.5. Intestinal Morphometry:

Seven cross sections for each sample were prepared after staining with hematoxylin and eosin using standard paraffin embedding procedures. Villous height was measured from the tip of the villous tothevillouscrypt junction; crypt depth was defined as the depth of the invagination between adjacent villous using light microscopes according to **(Brancroft *et al*., 1996).** Morphological indices were measured using image processing and analysis system (Version 1, Leica Imaging System Ltd, and Cambridge).

2.4. **Statistical Analysis:** Data were analyzed by one way analysis of variance (ANOVA) test according to **Snedecor and Cochran (1980).** Treatment means were compared by the least significance difference (LSD) at 5% level.

RESULTS

3.1. Productive Performance:

The results in (Table 2) illustrate that, there was a significant decrease of body weight gain of groups (CE) and (ACE) as compared with (O) during (12-15) week old. Feed intake of groups supplemented with (C), (O) and (OCE) was lower than all other groups during (9-15) weeks old at P ≤0.05. The feed conversion ratio (FCR) for (C) group was lower significantly than control group during (9-11) week old.

Table (2): Effect of *aloe vera*(A), olive pomace (O),vitamin C (C), vitamin E (E),vitamin C + vitamin E(CE),*aloe vera*+vitamin C+vitamin E(ACE) and olive pomace+ vitamin C+vitamin E(OCE) supplementation on body weight gain per week (BWG /Kg), feed intake per week (FI/Kg) , feed conversion ratio (FCR) in broiler rabbits.

Parameters \ Group		Control	(A)	(C)	(E)	(O)	(CE)	(ACE)	(OCE)	LSD
6-8 week old	BWG	0.16	0.14	0.18	0.17	0.12	0.14	0.16	0.16	0.05
	FI	0.43	0.45	0.37	0.44	0.39	0.42	0.42	0.36	0.09
	FCR	2.84	3.30	2.40	2.80	3.82	3.21	3.01	2.76	1.22
9-11 Week old	BWG	0.16	0.18	0.19	0.19	0.16	0.18	0.20	0.19	0.06
	FI	0.73	0.73	0.59	0.71	0.61	0.73	0.73	0.61	0.10
	FCR	4.73	4.32	3.45	3.80	4.48	4.02	4.11	3.63	1.24
12-15 week old	BWG	0.16	0.17	0.19	0.19	0.21	0.15	0.15	0.17	0.05
	FI	0.99	0.97	0.86	0.98	0.85	0.99	1.00	0.85	0.07
	FCR	5.75	5.22	4.62	5.59	5.58	5.32	5.76	4.65	1.15

Data indicates mean, n = 6 rabbits /group. LSD (least significant difference) atP ≤0.1

3.2. Metabolic Parameters:

Data analysis tabulated in table (Table 3) shows that, protein level had a lower significant value for group (A), (E), (O), (ACE) and (OCE) as compared with that of the control, with a lower significant values of albumin levels of (A) group than that of group (C), (E), (O), (CE) and (ACE) at $P \leq 0.05$.There was no significant differences found in AST and ALT serum activity between all groups at $P \leq 0.05$. Rabbits had higher significant values of serum glucose levels of group supplemented with vitamin C (C) when compared with all other groups, but groups (A), (O), (E), (CE), (ACE) and (OCE) showed hypoglycemic effect as compared with that of control group. Serum creatinine and urea levels had no significant differences between all experimental groups. A significant decrease of cholesterol levels of groups (O), (CE), (ACE), (OCE) and a higher significant value of (C) group as compared with its level of control group. Moreover, serum triglycerides levels of (A), (E), (O), (CE), (ACE) and (OCE) were lower significantly than its value of control group. While serum triglycerides value of (C) group was higher than the other experimental groups at $p \leq 0.05$.

Table (3): Effect of *aloe vera* (A),olive pomace (O),vitamin C (C), vitamin E (E),vitamin C + vitamin E (CE),*aloe vera* +vitamin C+vitamin E (ACE) and olive pomace+ vitamin C+vitamin E (OCE) supplementation on serum biochemical parameters of broiler rabbits.

Group / Parameters	Control	(A)	(C)	(E)	(O)	(CE)	(ACE)	(OCE)	LSD
Total protein (g/dl)	10.58	8.88	9.86	7.78	7.78	9.48	8.62	7.12	1.21
Albumin(g/dl)	2.80	2.41	2.98	3.00	2.88	2.92	2.98	2.58	0.40
Globulin (g/dl)	8.10	7.10	6.90	4.90	4.80	7.40	5.40	5.00	1.32
ALT(units/ml)	20.72	20.74	21.10	20.06	21.64	21.48	21.12	20.78	2.06
AST(units/ml)	16.28	16.74	15.32	15.82	16.43	16.38	16.12	16.88	1.41
glucose(mg/dl)	177.93	149.20	198.48	121.87	142.15	152.95	136.30	134.18	4.88
Creatinine(mg/dl)	0.55	0.41	0.36	0.47	0.53	0.76	0.46	0.73	0.22
Serum urea(mg/dl)	16.28	16.74	15.32	15.82	16.43	16.38	16.12	16.88	1.41
Cholesterol (mg/dl)	76.9	70.6	99.6	69.2	60.4	61.0	57.1	62.0	8.6
Triglycerides (mg/dl)	123.08	98.78	135.56	103.76	102.08	89.7	91.02	102.04	5.3

Data indicates mean, n=6 rabbits /group. LSD (least significant difference) at P ≤0.05.

3.3. Antioxidant Parameters:

Results of (Table 4) demonstrate that, serum total antioxidant capacity (TAC) was higher in rabbits treated with (A), (C), (E), (CE), (ACE) and (OCE) as compared with that of the control group at P≤ 0.05. Catalase (CAT) level increased significantly in groups treated with (A), (C), (CE), and (ACE) and (OCE) as compared with its value of control group P≤0.05. Serum Glutathione-S-Transferase (GST) levels of rabbits revealed a higher significant values of (A), (E), (O), (CE), (ACE) and (OCE) as compared with its value of control group. GST (mg/dl) level of group (A) was significantly lower than that of (ACE) and (OCE) groups. Serum Malonaldehyde levels (MDA) was lower significant values in all experimental groups compared with that of control group. Although, the level of MDA (nmol/ml) of (O) group was the same as that of control at P ≤ 0.5.

Duodenum tissue antioxidant Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) concentration showed a significant increase in all experimental groups versus control group.

Table (4): Effect of *aloe vera* (A), olive pomace (O), vitamin C (C), vitamin E (E), vitamin C + vitamin E (CE), *aloe vera* +vitamin C+vitamin E (ACE) and olive pomace+ vitamin C+vitamin E (OCE) supplementation on serum and duodenum tissue antioxidants of broiler rabbits.

Group / Parameters	Control	(A)	(C)	(E)	(O)	(CE)	(ACE)	(OCE)	LSD
Serum TAC (mM/L)	**1.04**	**1.32**	**1.18**	**1.22**	**1.11**	**1.41**	**1.85**	**1.45**	**0.12**
Serum CAT(U/L)	**684.27**	**723.69**	**730.46**	**672.65**	**662.67**	**736.26**	**743.05**	**697.59**	**3.99**
Serum GST(mg/dl)	**2424.9**	**2458.0**	**2425.4**	**2429.2**	**2431.6**	**2433.4**	**2484.4**	**2462.3**	**3.63**
Serum MDA(nmol/ml)	**17.07**	**7.37**	**9.77**	**9.27**	**15.86**	**12.99**	**6.88**	**16.63**	**1.6**
Tissue SOD(U/gT)	**157.82**	**179.15**	**161.05**	**170.51**	**175.32**	**178.43**	**194.77**	**188.48**	**3.80**
Tissue Gpx(U/g T)	**3.10**	**8.36**	**11.29**	**8.62**	**7.14**	**10.62**	**14.39**	**9.01**	**1.04**

Data indicates mean, n=6 rabbits /group. LSD (least significant difference) at P≤0.05.

3.4. Tissue DNA and protein concentrations:

Data tabulated in (Table 5) expose that the total DNA concentration in duodenum tissue of group (ACE) (230.5 ng/µl) was higher than all other experimental groups. While, group supplemented with (O) had the lowest value of DNA (137.4 ng/µl). The total DNA concentration in pancreatic tissue increased significantly in groups supplemented with (ACE), (A), (OCE), (CE), (C) and (O) which were (929.2, 833.8, 739.2, 719.0, 705.6 and 694.0 ng/µl respectively). Meanwhile, a significant decrease in total DNA of groups supplemented with

(E) (441.5 ng/µl) as compared with control (690.1ng/µl).At the same time the protein concentration of duodenal tissue showed a significant higher value in group (OCE) (0.085 ng/µl) than all other experimental groups. Meanwhile, group supplemented with (A) revealed the lowest protein concentration (0.018 ng/µl). The protein concentration of pancreatic tissue showed a significant higher value in group (CE), (OCE) and (O) which were (0.083, 0.081 and 0.069 ng/µl). While, group supplemented with (A), (C), (E) and (ACE) revealed lower values (0.025, 0.050, 0.062 and 0.048 ng/µl respectively) as compared with control (0.063 ng / µl). DNA to Protein ratio of duodenum pointed a significant increase in groups supplemented with (A), (E) and (C) (10306.9, 6919.5 and 5846.2 respectively). Meanwhile, a significant decrease of the ratio for groups supplemented with (O), (CE), (OCE) and (ACE) (1655.4, 2542.4, 2568.2 and 4349.1) as compared with control (3315.2). DNA to Protein ratio of pancreas recorded a significant increase in groups supplemented with (A), (C) and (ACE) which were (33352.0, 13944.7 and 19358.3 respectively). Meanwhile, a significant decrease of DNA to protein ratio of groups supplemented with (E), (O), (CE), and (OCE) (7120.9, 10057.9, 8662.6 and 9125.9) as compared with control (10867.7).

Protein to DNA ratio of duodenum revealed the highest level in group supplemented with (O) (0.0006) and lower value in group supplemented with (A) and (E) (0.0001). Protein to DNA ratio of pancreas revealed no significant difference between all experimental groups.

Table (5): Effect of *aloe vera* (A), olive pomace (O), vitamin C (C),vitamin E (E),vitamin C + vitamin E (CE), *aloe vera* + vitamin C+vitamin E (ACE) and olive pomace + vitamin C+vitamin E(OCE) supplementation on duodenal and pancreatic tissue DNA and protein concentrations.

Group / Parameters	Control	(A)	(C)	(E)	(O)	(CE)	(ACE)	(OCE)
Duodenal Tissue								
DNA (ng/µl)	**152.5**	**194.8**	**159.6**	**163.3**	**137.4**	**205.2**	**230.5**	**216.1**
Protein (ng/µl)	**0.046**	**0.0189**	**0.0273**	**0.0236**	**0.083**	**0.0799**	**0.053**	**0.085**
DNA/Protein	**3315.22**	**10306.9**	**5846.2**	**6919.5**	**1655.4**	**2568.2**	**4349.1**	**2542.4**
Protein/DNA	**0.0003**	**0.0001**	**0.0002**	**0.0001**	**0.0006**	**0.0004**	**0.0002**	**0.0004**
Pancreas Tissue								
DNA (ng/µl)	**690.1**	**833.8**	**705.6**	**441.5**	**694**	**719**	**929.2**	**739.2**
Protein (ng/µl)	**0.0635**	**0.025**	**0.0506**	**0.062**	**0.069**	**0.083**	**0.048**	**0.081**
DNA/Protein	**10867.7**	**33352.0**	**13944.7**	**7120.9**	**10057.9**	**8662.7**	**19358.3**	**9125.93**
Protein/DNA	**0.0001**	**0.0000**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

3.5. Intestinal Morphometry:

Data shown in (table 6) and figure1 (a, b, c) proved that, the villous length had a significant decrease in groups (C), (E), (CE), (ACE) and (OCE) in comparison to control group. The crypt depth showed the highest depth in group (O) than all groups. Moreover, calculated ratio of villous height to crypt depth revealed a significant increase of that ratio in group (A) than that of (C), (E), (O), (CE), (ACE) and (OCE) groups. The gland area in group (E) was larger than all other groups (272.6 µm).While group (C), (CE) and (ACE) recorded (167.6, 145.4 and118.9 µm respectively) with no significant difference. Meanwhile groups (A), (O) and control recorded the lowest values (87.2, 75.4 and 60.4 µm respectively) as shown in Fig. (2 a, b).

Table (6): Effect of *aloe vera* (A), olive pomace (O), vitamin C (C), vitamin E (E), vitamin C + vitamin E (CE), *aloe vera* + vitamin C + vitamin E (ACE) and olive pomace + vitamin C + vitamin E (OCE) supplementation on duodenal villous length (µm), crypt depth (µm) , villous length to crypt depth ratio and gland area(µm).

Group / Parameter	Control	(A)	(C)	(E)	(O)	(CE)	(ACE)	(OCE)	LSD
V.L /µm	380.4	380.9	317.2	218.8	395.8	299.4	322.6	308.7	57.04
Minimum	251.0	309.0	247.0	169.0	342	211.0	277.0	170.0	
Maximum	464.0	438.0	418.0	293.0	463	386.0	389.0	453.0	
C.D /µm	112.8	83.7	120.4	70.6	152.3	90.8	110.1	128.4	36.2
Minimum	46.0	55.0	69.0	56.0	91.0	61.0	85.0	64.0	
Maximum	172.0	114.0	145.0	89.0	281.0	142.0	131.0	163.0	
V/C Ratio	3.64	4.64	2.98	3.00	2.91	3.35	3.93	2.60	1.24
Gland area/µm	60.6	87.2	167.6	272.6	75.4	145.4	137.6	118.9	53.07
Minimum	39.0	45.0	78.0	131.0	51.0	87.0	113.0	48.0	
Maximum	87.0	159.0	343.0	366.0	131.0	210.0	206.0	196.0	

Data indicates mean, n=7 sections rabbits /group. LSD (least significant difference) at P≤0.05.

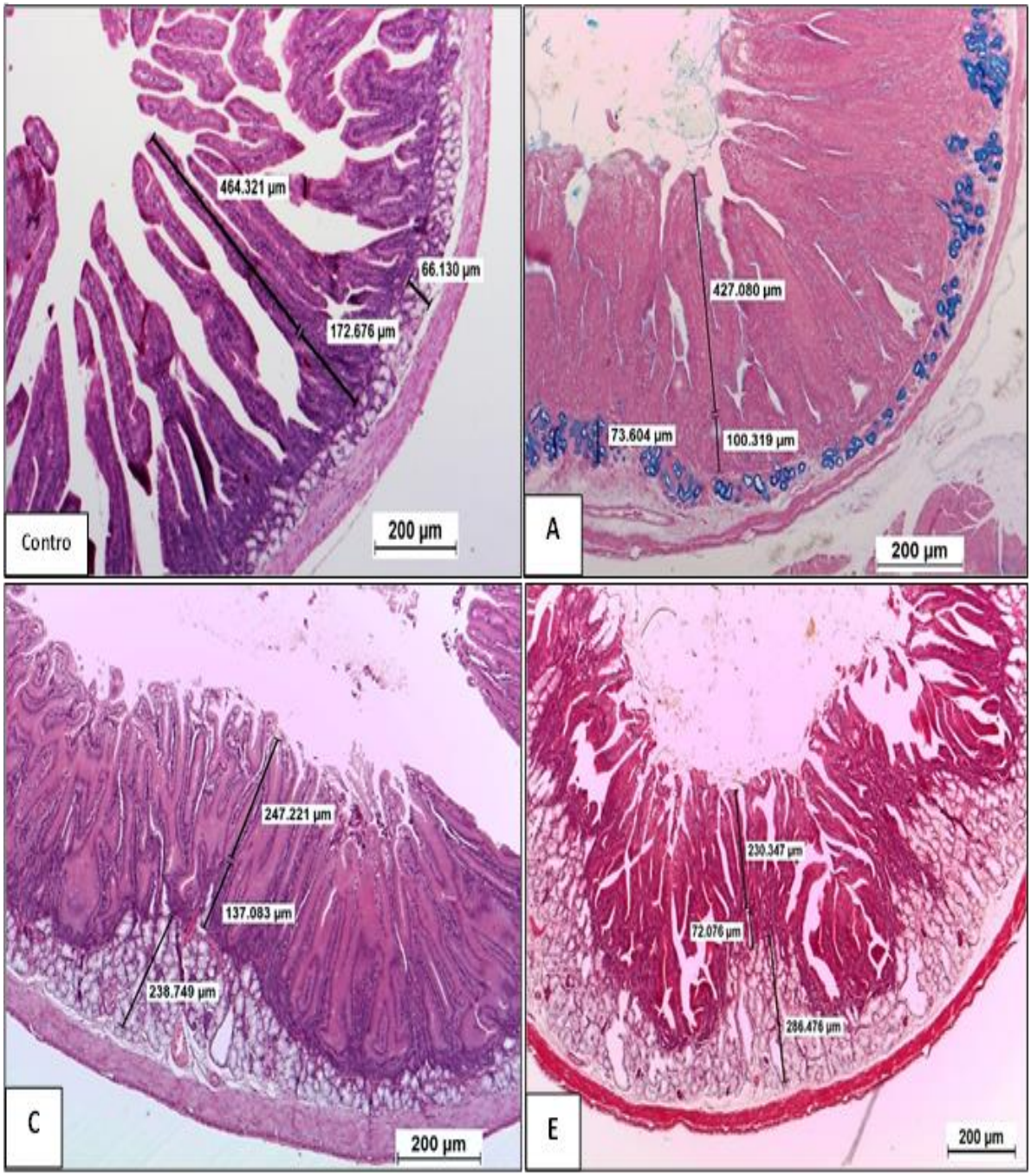

Fig.(1a): Photomicrographs of the small intestine (duodenum) from new zealand white rabbits showing the variation in duodenal villous length, crypt depth and gland area in control group and supplemented groups; (A) *aloe vera* group stained by alcian blue; (C) vitamin C group and (E) vitamin E group. (H&E) stains.

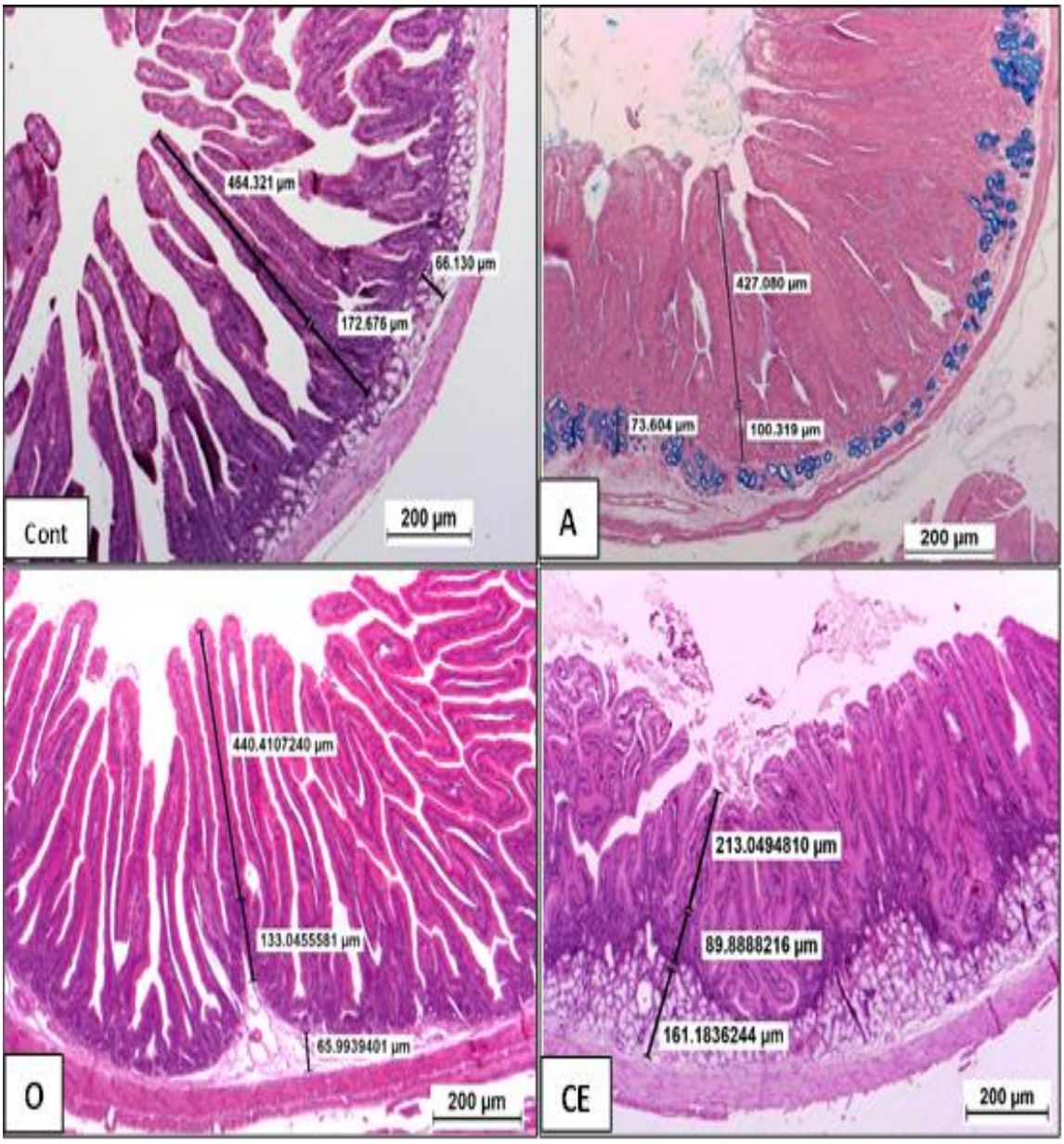

Fig.(1b): Photomicrographs of the small intestine (duodenum) from new zealand white rabbits showing the variation in duodenal villous length, crypt depth and gland area in control group and supplemented groups; (A) *aloe vera* group stained by alcian blue; (O) olive pomace group and (CE) vitamin C+ vitamin E group. (H&E) stain.

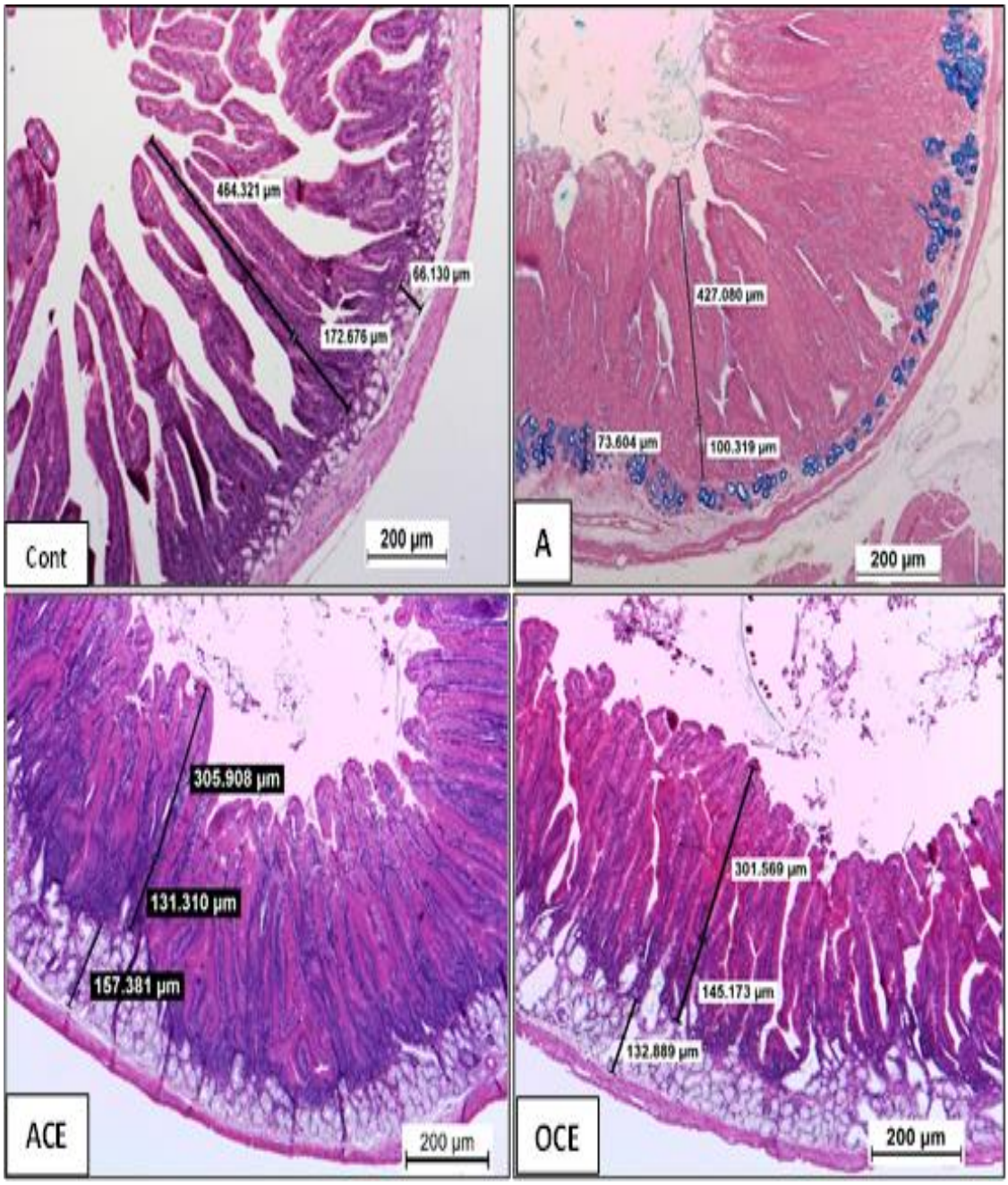

Fig. (1c): Photomicrographs of the small intestine (duodenum) from new zealand white rabbits showing the variation in duodenal villous length, crypt depth and gland area in control group and supplemented groups; (A) *aloe vera* group stained by alcian blue; (ACE) *aloe vera* + vitamin C + vitamin E group and (OCE) olive pomace + vitamin C+ vitamin E group. (H&E) stain.

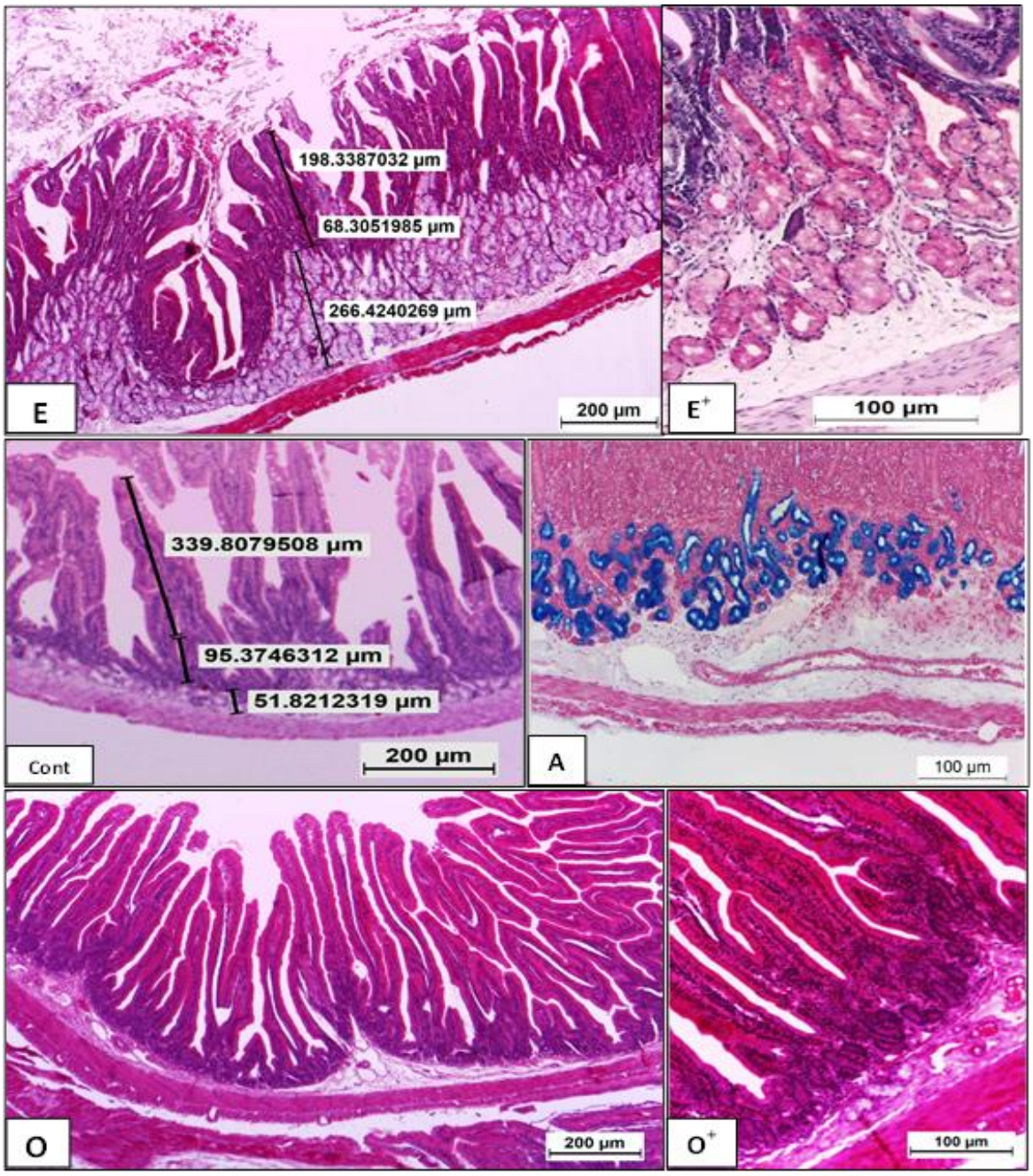

Fig (2a) : Photomicrographs of the small intestine (duodenum) from new zealand white rabbits showing the largest gland areas in (E) vitamin E group, (E^+) a higher magnification of E; the normal values in control group and (A) *aloe vera* group; but little or no glands occurred in (O) olive pomace group (O^+) a higher magnification of O. (E^+) PAS stained (A) alcian blue stain and the other stained by (H&E).

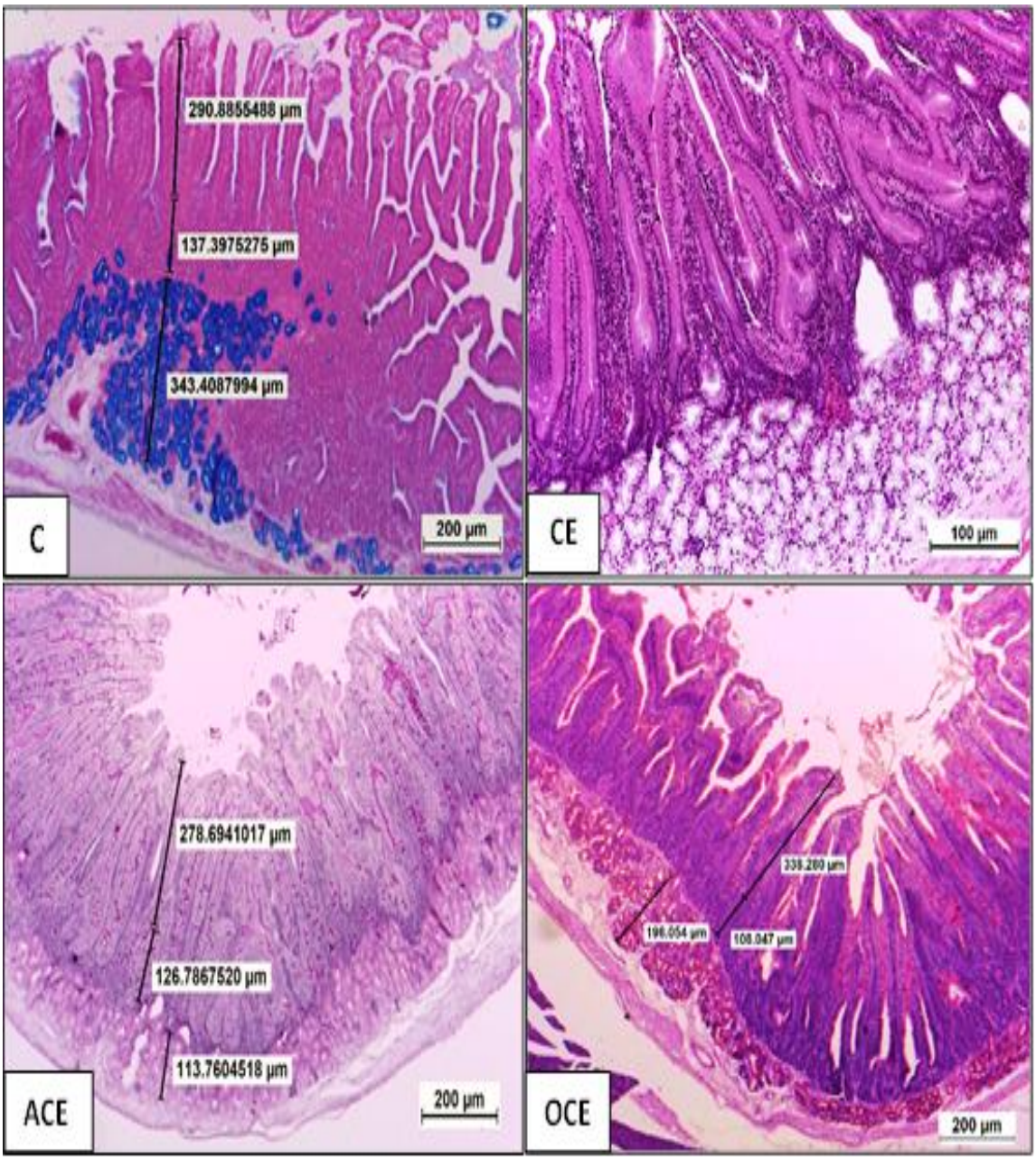

Fig.(2b): Photomicrographs of the small intestine (duodenum) from new zealand white rabbits showing variation of the gland areas in (C)vitamin C group (alcian blue) stain; (CE) vitamin C+vitamin E group (H&E) stain;(ACE) *aloe vera*+vitamin C+ vitamin E group and (OCE) olive pomace+vitamin C+vitamin E group,(PAS) stain.

DISCUSSION

According to the World Organization for Animal Health, lethality due to animal loss of at least 20% of livestock production worldwide **(Kerasioti *et al.*, 2017).** Antioxidant supplementation through nutrition is a must, in order to enhance animal's antioxidant defense system and prevent oxidative stress**.** Among these possible alternatives, herbal products are considered interesting because they have acquired more reliability and acceptability among consumers as safe and natural additives **(Loh *et al.*, 2010 Salim *et al.*, 2013 and Pourhossein *et al.*, 2015).Record *et al.* (2001)** illustrated that, Antioxidants may be classified into enzymatic antioxidant and non-enzymatic antioxidant (vitamin E, vitamin C, *aloe vera* gel and phenolic compound as olive pomace). The present study shows that, the group supplemented with *aloe vera* gel had no difference in live body weight, body weight gain and feed conversion ratio in comparison to all other groups. Similar results were obtained by **Anilakumar *et al*. (2010), Manoj *et al*. (2013) and Safa and Zeineb, (2016)** who found that, feeding 2% *aloe vera* gel not affect live body weight and body weight gain in rats**.** Other studies on herbs revealed that, supplementation of feed with essential oils, plant extracts and herbs powders did not improve feed conversion ratio **(Toghyani *et al.*, 2010)**.However, **Eevuri and Putturu (2013)** found that *aloe vera* supplementation in broilers improved growth indirectly due to anti-bacterial, anti-oxidant, immune-modulatory, antiviral and anti-inflammatory properties of *aloe vera* which lead to better immunity in supplemented group **(Yadav *et al.*, 2017)**. Moreover, **Yim *et al*. (2011)** reported that *aloe vera* gel contains several beneficial ingredients including vitamins, minerals, enzymes, organic acids, and carbohydrates which could improve performance criteria of broilers**.**

Concerning vitamin C & E groups, the present results shows that, rabbits treated with either vitamin C (C) or (E) had mathematical improvement but not significant when compared with control and other groups, which was in agreement with **Selim *et al*. (2004)** who found that, vitamin C supplementation (250mg/kg diet) resulted in a mathematical improvement of growth rate which could be attributed to the enhancement of the total antioxidant status. The lowering feed intake from (9 to 15 weeks old) are in contrary to the results obtained by **Dauda *et al*. (2015)** who indicated that, there was no significant effect of vitamin C on feed intake in rabbits, however there was a significant effect of vitamin C on final weight , weight gain and feed conversion efficiency. The same author suggested that, the beneficial effect of ascorbic acid can

be attributed to the antioxidant effects of vitamin C which is a scavenger of oxygen free radicals which are toxic byproducts of many metabolic processes**,** contrary to **Abu El-Hamd *et al.* (2013) and Hasan, (2014)** who recorded that, the total dry matter intake (DMI),was significantly higher in group receiving 25 or 50 mg vitamin C/rabbit/day in drinking water than those in control group, Concerning the effect of vitamin E, **Niu *et al* .(2009)** indicated that, dietary vitamin E (200 mg/kg) did not significantly influence body weight BW which are proud with the present results. In contrary to these **Ebeid *et al*. (2013)** reported that dietary vitamin E supplementation increased the final body weight. The possible reasons are that, natural antioxidants can protect intestinal mucosa against oxidative damage and pathogens and limit peristaltic activity in digestive disorders preventing diarrhea **(Kermauner and Laurenčič, 2008)** as well as to the immunomodulating properties of vitamin E**.** Olive pomace supplementation lead to no improvement in body weight of broiler rabbit. The former result assured the studies of **Mehrez and Mousa, (2011)** who recorded that, olive pomace could be considered as a source of high fiber content but, it contain low amount of crude protein as obtained by **Dorbane *et al*. (2016)**. The present investigation indicates that, the combination of vitamin C and E (CE) revealed no effect on FCR and body weight gain. Some factors cannot be ignored; e.g. the experimental conditions under which the study was performed (environmental condition, dose of the vitamin or way of introduction either with diet or water, oral or injection) **(Selim *et al*., 2008).** In contrary, **Yoo *et al*. (2016)** stated that, (vitamin E &C) as an antioxidants when added to drinking water, increase growth performance, intestinal morphology, and meat quality under moderate heat stress, suggesting that, supplementation of dietary antioxidants could ameliorate the raising and productivity of broiler chickens.

Moreover, the addition of vitamin C and E to *aloe vera* gel (ACE) showed mathematical improvement of the final body weight as compared with *aloe vera* gel alone (A) which confirm the fact that, *aloe vera* gel improve the bioavailability of the vitamins which was explained to be a possible protection effect against the degradation of the vitamins in the intestinal tract as well as binding of the polysaccharides to the vitamins and thereby slowing down the absorption rate **(Vinson *et al*, 2005).**The group supplemented with (OCE) had no effect on the final live body weight, while feed intake decreased during the period (12-15 week old) significantly as compared with control which could be attributed to the high fiber content (23.1%) in the chemical composition of olive pomace &low protein content as

reported by **Dorbane *et al*. (2016).**The present results revealed that, serum Total protein showed a low significant values of (A), (E), (O), (ACE) and (OCE) groups as compared with that, of the control group. Similar results were obtained by **Sultana and Najam (2013)** which recorded that, given rabbits 500 mg /kg *aloe vera* gel orally decreased the level of serum protein and protein fractions which may be due to the low protein content of *aloe vera* gel. Also olive pomace supplement revealed decreased protein level and assured the results which recorded by **Mehrez and Mousa (2011).** Who indicated that, total protein slightly decreased with level 30% olive pulp, this might be due to the lower digestibility of crude protein in the ration. The liver functions represented by serum AST and ALT activity had no significant differences between all groups at $P \leq 0.05$ indicated that, these supplements are safe to animals and has no harmful effects on liver. Moreover, the present data showed a significant hypoglycemic effect of *Aloe vera* gel in rabbits. Proud with a similar result which was obtained by **Sethi *et al*. (2012) who** found that, supplementation of *aloe vera* gel extract 300mg/kg in aqueous solution for 21 days to alloxan induced diabetic rabbits reverse blood glucose near the normal level. The hypoglycemic effect of *aloe vera* gel may be due to *aloe vera* extract can stimulate the regeneration of pancreatic β-cells increases the resistance of the cells, mitochondrial activity, and insulin production and improve serum antioxidant enzyme levels, thus preventing pancreatic cell damage in diabetics rats **(Mohapatra *et al*., 2013).** The lipid profiles results of the study indicated that, *aloe vera* supplementation led to significant decrease of serum triglycerides level**.** Similar results were obtained by **Rajasekaran *et al*. (2006)**.Plasma concentrations of creatinine and urea could be used as indicators of kidney functions. The present results demonstrate that, *aloe vera*, olive pomace, vitamin C and E administration to broiler rabbit had no effect on serum creatinine and urea levels which means normal function of the kidney. Current study with olive pomace showed a decrease in serum **cholesterol and triglycerides** which in agreement with the study of **Gorinstein *et al*. (2002)** who reported that, polyphenols from olive oil decrease plasma LDL-C levels and prevent their oxidation *in vivo*. **Fki *et al*. (2005)** assured the former studies that, the phenol-rich extract of olive has hypocholesterolemia effect which might be due to its ability to lower serum cholesterol level as well as to retard the lipid peroxidation process and to enhance the antioxidant enzyme activity .The mechanism of this hypocholesterolemia action may be due to the inhibition of dietary cholesterol absorption in the intestine (**Krzeminski *et al*., 2003)** or

stimulation of the biliary secretion of cholesterol and cholesterol excretion in the faces **(Prasad *et al*., 1993). Coni *et al*. (2000)** concede that, addition of 10% extra-virgin olive oil and 7 mg/kg oleuropein to the standard diet reduces plasma levels of total cholesterol and increases the ability of LDL to resist oxidation in the rabbits. However, blood total lipids and cholesterol significantly increased for growing lambs fed 25% olive pulp than the control group **(Mousa, 2001).** The group receiving olive pomace or (OCE) had potent lowering of glucose, cholesterols and triglycerides which go in accordance with **Yousaf *et al*. (2014)** who reported that, olive pomace containing some residual oil after refining was done, this oil contains monounsaturated fatty acids (MUFA) and chemically its fat composition is quite similar to regular olive oil. It is cheaper than extra virgin olive oil whose effect on glycemic status and lipid profile is well documented which may regulate blood glucose level by enhancing secretion of glucagon-like peptide-1 (GLP-1) from intestinal cells **(Gorinstein *et al*., 2002)**. GLP-1 is the potent anti-hyperglycemic hormone, which stimulates the proliferation and differentiation of insulin secreting β-cells, insulin biosynthesis, glucose - dependent insulin secretion, restores glucose sensitivity of pancreatic β-cells and suppress glucagon secretion **(Doyle and Egan, 2007).**The obtained data showed that, *aloe vera* gel administration resulted in significant increase of serum total antioxidant capacity (TAC), catalase (CAT), glutathione S- transferase (GST) with reduction of malondialdehyde (MDA). this results go hand by hand with previous study of **Prueksrisakul *et al*. (2015)** who demonstrated that, daily consumption of *aloe vera* gel extract significantly increased plasma total antioxidant activity, there are several active compounds in *aloe vera* that, reported the evidence for antioxidant capacity such as acemannan, aloin, aloe-emodin and aloesin in vitro **(Harlev *et al*., 2012)** therefore, the antioxidant activity may be the sum of several active compounds in *aloe vera* rather than from a single active component. The synergy of a number of components such as, glucomannans, acemannan, minerals, flavonoids, tannic acid, c-glucosylc hormone, etc. present in the aloe vera may be responsible for the observed reduction in Azoxy methane (AOM) induced oxidative stress and toxicity. Supplementation of *aloe vera* gel extract (300mg /kg) in aqueous solution nearly normalized the levels of antioxidant enzymes as reduced glutathione (GSH), superoxide dismutase (SOD), with reduction of MDA in alloxan induced diabetic rabbits **(Sethi *et al*., 2012).**The current results showed increase in TAC, CAT, SOD and GPx with decrease in MDA in rabbits supplemented

with vitamin C. Concede the results of **Yousef *et al*. (2007)** who found that, treatment of rabbits with vitamin C caused increased activity of superoxide dismutase and catalase when compared to control besides, a decrease in plasma MDA level. Also **Khaki *et al*. (2011)** recorded that, treatment of rats with vitamin C resulted in significant increase in concentration of TAC, SOD and significant decrease in serum MDA. Our results indicate that, vitamin E as a supplement to rabbits showed increase in concentration of TAC, GST, SOD, GPx and MDA serum level versus control. Similar results obtained by **Perez-Matute*et al.* (2012)** who found that vitamin E increased the activity of several antioxidants enzymes such as SOD, glutathione peroxidase (GPx) and TAC, whereas, it is able to decrease the levels of isoprostane 8-epi PGF2 alpha , which is product of oxidative stress. Also, **Aksoy *et al*. (2005)** found that vitamin E potentiates the antioxidant system by increasing glutathione and antioxidant enzymes, such as superoxide dismutase and glutathione peroxidase**.** Also vitamin E supplementation in broiler birds improve activity of antioxidant enzymes by increasing GSH-Px and SOD activities and decrease MDA concentration **(Ebrahimzadeh *et al*., 2018).**The present results showed that olive pomace supplementation group had increased in serum glutathione -s-transferase GST level significantly as compared with control. Similar to the present results **Kerasioti *et al*. (2017)** found increased GST in sheep supplemented with olive mill waste, the beneficial effect of olive pomace derived from phenolic compounds have been attributed to antioxidant activity. The antioxidant activity of phenolic compounds is due to scavenge free radicals, donate hydrogen atoms or electron and chelate metal cation **(Dai and Mumper, 2010).** The protective effects of oleuropein on lipid oxidation was demonstrated through the evaluation of the decreased formation of lipid peroxides by-products, such as malondialdehyde (MDA**) (Visioli *et al*., 1995).**Studies suggest that, vitamin C and E act synergistically **(Gey, 1998).** Vitamin E acts as primary antioxidant quenching lipid peroxide radicals. The resulting vitamin E radicals is regenerated by vitamin C. In this respect vitamin C and vitamin E work together such that vitamin E is the major chain breaking antioxidant in lipid phase such as cellular membrane or low density lipoprotein, and oxidizing free radicals in aqueous compartments via vitamin C as terminal reductant **(Packer, 1991 and Macpherson, 1994).**The results of the present study indicated that combination of *aloe vera* , vitamin C and vitamin E (ACE) had the highest levels of antioxidant enzymes (TAC, CAT, GST, SOD and GPx) with lowest level of MDA which attributed to presence of polysaccharides of natural origin as chitosan in *aloe vera* gel are capable of enhancing the

intestinal absorption of co-administered drugs by means of a transient opening of the tight junctions between adjacent epithelial cells to allow for par cellular transport across the intestinal epithelium and decrease the trans epithelial electrical resistance of intestinal epithelial cell monolayers, thereby indicating opening of the tight junctions between adjacent epithelial cells. **(Hamman and Viljoen, 2008 and Chen, 2008)**. Also, *aloe vera* gel improve the bioavailability of the vitamins which was explained to be a possible protection effect against the degradation of the vitamins in the intestinal tract as well as binding of the polysaccharides to the vitamins and thereby slowing down the absorption rate **(Vinson *et al.*, 2005).**The recent science (Nutritional Genomics) which study the interactions of food and its components with the genome at the molecular, cellular and systemic levels and study how dietary nutrients can interact with genes affecting transcription factors, RNA and protein expression, cellular homeostasis and metabolite production **(Benitez *et al.*, 2017)**. Therefore the determination of DNA and protein concentrations of intestinal tissue, together with morphological measurements, is a must to provide new knowledge about the pattern of intestinal development. The present study showed a significant increase of the DNA concentration in both duodenal and pancreas tissue of groups (CE), (ACE) and (OCE) versus that control. While, the former groups revealed significant increase in protein concentration in duodenal tissue; the pancreas tissue pointed a higher protein concentration in groups (CE) and (OCE) only versus to control group (table, 5).The former results indicate that, the combination of the feed additives (CE and OCE) resulted that, there was an increase of the cell population in both duodenal and pancreas tissue, which concede with **Jin *et al*. (1998) and Uni and Sklan, (1999)** who recorded that**,** the DNA concentration in a tissue reflect its rate of mitosis in a cell population. Concerning DNA to protein ratio of the present study which recorded a highly significant increase of (A) group compared to all groups. In addition, a lower ratio of control compared to (C), (E) and (ACE) groups (table, 5) .The former ratio (DNA/protein ratio) of the pancreatic tissue revealed that, combination of *aloe vera*, vitamins C and E (ACE) as a feed additives has a highly significant DNA/protein ratio than all other groups, these indicate decrease in the cell size of pancreatic tissue **(Uni and Sklan, 1999).** The present results of protein/DNA ratio in groups (O), (CE) and (OCE) recorded a significant increase in duodenal tissue than all other groups, these proud with **Uni and Sklan, (1999)** who recorded that**,** the DNA concentration in a tissue reflect its rate of mitosis in a cell

population with the protein to DNA ratio indicating the cell size. The former results are concede with results of **Jin *et al*. (1998)** who found that, concentrations of DNA and its ratio to protein content, undergo natural variation during the development of the gastrointestinal tract with the pattern of changes described by **Uni *et al*. (1998), and Iji (1999).** However, the patterns reported have not always been consistent, and differences between species and breeds have been seen. Increased values of villous height and cell mitosis numbers as well as protuberated cells were found in activated function of intestine **(Yamauchi *et al*., 2010).** Long intestinal villous mean a greater surface area for nutrient absorption **(Onderci *et al*., 2006)**. Therefore, intestinal histological is thought to be able to assess the supplemented feed ingredients **(Maneewan *et al*., 2012).** Furthermore, increased villous size was also related to raised cell proliferation in the crypt **(Lauronen *et al*., 2000)** and provided more surface area for nutrient absorption and thus improved nutrient digestibility **(Onderci *et al*., 2006).** Although villous height is correlated positively with body weight gain and feed intake **(Kelly *et al*., 1991),** the same cannot be observed in this study because the performance parameters body weight gain, feed intake, and FCR did not present significant effect in relation to the treatments. The present investigation revealed that, broiler rabbits supplemented with *aloe vera* gel with dose 500mg/L drinking water did not increase the villous height than control with a no significant decrease of crypt depth, with mathematical improvement of villous height /crypt depth ratio. Similar results obtained by **Shokri *et al*. (2017)** who found that, bird fed with *aloe vera* powder 2.5 g/kg not increase villous height (VH) or crypt depth (CD) with slight improved VH to CD ratio, where broiler birds fed with *aloe vera* powder 5 or 7.5 g/kg had significant increase of villous length and significant decrease of crypt depth, thus higher VH to CD ratio. The improvement of VH to CD ratio was related to epithelial cells turnover **(Shokri *et al*., 2017)** since improvements in VH to CD ratio indicates better nutrient absorption. Contrary to the present results **Darabighane *et al*. (2011)** found that, adding *aloe vera* gel to diet of bird by 2% had higher villous height, lowest crypt depth with the highest VH to CD ratio. Lower crypt depth in *aloe vera* gel groups than that, of the control an observation that, can be explained based on reduced bacteria count in intestine and therefore, reduction in turned over mucous- generating cells. Reduced villous height to crypt depth ratio can also indicate presence of toxins, reduced absorption of nutrients, increased secretion in gastrointestinal tract, diarrhea, reduced disease resistance and lower overall performance

(Xu *et al*., 2003), who observed that, the villous height to crypt depth ratio in the 2% and 2.5% *aloe vera* gel groups was significantly different from other groups, with the 2% *aloe vera* gel group having the largest ratio, which can indicate better performance of this group. Vitamin C lead to significant decrease of villous length but crypt depth not influenced, also the VH to CD ratio showed no significant difference. Contrary to this results **Moghaddam *et al*. (2009)** reported that, duodenum villous height, width, lamina propria thickness and surface area were greater in vitamin C treated group .Also **Hajati *et al*. (2014)** reported that, ascorbic acid supplementation lead to significant increase of duodenum villous height, width and VH to CD ratio, these studies confirm that, proliferative, anti-apoptic and antioxidative effects of vitamin C may be the factors responsible for increased villous dimensions. However**, Yoo *et al*. (2016)** found that, addition of vitamin C with vitamin E (150ppm vitamin C and 75ppm vitamin E) to broiler diet results in higher villous height, lower crypt depth and higher villous height to crypt depth ratio as compared by control group. Significant decrease of villous length and crypt depth in duodenum of rabbits supplemented with vitamin E (1ml/l drinking water) versus that, of the control group, the VH to CD ratio showed no significant difference, however there are a significant increase of gland area than all groups. Similar to the obtained data **Ebrahimzadeh *et al*. (2018)** found that, supplementation of broiler diet with vitamin E (200 mg/kg α-tocopherol acetate) results in significant decrease of the villous height to crypt depth ratio and muscular thickness of chicken duodenum as compared with control group**.** Contrary to the present results **Shirpoor *et al*. (2006)** observed that, the small intestine villous length and crypt depth were not altered. While **Murakami *et al*. (2006)** reported that, broiler chickens supplemented with 10 mg Vitamin E/kg of diet had a higher villous height as compared with that of control group. The present data revealed that, **olive pomace** fed rabbits (10% of feed) had statistically higher villous height, where crypt depth increased significantly than control while the villous height **to** crypt depth ratio was not affected. Similarly, **Ebrahimzadeh *et al*. (2018)** recorded that, broiler chicken fed grape pomace (polyphenol rich by-product) had no effect on villous height or crypt depth. Contrary to the present results, **Viveros *et al*. (2011)** observed a reduced villous length of bird fed polyphenol rich by-product (grape pomace concentrate) and reduced crypt depth with significant increased VH to CD ratio. It is obvious in the present study that the supplemented rabbits groups revealed a significant larger (great scale) of sub-mucosal gland area of groups

(C, E, CE, ACE, OCE), these compensate the decrease in villous length of the same groups. The gland secretes more mucous lining which lubricate and protect mucosal epithelia from damage caused by food, digestive secretions, and microorganisms. Mucous also serves as a selective barrier for absorption across the small intestine **(Guyton and Hall, 1997).**

CONCLUSION

In conclusion, supplementation of rabbits with *aloe vera* gel mixed with vitamin C and E had the best antioxidant activity and the used supplements have no harmful effects on liver and kidney. Additionally, olive pomace supplementation could be considered as a beneficial tool for maintaining health and performance parameters with reduced production costs (13% less than the control).

Table (7): Cost of Production of Rabbits under Different Treatments at the End of Experiment.

Group / Parameters	Control	(A)	(C)	(E)	(O)	(CE)	(ACE)	(OCE)
LBW at 15weeks old/group /kg	**16.52**	**13.68**	**14.88**	**14.88**	**14.52**	**13.80**	**14.4**	**13.98**
Total feed consumption (/group of rabbit)	**44.64**	**44.52**	**37.86**	**44.46**	**37.86**	**44.46**	**44.7**	**37.86**
Aloe vera cost (group/LE)		**10**					**10**	
Vitamin E cost (group/LE)				**26.25**		**26.25**	**25.5**	**33.0**
Vitamin C cost (group/LE)			**17.5**			**17.5**	**17.5**	**22.0**
Total cost (group/LE)	**140.66**	**150.22**	**136.96**	**165.57**	**109.2**	**183.79**	**193.82**	**164.2**
Total cost (Rabbit/LE)	**23.44**	**25.03**	**22.8**	**27.59**	**18.2**	**30.6**	**32.3**	**27.37**
Total cost /kg	**8.51**	**10.98**	**9.20**	**11.12**	**7.52**	**13.31**	**13.45**	**11.74**
± Cost % of kg in relation to control		**< 29%**	**< 8%**	**< 31%**	**>13%**	**< 56%**	**< 58%**	**< 37%**

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