

Research Article



Impact of Dietary Addition of Aloe Vera Leaves Powder on Productive Performance, Caecal Ecosystem and Some Physiological Parameters of Growing Rabbits

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Abstract | The inclusion of phytochemicals in rabbit diets is a strategy that has been used to improve animal productivity by protecting against oxidative stress which in turn increasing the production yield. This study hypothesized that using *Aloe vera* (AV) leaves powder may provide protection against stress and enhance animal physiological responses. Thus, this study aimed to investigate the effect of dietary AV addition on growth traits, caecal ecosystem and some physiological and biochemical responses in growing rabbits. APRI rabbits (age = 6 weeks, n = 60) were split equally into four groups (n = 15 in each group). First group was fed a basal diet only (control group, G1), while the other three groups fed a basal diet along with 0.5 (G2), 1.0 (G3) and 2.0 g (G4) AV leaves powder/kg diet. The results revealed that, AV addition at its highest levels (G3 and G4) significantly ($P \leq 0.05$) increased the final body weight, daily weight gain and feed efficiency. The AV inclusion, especially in high dose "G4", lead to significant ($P \leq 0.05$) reduction in bacteria count of *E. coli* and clostridium spp. and increased total bacterial count in caecal content. The caecal NH_3 concentration significantly ($P \leq 0.05$) decreased when the dietary AV level increased. Haematological, plasma biochemical and antioxidant enzymes (SOD, GPx and CAT) were significantly elevated ($P \leq 0.05$) by the highest dietary AV inclusion (G4). The addition of AV significantly enhanced immune- responses; lymphocyte percentage, IgG and IgM increased when growing rabbits were fed G4 compared to the control group. In conclusion, dietary addition of 1 or 2g AV may serve as an enhancer of growth and immune response in growing rabbits.

Keywords | Aloe vera, Antioxidants, Blood, Growth, Immunoglobulin, Rabbits.

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INTRODUCTION

Scientists' focus has recently shifted from antioxidant vitamins to antioxidant phytochemicals, which can shield people from a variety of illnesses and hence lengthen their lives (Yeung et al., 2019). Also in rabbit production, antioxidant phytochemicals are being studied due to their ability to reduce oxidative stress which in turn lead to improve

antioxidant status and productive performance (Attia et al., 2019; El-Kholy 2019; 2021).

In livestock production, several nutritional strategies have been devised to alleviate antioxidant defense deterioration (Albonetti et al., 2017; El-Kholy et al., 2018a,b). Due to their antioxidant effects and safety, herbal supplements are widely utilized as feed additives to benefit both human and

animal health (El-Shall et al., 2021). Aloe vera (AV) is an old medicinal plant that is grown mainly in the dry regions of Africa, Asia, Europe and America (Akinyele et al., 2007). The name “aloe vera” comes from the Arabic word “alloeh,” which meaning brilliant and bitter while “vera” in Latin means “true” (Surjushe et al., 2008). Aloe vera have different medical applications due to its contains 75 potentially active constituents as it has antibacterial activity, antiseptic, antiviral, antifungal, and anti-inflammatory agent, and immune stimulants (Babak and Nahashon, 2014). By functioning as a powerful antiviral agent against the human immune deficiency virus (HIV) and hepatitis, it is also used to treat numerous viral infections and strengthen the immune system (Rabe et al., 2005). Barbaloin (one of the AV components) and other products of the phenylpropanoid pathway are commonly referred to as polyphenolic compounds (Khalid et al., 2020). These are derived from the precursor phenolic acids, and they may act as antioxidants to inhibit free radical-mediated cytotoxicity and lipid peroxidation (Cook and Samman, 1996). The inclusion of AV in poultry feed as a natural supplement can improve poultry productivity and health (Ebrahim et al., 2020). Thus, the phytochemicals found in AV may be responsible for their in vitro antioxidant effects. Acemannan is one of the highly significant physiologically active chemicals (found in AV) that is thought to be the primary cause of its medicinal effects (Mascolo et al., 2004). Aloe vera also contains anthranoids, polyphenols, and Pyron derivatives, steroids, saponins, salicylic acid, fiber and mineral components (calcium, manganese, potassium, chromium, iron, phosphorus, zinc and natrium), as well as vitamins (ascorbic acid, E, β -carotene, B₁, B₂, B₃, B₆, choline, B₁₂, folic acid) (Sahu et al., 2013; Khalid et al., 2020).

Thus, there is an urgent need to explore promising alternatives to natural antioxidants that may increase rabbit productivity. Therefore, this study aimed to assess the *in vivo* antioxidant activity property of AV grown in Egypt and its effects on growth performance, caecal ecosystem and some physiological parameters in growing rabbits.

MATERIAL AND METHODS

This research work was conducted in Rabbits Research Unit, at Sakha Research Station, Kafr El-Shiekh governorate, Animal Production Research Institute, (APRI) Agricultural Research Center (ARC), Egypt. All the growing rabbits were raised according to rabbit's house management standard operating procedures.

ETHICAL APPROVAL

The scientific committee of the APRI, the Agriculture Research Center, the Ministry of Agriculture and Land Reclamation, Egypt, approved the experimental procedure

(protocol No. 01-05-03-37).

EXPERIMENTAL DESIGN AND ANIMALS

A total number of 60 mixed-sex growing APRI rabbits aged 6 weeks and initial body weight (610.83 g \pm 38.43) were divided randomly into four groups (n= 15). The treated groups were: The 1st group (G1) received basal diet without any supplementation as a control group. While the 2nd (G2), 3rd (G3) and 4th (G4) groups were received basal diet supplemented with 0.5g, 1.0g and 2.0g AV leaves powder (g/kg diet), respectively. Rabbits were housed in individual galvanized wire pyramidal batteries (30 \times 25 \times 35 cm) with feeder and automatic nipple drinkers. The batteries were arranged in rows in a windowed house naturally ventilated. All rabbits were kept under the same management conditions. Feed and water were supplied ad libitum. From 6 to 14 weeks of age, rabbits were fed a commercial growth diet, as shown in Table 1, designed to meet the National Research Council's (NRC, 1977) recommendations for post-weaning rabbits' nutritional needs.

Table 1: Ingredients and chemical analyses of the basal diet.

Ingredients %		Chemical analysis %	
Alfalfa hay (12%)	28.55	Crude protein	17.10
Barley	34.40	Crude fiber	12.74
Wheat bran	11.00	Crude fat	1.99
Soybean meal (44%)	17.00	Digestible energy (kcal/kg)	2500
Molasses	5.00	Calcium	1.30
Limestone	0.85	Total Phosphorus	0.80
Di-calcium phosphate	2.10	Methionine	0.63
Sodium chloride	0.40	Lysine	0.86
		Meth.+Cyc.	0.91
Mineral and vitamin premix ⁽¹⁾	0.30		
DL-Methionine	0.40		
Total	100		

⁽¹⁾ Each 3 kg contain: 6000000 IU Vit. A; 900000 IU Vit. D3; 40000 mg Vit. E; 2000 mg Vit. K3; 2000 mg Vit. B1; 4000 mg Vit. B2; 2000 mg Vit. B6; 10 mg Vit. B12; 50 mg Biotin; 10000 mg Pantothenic acid; 50000 Niacin; 3000 mg Folic acid; 250000 mg Choline; 8500 mg Mn; 50000 mg Zn; 50000 mg Fe; 200 mg I; 100 mg Se, 5000 mg Cu, and 100 mg Co.

PREPARATION OF ALOE VERA LEAVES POWDER

The full, fresh AV leaves were purchased from a nearby market, cleaned under running tap water, dried in a hot air oven at 50°C and then 105°C until they reached a constant weight, and then ground into fine powder using a manual grinder as described by Mohamed et al. (2017). Resulting powder of AV leaves was stored in the dried bottles till use by mixed with commercial pelleted diet in the presence of

molasses to avoid the loss of additives' functionality and concentration.

EXPERIMENTAL PROCEDURES

Growth performance: The body weight gain and feed intake were recorded weekly for each rabbit during the growing period from 6 to 14 week. Accordingly, the average of both daily body weight gain (BWG, g/d) and daily feed intake (FI, g/d) were calculated. The feed conversion ratio (FCR) was determined as a ratio of (g feed/g weight gain).

Carcass traits: At the end of the experiment period, 14 weeks of age 3 rabbits/ treatment were randomly taken and fasted for 12 hours from each group before being slaughtered (to complete bleeding) to estimate carcass traits according to [Biasco and Ouhayoun \(1996\)](#).

Microbiological measurements: Cecum contents were collected from three slaughtered rabbits/treatment immediately to investigate total bacterial counts ($\times 10^6$ CFU/g caecal digesta) *Cholsterida* spp. ($\times 10^5$ CFU/g caecal digesta), *E. coli* spp. ($\times 10^5$ CFU/g caecal digesta), *Lactobacillus* bacteria ($\times 10^5$ CFU/g caecal digesta) and urealtic bacteria ($\times 10^5$ CFU/g caecal digesta) after slaughtering by Pour Plate Count Technique according to [British Standard Institution \(1991\)](#). Also, the pH of the caecal digesta was taken immediately after laparotomy, with a digital pH meter (AD130 Proof PH-ORP-TEMP Portable Meter ADWA). In addition, portions of the caecal digesta sample (5–10 g fresh matter) were put in tubes containing (2%, (v/v)) either H_3PO_4 or H_2SO_4 storage solution (1 and 2 ml per tube), respectively, for further analyses of total volatile fatty acid (VFA) and ammonia (NH_3) and stored at $-18^\circ C$. Ammonia (NH_3) concentrations were measured using the procedure outlined by [Searle \(1984\)](#). Total VFA were measured by gas chromatography method (Thermo-Electron mod. 8000top) by the method of [Bovera et al. \(2008\)](#) adapted to a fused silica capillary column.

Blood constituents: Blood samples of growing rabbits were collected immediately after slaughtering in heparinized glass tubes from 3 rabbits / treatment. Plasma was separated by centrifugation at 3000 rpm for 15 min and kept at $-20^\circ C$ until analyzed. Heparinized blood samples were tested shortly after collection to determine blood pictures such as, red blood cells count (RBCs, $10^6/mm^3$), white blood cells count (WBCs, $10^3/mm^3$); different subclasses of WBC's (lymphocyte (L); neutrophils (N), hemoglobin (Hb, g/dl) concentration and hematocrite (Ht, %), according to [Drew et al. \(2004\)](#). The ratio of N to L was calculated. Quantitative colorimetric of total protein (TP, g/d), albumin (Alb, g/d), creatinine (CR, g/dl) levels in plasma were determined using commercial kits (Bio-Diagnostics Co., Cairo, Egypt). The concentration of globulin

(Glb, g/dl) was calculated by deducting the values of Alb from the equivalent values of TP. Albumin/ Globulin ratio (A/G ratio) was calculated. Plasma samples were analyzed for the activity of aspartate (AST, U/L) and alanine amino transferases (ALT, U/L) using commercial kits (Diamond Diagnostics, Egypt) according to the manufacturer procedure. Also, the plasma was assayed for cholesterol levels using standard protocol methods ([Vogel and Vogel, 1997](#)). Catalase (CAT, U/mg) activity, glutathione peroxidase (GPx, U/mg), superoxide dismutase (SOD, U/ml) activity, and plasma malondialdehyde (MDA, nmol/ml) levels were spectrophotometer "UV4802 (Unico Co., Dayton, USA) "–based calorimetrically measured using commercial kits (purchased from Bio-Diagnostic, Cairo, Egypt, according to the manufacturers' instructions). The plasma levels of immunoglobulin A (IgA, mg/dl), immunoglobulin G (IgG, mg/dl) and immunoglobulin M (IgM, mg/dl) were determined by ELISA kits (Kamiya Biomedical Company, USA) according to [Van der Zipp et al. \(1983\)](#).

Statistical analysis: The data analyzed by using the general linear model (GLM) procedures of [SAS \(2004\)](#), by applying one-way analysis of variance (ANOVA). The following statistical model was: $Y_{ij} = \mu + T_i + e_{ij}$ Where: Y_{ij} = the observation of the j_{th} rabbit in the treatment i ; μ = overall mean; T_i = effect of treatments, ($i = 1, 2, 3$ and 4) and e_{ij} = random error component assumed to be normally distributed. The differences among groups' means were separated by Duncan's multiple range ([Duncan, 1955](#)).

RESULTS

GROWTH PARAMETER

A significant ($P \leq 0.05$) increase in final body weight (FBW) and BWG were observed in G3 and G4 while G2 showed insignificant differences in compared to G1 ([Table 2](#)). Best FCR was observed in G4 which was followed by G3, G2, and G1. Also, [Table 2](#) shows a significant ($P \leq 0.05$) decrease in FI in G3 and G4 while G2 showed insignificant differences in compared to G1.

CARCASS CHARACTERISTICS

Dietary addition of AV did not affect ($P \leq 0.05$) dressing % and percentages of liver, heart, spleen, edible giblets, total edible parts and shoulder fats, while abdominal fat decreased significant in all treated groups compared to the control. However, carcass weight was significantly improved by AV powder ([Table 3](#)). High carcass weight was observed in the group fed G4 diet than other groups. A significant ($P \leq 0.05$) decrease in kidney percentage was observed in G4 while G2 and G3 showed insignificant differences in compared to G1.

Table 2: Growth performance of growing APRI rabbits as affected by dietary addition of Aloe vera leaves powder.

Item	Control G1	Aloe vera leaves powder (g/kg)			±S.E
		G2(0.5)	G3(1.0)	G4(2.0)	
Average body weight (g)					
Initial body weight (g)	610.83	610.83	610.83	610.83	38.43
Final body weight (g)	2335.8 ^c	2373.7 ^{bc}	2420.4 ^{ab}	2464.5 ^a	27.48
Average daily body weight gain (g)					
Weeks 6-10	32.5 ^b	36.3 ^a	37.08 ^a	37.41 ^a	0.865
Weeks 10-14	29.08	26.58	27.83	29.16	1.110
Weeks 6-14	29.25 ^b	29.83 ^{ab}	30.83 ^{ab}	31.41 ^a	0.533
Average daily feed intake (g/day)					
Weeks 6-10	71.25	71.08	69.75	67.08	2.497
Weeks 10-14	116.5 ^a	110.83 ^a	99.16 ^b	92.50 ^b	3.548
Weeks 6-14	93.83 ^a	91.00 ^a	84.50 ^b	79.83 ^b	2.257
Feed conversion ratio (g feed/ g gain)					
Weeks 6-10	2.21 ^a	1.98 ^{ab}	1.90 ^b	1.79 ^b	0.094
Weeks 10-14	4.06 ^{ab}	4.37 ^a	3.59 ^{bc}	3.20 ^c	0.233
Weeks 6-14	3.20 ^a	3.08 ^{ab}	2.77 ^{bc}	2.55 ^c	0.108

^{a, b and c} Means in the same row with different superscripts are significantly different at (P≤0.05). SE = Standard error of means.

Table 3: Carcass characteristics of growing APRI rabbits as affected by dietary addition of Aloe vera leaves powder.

Item	Control G1	Aloe vera leaves powder (g/kg)			±S.E
		G2(0.5)	G3(1.0)	G4(2.0)	
Pre-slaughter weight(g)	2341.67 ^b	2391.67 ^b	2358.33 ^b	2575 ^a	38.67
Carcass weight (g)	1211.67 ^b	1293 ^{ab}	1280 ^{ab}	1356 ^a	27.30
Dressing (%)	51.78	54.08	54.26	52.70	1.09
Liver (%)	3.85	3.73	2.98	5.25	0.946
Heart (%)	0.45	0.41	0.37	0.29	0.054
Kidney (%)	0.89 ^a	0.89 ^a	0.82 ^a	0.61 ^b	0.055
Spleen (%)	0.098	0.092	0.115	0.071	0.021
Edible giblets (%) ¹	5.20	5.03	4.17	3.94	0.378
Total edible parts (%) ²	56.98	59.12	58.44	56.64	1.260
Cecum weight (g)	119.73	155.93	123.47	129.23	14.37
Shoulder fat(%)	7.43	6.2	5.56	5.46	0.672
Abdominal fat(%)	28.5 ^a	13.63 ^b	11.03 ^b	9.23 ^b	1.595

^{a and b} Means in the same row with different superscripts are significantly different at (P≤0.05). SE = Standard error of means. ¹Edible Giblets %=(liver+ kidney+ heart)/Pre-slaughter weight (g)×100. ²Total edible parts%=(carcass wt.+ edible giblets wt.)/Pre-slaughter weight (g)×100.

Table 4: Microbial count and activity in caecum of growing APRI rabbits as affected by dietary addition of Aloe vera leaves powder.

Item	Control G1	Aloe vera leaves powder (g/kg)			±S.E
		G2(0.5)	G3(1.0)	G4(2.0)	
Ph	6.43	6.26	6.46	6.26	0.084
NH ₃ (mmol/l)	4.10 ^a	3.74 ^{ab}	3.34 ^b	3.44 ^b	0.168
Total bacterial count (10 ⁶)	20.44 ^b	22.10 ^b	25.74 ^a	25.87 ^a	0.557
Lactobacilli (10 ⁵)	9.65 ^c	10.56 ^b	10.55 ^b	11.51 ^a	0.247
Escherichia coli (10 ⁵)	2.33 ^a	2.14 ^a	1.50 ^b	1.09 ^b	0.167

Ureolytic bacteria (10^5)	1.37	1.56	1.59	1.48	0.193
Clostridium spp.	5.18 ^a	4.51 ^b	4.56 ^b	3.32 ^c	0.138
TVFA (mmol/l)	57.63 ^c	61.27 ^c	70.67 ^b	77.86 ^a	1.750

^{a, b and c}Means in the same row with different superscripts are significantly different at ($P \leq 0.05$).

SE = Standard error of means.

Table 5: Some blood hematological values of growing APRI rabbits as affected by dietary addition of Aloe vera leaves powder.

Item	Control G1	Aloe vera leaves powder (g/kg)			±S.E
		G2(0.5)	G3(1.0)	G4(2.0)	
Hemoglobin (g/dl)	9.76 ^d	11.00 ^c	11.83 ^b	13.23 ^a	0.236
Hematocrite %	30.20 ^b	32.70 ^a	33.46 ^a	34.10 ^a	0.637
RBCs ($\times 10^6/\text{mm}^3$)	5.23 ^b	5.46 ^b	6.03 ^{ab}	6.76 ^a	0.257
WBCs ($\times 10^3/\text{mm}^3$)	32.00 ^c	47.33 ^{bc}	61.00 ^{ab}	65.66 ^a	4.722
Differential leukocyte count (%)					
Lymphocyte (N)	36.60 ^c	42.40 ^b	52.66 ^a	53.30 ^a	1.548
Neutrophil(L)	43.60 ^a	38.40 ^b	37.50 ^b	28.66 ^c	1.301
N/L ratio	1.18 ^a	0.90 ^b	0.71 ^c	0.53 ^d	0.024

^{a, b, c and d}Means in the same row with different superscripts are significantly different at ($P \leq 0.05$). SE = Standard error of means.

Table 6: Some blood constituents of growing APRI rabbits as affected by dietary addition of Aloe vera leaves powder.

Item	Control G1	Aloe vera leaves powder (g/kg)			±S.E
		G2(0.5)	G3(1.0)	G4(2.0)	
Total protein (g/dl)	6.47 ^c	7.13 ^b	7.40 ^b	7.74 ^a	0.096
Albumin (A, g/dl)	4.43 ^a	4.32 ^{ab}	4.19 ^{ab}	4.11 ^b	0.077
Globulin (G, g/dl)	2.04 ^c	2.81 ^b	3.21 ^{ab}	3.63 ^a	0.133
A/G ratio	2.24 ^a	1.53 ^b	1.30 ^b	1.12 ^b	0.156
Creatinine (mg/dl)	0.93	1.00	1.01	1.03	0.032
Cholesterol (mg/dl)	46.60 ^a	44.76 ^a	40.33 ^b	36.27 ^c	0.773
AST(U/L)	44.01 ^a	40.62 ^b	37.70 ^b	34.63 ^c	0.936
ALT(U/L)	54.00 ^a	52.12 ^b	50.54 ^c	49.63 ^c	0.422

^{a, b and c}Means in the same row with different superscripts are significantly different at ($P \leq 0.05$).

SE = Standard error of means.

Table 7: Oxidative enzyme activity in plasma of growing APRI rabbits as affected by dietary addition of Aloe vera leaves powder.

Item	Control G1	Aloe vera leaves powder (g/kg)			±S.E
		G2(0.5)	G3(1.0)	G4(2.0)	
CAT(U/mg)	1752.3 ^c	1838.0 ^b	1870.3 ^{ab}	1909.6 ^a	18.61
GPx (U/mg)	1304.3 ^c	1345 ^b	1448.6 ^{ab}	1547.6 ^a	33.07
SOD (U/mg)	434.3 ^c	462.0 ^c	619.6 ^b	675.6 ^a	9.06
MDA (nmol/ml)	6.4 ^a	5.4 ^b	4.5 ^c	4.5 ^c	0.21

^{a, b and c}Means in the same row with different superscripts are significantly different at ($P \leq 0.05$). SE = Standard error of means.

Catalase = CAT, Glutathione peroxidase = GPx, Super oxide dismutase = SOD and Malondialdehyde = MDA

Table 8: Immunological assay of growing NZW rabbits as affected by dietary addition of Aloe vera leaves powder.

Item	Control G1	Aloe vera leaves powder (g/kg)			±S.E
		G2(0.5)	G3(1.0)	G4(2.0)	
IgM (mg/dl)	18.96 ^b	20.59 ^b	22.40 ^a	23.68 ^a	0.511

IgA (mg/dl)	00.47	00.59	00.66	00.73	0.101
IgG (mg/dl)	186.39 ^d	197.89 ^c	232.98 ^b	245.93 ^a	3.376

^{a, b, c and d}Means in the same row with different superscripts are significantly different at ($P \leq 0.05$).

SE = Standard error of means. IgM: Immunoglobulin's M, IgA: Immunoglobulin's A, IgG: Immunoglobulin's G.

THE CAECAL MICROBIAL ACTIVITY

The cecum pH, bacteria count of *E. Coli*, total bacteria, lactobacilli, ureolytic, clostridium spp., and total VFA concentrations (mmol/l) of cecum contents are presented in Table 4. Concerning the values of caecal pH and ureolytic bacteria count, there were insignificant ($P \leq 0.05$) differences among experimental groups. The AV inclusion, especially in high dose "G4", lead to significant ($P \leq 0.05$) reduction in bacteria count of *E. coli* and *Clostridium* spp. and increased in total bacterial count and lactobacilli. The NH_3 concentration significantly ($P \leq 0.05$) fell when the dietary AV level increased. In rabbits belongs to G3 and G4, TVFA significantly ($P \leq 0.05$) increased in compared to G1.

HEMATOLOGY AND BLOOD BIOCHEMISTRY

Dietary addition of AV significantly ($P \leq 0.05$) affects all hematology and blood biochemistry (Tables 5 and 6). Hemoglobin was high in G4. Similarly, RBCs and WBCs were at the highest level in G4. A significant ($P \leq 0.05$) increase in lymphocyte percentage was observed in G2, G3 and G4 in compared to G1 (Table 5). Rabbits received AV recorded significant ($P \leq 0.05$) increase in TP and Glb compared to control group (Table 6). These increases were pronounced with the high levels of AV (G4). The albumin/globulin ratio, cholesterol and liver function (AST and ALT) were significantly ($P \leq 0.05$) decreased by AV dietary addition where the lowest value for G4 diet. Likewise, low ALT and AST levels were observed in G3 and G4 than in G2 and G1 (Table 6). Observation showed that AV had no significant influence on creatinine value. The values in final plasma metabolites in this experiment tended to match the pattern of the rabbits' growth performance records in this study

PLASMA MALONDIALDEHYDE AND ANTIOXIDANT ENZYMES

Table 7 shows the effect of AV on plasma CAT, GPx, SOD and MDA of growing rabbits. The plasma CAT, GPx and SOD of the rabbits in the groups that received AV were higher than that of control group. The values of CAT, GPx and SOD were at the highest level in G4. The levels of MDA of the AV-treated rabbits was significantly ($P \leq 0.05$) lower than that of control group. The MDA level was lowest for G3 and G4 diets.

IMMUNOGLOBULINS CONCENTRATION:

A significant ($P \leq 0.05$) increase in IgM was observed in G3 and G4 while G2 showed insignificant differences in compared to G1 (Table 8). Data in Table 8 were also clear-

ly that, different dietary AV level caused to increase values ($P \leq 0.05$) of plasma IgG concentrations than that of the control group. However, there were insignificant differences in IgA concentrations among all treated groups.

DISCUSSION

Dietary addition with antioxidants from plants is one of the simplest strategies to support antioxidant defense. The improvements of growth traits in current study due to dietary AV powder addition agreed with studies of El-Kholy et al. (2018b) and Mohamed and Hassan (2022) who used phytochemical substances as safe growth promoters in poultry nutrition. Also, these results are agreed with findings of Khan et al. (2014) and Arif et al. (2022), who observed that at 1 and 0.5% dietary inclusion of AV leaves powder meal in Fayoumi chicks and quails diets significant increased in FBW and BWG in compared to control. The yielded improvement of productive performance of growing rabbits may be attributed to that AV is rich in flavonoids, terpenoids, lectins (Boudreau and Beland, 2006; Harlev et al., 2012) fatty acids, anthraquinones (Surjushe et al., 2008), mono- and polysaccharides (pectins, hemicelluloses, glucomannan), tannins, sterols (campesterol, β -sitosterol), enzymes, salicylic acid, minerals (calcium, chromium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium and zinc) and vitamins (A, C, E, β -carotene, B₁, B₂, B₃, B₆, choline, B₁₂, folic acid) (Sahu et al., 2013; Rodrigues et al., 2018). Moreover, Danhoff and McAnally (1988) also reported that AV accelerated the growth of new cells, thereby resulting to increased body weight. Durrani et al. (2008) reported that the higher BWG and improved FCR values of the broilers given 10 mL of aqueous extract of AV gel/liter of drinking water could be due to better performance of the broilers and the diversified antimicrobial activities of Aloe gel. In growing rabbits, the significant effect on improving growth performance due to antioxidants addition has been previously reported (Attia et al., 2019; Shehata et al., 2022). On the hand, the adverse significant ($P < 0.05$) reduction in FI in G3 and G4 compared to G1 could be contributed to better nutritional availability and improvement in nutrients absorption in rabbit's intestine.

Statistical analysis outcome of current study regarding to carcass weight of growing APRI rabbits are in line with the results of another poultry species (because lack of studies in AV on rabbits) such as Fallah (2015) who reported that carcass weight was improved by using AV in broiler feed. The insignificant differences in dressing % among

current experimental groups are in contrary with results by Tariq et al. (2015) who reported that dressing yield were affected by the supplementation of AV powder in Japanese quail diets. Mansoub (2011) found that herbal plants cause good effect on carcass quality by higher absorption of amino acid, nutrient utilization and improved protein metabolism which result in higher percentage of carcass weight.

In the current investigation, two methods were combined to describe caecal microbial activity: assessment of the caecal fermentative activity (pH, NH_3 , TVFA), and measurement of the bacterial ureolytic activity, which reveals the capacity of the caecal flora to break down a substrate for a cell wall. It so clear that AV inclusion could be positively modified activity of the caecal ecosystem especially with the highest level (G4). In the line with current results, Benlemlih et al. (2014) showed reduction counts of *E. coli* and increased in total VFA concentration during the application of dried fennel and thyme to rabbit. Actually, polysaccharides contained in AV (particularly acemannan) have similar effects to those of prebiotics; that is, they increase the number of *Lactobacillus* colonies and reduce gram-negative bacteria (Darabighane et al., 2012). Previous studies show that acemannan added to the broiler diet decreased the number of intestinal *E. coli* colonies (Lin et al., 2005). In fact, short-chain fatty acids, as the final product of *Lactobacillus* fermentation, can decrease intestinal pH and make the environment unfavorable for gram-negative bacteria. In addition, AV forms a protective film on the mucous epithelia of the gastro-intestinal tract against pathogens and toxins (Nalge et al., 2017) as well as the primary active principal in AV is acemannan polysaccharide which is responsible for the increased BWG in treated groups due to its antibacterial properties and immune stimulant effect (Nalge et al., 2017). Lee and Shibamoto (2002) demonstrated reduced population of pathological microbes by using herbs and they also reported reduction in degradation of amino acid so, it improves FBW and BWG which might be due to availability of more amino acids. Thus, in the present study dietary addition of AV resulted in better FBW and BWG of rabbits.

Moreover, polysaccharides (prebiotics) present in AV powder are reported to have the ability to sustain the homeostasis of gut microbial community as well as host health (Tremaroli and Bäckhed, 2012) either by reducing the bacterial and viral infection (Chen et al., 2003) or by directly affecting pathogenic gut microflora (Yu et al., 2018). This result improves feed digestibility and availability of nutrients from feed stuffs, and shortens the feed transit time, which might have beneficial influence on digestive enzymes (Platel and Srinivasan, 2004), and reducing the amount of feed substrate available for proliferation of pathogenic bacteria (El-Kholy et al., 2018b; 2019).

Also, decreasing NH_3 concentration and the increase of VFA in the cecum, accordingly, improving the nutrients utilization (Table 4) may be attributed to the conversion of ammonia-N into microbial protein for the benefit of rabbits which characterized by the pseudo-rumination. The phytogenic compounds of AV powder have positive effects, such as the gut microflora regulation through stimulating macrophages for growing rabbits. According to Singh et al. (2021), phytochemicals including flavonoids, phenols, and alkaloids can reduce the activity of harmful bacteria through the process of competitive exclusion and promote the growth of helpful bacteria like *Lactobacillus* sp., acting as probiotics. So, AV leaves powder as phytogenic have provided sufficient evidence to be safe and natural antioxidant in growing rabbits diets to prevent microorganisms' contamination of human food and to avoid diseases.

Hematology is the professional tools of diagnosing and treatment of a wide range of illnesses and diseases (Terzungwe et al., 2013). Our findings showed that all hematological parameters were within the normal range. The addition of different levels of AV to rabbit diets increased the hematological parameters (RBCs, Hb%, Ht%, and erythrocyte indices) and had a direct impact on the blood characteristics. The trends resulting from adding AV in the present study agree with study involving rabbits fed AV extract (Channa et al., 2014).

The erythropoietin effect of AV of hemopoietic cells of bone marrow have been reported by Iji et al. (2010). Similar findings have been reported by Hamman (2008), who has attributed the effect of increasing hematological parameters to essential vitamins, e.g. riboflavin, thiamine and folic acid; and essential and non-essential amino acids in AV, that are required for synthesis of haemoglobin. Research has also been investigated that this increase in erythropoiesis is due to the presence of polysaccharides in AV (Ni et al., 2004). The presence of thiamine has been found to be the only factor contributing to the hemopoietic characteristics of AV, that is responsible for formation of α -ketoglutarate dehydrogenase and pyruvate dehydrogenase complex in Krebb's cycle (Oishi et al., 2002).

Concerning the effects of AV leaves powder on TP and Glb, it can be explained and interpreted the improvements in their profile in the present study especially in the highest levels (G3 and G4). These improvements may be partially due to the increasing animal resistance to any physiological or physical stress. Furthermore, a general indicator of immunological status is plasma TP level (White et al., 2002). Also, increased globulin concentration with AV leaves powder inclusion as observed in the present study may be a sign of increased rabbit's immunity because the liver will be able to produce enough globulins to have an immu-

nological effect as mentioned by Sunmonu and Oloyede (2007). The level of globulin has been utilized as a measure of immunological responses and as a source for the creation of antibodies. El-Kholy et al. (2014) stated that high globulin level signifies better disease resistance and immune response. This result is in harmony with high percentage of plasma total immunoglobulin in growing rabbits given AV than the control ones as mentioned in Table 8. This established enhancement of immune response associated with AV leaves powder addition.

The increase in plasma TP and Glb concentrations in the rabbits supplemented with the highest AV level (2.0 g/kg; G4) increased protein synthesis in these supplemented groups over the control group, which resulted in highly live BW and weight gain, as compared to the control group.

In agreement with the present results, El-Kholy et al. (2018a; 2021) showed significant decreasing cholesterol concentrations in rabbits treated with photobiotic than in untreated group. Five phytosterols (plant sterols) have been isolated from AV gel as mentioned by Tanaka et al. (2006) have been shown to decrease plasma cholesterol concentrations (Moghadasian and Frohlich, 1999).

Despite overwhelming evidence that aloe vera is the most effective therapeutic plant, several investigations have raised concerns about its toxicity (Liang et al., 2020). For this reason, it is necessary to monitor biomarkers of general toxicity. As can be seen in Table 5, significant ($P < 0.05$) decreases were found in AV treated rabbits in compared to the control one for all liver enzymes (ALT and AST). Also, the insignificant differences were found among all experimental groups including the control one for creatinine level. In otherworld, kidney function seemed to be not affected by any levels of AV leaves powder addition. These results are similar with that obtained by Sinha et al. (2017) and Arif et al. (2022) who reported that supplementing the diet of birds with AV in extract or powder form reduced lipid peroxidation, improved the antioxidant state, and offered protection to the liver and kidney. In spite of literature data for the effect of dietary addition of AV leaves powder on liver and kidney functions are sparse but current results pointed out that rabbits could tolerate the addition of AV leaves powder up to 2.0 g/kg diet without neither deleterious effects on liver nor kidney functions.

A. vera has immunomodulatory effect that has been observed in the results obtained by Channa et al. (2014) in rabbits. In current study, treatment of varying doses of AV powder to rabbits significantly increased ($P \leq 0.05$) the total lymphocyte concentration. Lymphocytes are the most important blood cells that play a vital role in developing humoral and T cell immune response against foreign

antigens such as bacterial, viral, etc. It is thought that a molecule in the AV, known as acemannan, impulses the body to produce disease-fighting WBCs, particularly macrophages. Macrophages consume and digest undesirable elements like bacteria and viruses. In addition, research has investigated that AV has initiated phagocytic activity of reticuloendothelial system (Im et al., 2005). It has been demonstrated that it can enhance both cellular and humoral immunity by proliferation of myeloid and erythrocyte colony forming cells, macrophage colony forming cells and pleuri-potent hemopoietic cells (Boudreau and Beland, 2006). It has been observed that it significantly increases B lymphocyte that is responsible for formation of both immunoglobulin's M and G (Yates et al., 2019). This scientific theory is completely consistent with the results obtained in the current study. It has also been investigated that *A. vera* can increase CD4 and CD8 receptor of T lymphocytes (Ghasem et al., 2011). Thus, increase in lymphocyte concentration might be due to the presence of low molecular weight proteins and glycoproteins that have mitogenic effect causing proliferation of immune cells, that is, lymphocytes.

Evaluation of the redox status offers more biologically relevant information than the individual levels of specific antioxidants of a given body fluid such as plasma or serum. The activities of endogenous antioxidant enzymes influence the cumulative effect of all antioxidants present in the plasma and is used to evaluate the impact of several physiological responses (Ghiselli et al., 2000). Free radicals are formed by stress, and they can harm cell membranes by causing lipid peroxidation of polyunsaturated fatty acids surrounding cell membrane (Rehman et al., 2018). Data presented in the Table 7 revealed the different dietary levels of AV leaves powder influences significantly the lipid peroxidation (LPO) by decreasing MDA values. Lipid peroxidation causes a negative effect in membrane fluidity, which, in turn, impact on immunological responses (Van der Paal et al., 2016). The SOD shields the cells from oxygen-free radicals by catalyzing the elimination of superoxide radical, which harms the membrane and biological structures. The CAT was shown to be charge for the detoxification of H_2O_2 (Mahboob et al., 2005). In this study, dietary AV levels were observed in the activities of SOD and CAT, which were significantly higher in G3 and G4 rabbits than the G1 and G2 rabbits. Phenolic compounds in AV leaves powder may be play a crucial role in homeostatic animal antioxidant reactions and antioxidant activities (SOD, CAT and GPx) which were significantly increased in treated rabbits with AV. The observed increase in antioxidant activities and decline in the activities of MDA in AV leaves powder-treated rabbits suggest its potential anti-lipid peroxidative and antioxidant effects. Potent antioxidant effects of AV, including the ability to scavenge super-

oxide anions, have been attributed to the caffeoyl group of isorabaichromone, a derivative of aloesin (C-glycosylated 5-methylchromone) (Boudreau and Beland, 2006).

Regarding humoral immune responses, our results indicated that rabbits fed dietary AV (G3 or G4) had the highest values of IgM, and IgG. This could be related to the potent antioxidant constituents (such as flavonoids, terpenoids, lectins (Boudreau and Beland, 2006; Harlev et al., 2012); superoxide dismutase (Gao et al., 2018) found in aloe of AV inclusion. Therefore, AV have ability to affect oxidative damage in rabbits by increasing anti-oxidant enzymes activity, decreasing lipid peroxidation, free radicals generation, and suppression of ROS formation, consequently improving production and physiological indices.

CONCLUSION

It can be suggested that AV could be used at a level of 2.0 g/kg of the growing rabbit's diets with beneficial impacts on growth performance, carcass characteristics, gut integrity, immunological response and some hematological and biochemical parameters without any toxicity-effects on liver and kidneys functions. Therefore, improving production and physiological status of rabbits during fattening period by treatment with AV merits further attention.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

NOVELTY STATEMENT

We found that dietary addition with Aloe vera leaves powder especially at 2.0 g/kg growing rabbit's diet could be contributed to the improve of growth performance, carcass characteristics, gut integrity, immunological response and some hematological and biochemical parameters without any toxicity-effects on liver and kidneys functions.

AUTHORS CONTRIBUTION

K.H.E., E.H.A., S.A.B. , M.G.M and S.M.H. developed the concept of the manuscript. K.H.E. wrote the manuscript. All authors checked and confirmed the final revised

manuscript.

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