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Effect of apple cider vinegar and vitamin A on hematological parameters and total immunoglobulin G in sheep: a pilot study

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ABSTRACT

Apple cider vinegar is a fermented compound that contains acetic acid, flavonoids, phenolic compounds, organic acids, minerals, and vitamins. Vitamin A is involved in the development of the immune system and plays regulatory roles in cellular immune responses and humoral immune processes. The aim of this study was to investigate the effect of apple cider vinegar and vitamin A on hematological parameters and immunoglobulin G levels in Gezel lambs. 10 healthy Gazel lambs were used for the study. The lambs were randomly grouped into three groups (control (n = 3), Apple cider vinegar (n = 4), and Vitamin A (n = 3) group). Vitamin A was administrated at a dose of 44,000 IU/kg every ten days for four treatments. ACV was administered orally by drenching (0.5 ml/kg of 6% ACV solution (600 mg ACV), every day, for 40 days). Hematological parameters were determined using standard methods. Total immunoglobulin G concentration was assayed using the turbidimetric immunoassay method. The data obtained before and after drug administration were analyzed by paired T-test and the data of different groups were analyzed using Independent-sample T-test.White blood cells, lymphocytes, and IgG in lambs were significantly increased after administration of Apple cider vinegar ($p < 0.05$). IgG and lymphocytes were significantly higher in lambs under oral administration of Apple cider vinegar compared to the lambs in the control group ($p < 0.05$). White blood cells, neutrophils, and IgG were significantly increased in lambs after vitamin A administration ($p < 0.05$). Neutrophils and IgG were significantly higher in lambs under vitamin A injection compared to the lambs in the control group ($p < 0.05$). Administration of vitamin A and apple cider vinegar in sheep is safe. They also improve the immune system.

Keywords

apple cider vinegar, hematological parameter, immunoglobulin G, lamb, vitamin A

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Abbreviations

APC: apple cider vinegar
RBC: red blood cell
WBC: white blood cell
TP: total protein
PCV: packed cell volume
Hb: Hemoglobin
IgG: immunoglobulin G

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Introduction

Apple cider vinegar (ACV) is a fermented compound that contains acetic acid, flavonoids, phenolic compounds, organic acids, minerals, and vitamins [1, 2]. ACV is produced through a two-stage fermentation process. First, the sugar is converted into alcohol by yeasts, then the alcohol is converted into acetic acid by acetic acid bacteria[3].

Animal experiments have reported that ACV has a variety of pharmacological activities, including antioxidant, anti-inflammatory, anti-diabetic, antifungal, antimicrobial, anti-hypertensive, and anti-hyperlipidemic actions [4-8]. A study showed that using ACV in a fish diet improves immunological parameters and gene expression related to immunity, antioxidant system, and growth performance[9]. Also, a study showed that using ACV in a fish diet increases total immunoglobulin and lysozyme activity[10]. In addition, a study has reported that dietary supplementation with ACV has beneficial effects on performance and immune response in broiler chickens[11]. It has been shown that ACV has an anthelmintic impact against gastrointestinal parasites in sheep[12], and increases the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase[13].

Vitamin A is known as an anti-inflammation vitamin because of its critical role in enhancing immune function[14]. Vitamin A is involved in the development of the immune system and plays regulatory roles in cellular immune responses and humoral immune processes[14].

Thus, the aim of this study was to assess the effect of ACV and vitamin A on hematological parameters and immunoglobulin G in Gezel lambs.

Result

By administering ACV and Vitamin A at the mentioned doses, no disorders and lesions have occurred in the lambs.

ACV

RBC, PCV, Hb, fibrinogen, TP, neutrophils, band neutrophils, eosinophils, and monocytes in lambs before and after administration of ACV were not statistically different.

WBC, lymphocytes, and IgG in lambs were significantly higher after administration of ACV compared to the control group ($p < 0.05$).

RBC, PCV, Hb, fibrinogen, TP, WBC, neutrophils, band neutrophils, eosinophils, and monocytes were not statistically different between lambs under oral administration of ACV and lambs in the control group.

IgG and lymphocytes were significantly higher in lambs under oral administration of ACV than in lambs in the control group ($P<0.05$).

The results of the study are summarized in Tables 1 and 2.

Vitamin A

RBC, PCV, Hb, fibrinogen, TP, band neutrophils, lymphocytes, eosinophils, and monocytes in lambs before and after administration of vitamin A were not statistically different.

WBC, neutrophils, and IgG were significantly higher in lambs after vitamin A administration compared to the control group ($p < 0.05$).

RBC, PCV, Hb, fibrinogen, TP, WBC, band neutrophils, lymphocytes, eosinophils, and monocytes were not statistically different between lambs under vitamin A injection and lambs in the control group.

Neutrophils and IgG levels were significantly higher in lambs under vitamin A injection than in the control group ($p < 0.05$).

The results of the study are summarized in Tables 1 and 2.

Discussion

A study on the effects of ACV on haemato-biochemical parameters in rate showed that there are no significant variations between the groups in post-treatment values of PCV, Hb, RBC, and absolute eosinophil counts [15]. In our study, these mentioned parameters were not different before and after administration of ACV and also they were not different between the test article groups and the control group. Also, another study found no difference in RBC between control and case groups after adding a combination of onion, garlic, and ACV to broiler chicken diets[16]. A study showed that there is no significant impact of ACV supplementation on broiler serum total protein levels[17]. This finding is similar to our finding. Also, a study showed that ACV has no effect on RBC, Hb, and PCV in humans [18]. The same results are presented in our study.

A study on the effects of an herbal mixture (onion, garlic, and ACV) on hematological parameters of broiler chickens showed that the herbal mixture resulted in significant increases in PCV and Hb values[16]. This finding is contrary to our results, and probably the results obtained in the mentioned study were not caused by the effect of ACV, or maybe the difference between the results of our study and that study is due to differences between species.

Table 1.
Hematological parameters and IgG in lambs before and after administration of vitamin A and ACV

Parameter	A day before study			A day after study			P value
	Min	Max	Mean ±SE	Min	Max	Mean ±SE	
Case group under vitamin A injection							
RBC (104/µl)	1025	1190	1105 ± 47.69	1125	1155	1142 ± 9.06	0.464
PCV (%)	30	35	32.67 ± 1.45	33	34	33.34 ± 0.33	0.635
Hb (g/dl)	10	11.66	10.88 ± 0.48	11	12	11.44 ± 0.29	0.336
TP (g/dl)	6.2	6.66	6.42 ± 0.13	6.4	7.1	6.67 ± 0.21	0.134
Fibrinogen (mg/dl)	100	200	166.7 ± 33.34	100	400	266.7 ± 88.2	0.225
WBC/µl	4446	10349	7248.4 ± 1710	8511	14150	10917.7 ± 1679.3	0.006*
Neutrophil /µl	1269	3895	2421.4 ± 774.9	4514	5382	4898.7 ± 255.3	0.041*
Band neutrophil/µl	30	100	66.7 ± 20.2	112	207	143.7 ± 31.7	0.055
Lymphocyte/µl	2976	5842	4439.4 ± 827.89	3519	7320	5113 ± 1139.2	0.259
Eosinophil /µl	124	307	200.4 ± 54.96	244	724	449.4 ± 142.8	0.105
Monocyte/µl	47	205	120.7 ±45.9	122	517	313 ±114.2	0.107
IgG (g/l)	1.19	1.30	1.24 ± 0.031	1.34	1.40	1.36 ± 0.018	0.020*
Case group under oral administration of ACV							
RBC (104/µl)	952	1260	1128 ± 76.03	982	1342	1143 ± 74.95	0.804
PCV (%)	28	37	32.7 ± 1.89	29	40	35.5 ± 2.39	0.102
Hb (g/dl)	9.33	12.33	10.9 ± 0.61	9.6	13.33	8.5 ± 2.4	0.470
TP (g/dl)	5.7	6.5	6.25 ± 0.18	6.1	7.4	6.52 ± 0.29	0.340
Fibrinogen (mg/dl)	100	300	187.5 ± 42.6	100	100	100 ± 0	0.133
WBC/µl	3330	8702	5242 ± 1187.5	5458	12470	8811.5 ± 1459.2	0.01*
Neutrophil /µl	1239	2790	1732.2 ± 356.6	910	4810	3005 ± 813.1	0.105
Band neutrophil/µl	62	80	67.7 ± 4.2	60	100	80 ± 8.16	0.091
Lymphocyte/µl	1909	5460	3217.2 ± 780.1	4250	6900	5337.5 ± 571.3	0.007*
Eosinophil/µl	80	186	121.5 ± 22.9	130	400	232.5 ± 58.36	0.053
Monocyte/µl	40	186	104 ± 30.8	108	260	156.5 ± 35.8	0.063
IgG (g/l)	1	1.25	1.08 ± 0.059	1.55	1.90	1.73 ± 0.074	0.001*
Control group							
RBC (104/µl)	1021	1184	1118.4 ± 49.7	1187	1274	1220 ± 27.09	0.096
PCV (%)	30	35	33.4± 1.7	35	38	36 ± 1	0.208

Table 1 cont.

Hb (g/dl)	10	11.66	11 ± 0.54	11.66	12.66	12.1 ± 0.293	0.107
TP (g/dl)	5.8	6.6	6.3 ± 0.25	6.8	7.3	6.97 ± 0.16	0.081
Fibrinogen (mg/dl)	150	300	200 ± 50	200	300	266.7 ± 66.7	0.181
WBC /µl	3851	9960	6722.7 ± 1772.96	5484	7400	6561.4 ± 565.8	0.909
Neutrophil /µl	1692	3840	2677.4 ± 626.3	2557	3040	2765.7 ± 143.2	0.872
Band neutrophil /µl	47	60	51.4 ± 4.3	40	70	53.4 ± 8.8	0.724
Lymphocyte/µl	1909	5460	3623 ± 1026.9	2500	3750	3283.4 ± 394.05	0.675
Eosinophil/µl	141	360	217 ± 71.5	232	340	274 ± 33.4	.278
Monocyte /µl	62	240	154 ± 51.4	155	200	185 ± 15	0.507
IgG (g/l)	1.18	1.25	1.2 ± 0.023	1.20	1.29	1.24 ± 0.026	0.225

*$p < 0.05$, RBC: red blood cell, PCV: packed cell volume, Hb: hemoglobin, TP: total protein, WBC: white blood cell, IgG: immunoglobulin G, min: minimum, max: maximum, SE: standard error.

In our study, the significant increase in WBC count after the administration of ACV was mainly due to the substantial increase in lymphocyte count. However, the significant increase in the lymphocyte count in the ACV-administered group compared to the control group did not lead to an increase in WBC count in the ACV-administered group compared to the control group. A study showed that the addition of an herbal mixture (onion, garlic, and ACV) to the diet of broiler chickens increased lymphocyte counts [16]. The improvement of the immune system due to the administration of ACV has been mentioned in several studies.[9, 10, 19, 20]. Since lymphocytes play a role in humoral immunity, perhaps the improvement of the immune system caused by ACV is responsible for the increase in lymphocytes. However, this requires further study.

In our study, it was shown that immunoglobulin G increased after the administration of vitamin A and ACV. It also increased in the ACV and vitamin A-administered group compared to the control group. These findings show that vitamin A and ACV (as mentioned above) are useful in improving the immune system in lambs. An increase in total immunoglobulin has been reported in fish after administration of ACV [10, 20, 21]. In a study, it was shown that administration of vitamin A leads to an

Materials and Methods

Selection of lambs, grouping and sampling

To perform this study, ten healthy male Gazel lambs were selected. Their health was confirmed by veterinary examinations. The age (6 months) and weight (20 kg) of the selected lambs were similar. Before starting the study, the selected lambs underwent a week of adaptation to the new environment. During the study, lambs were fed alfalfa and hay ad libidum and were observed for any clinical signs of disease throughout the study.

The lambs were randomly grouped into three groups:

1- Control group: 3 lambs were assigned to this group, and no medication was administered to them.

2- Case group under vitamin A injection: 3 lambs were assigned to this group and vitamin A was administered to them (44,000 IU/kg Retinavit®, intramuscularly (IM), every ten days, 4 treatments).

3- Case group under oral administration of ACV: 4 lambs were assigned to this group, and ACV was administered orally by drenching (0.5 ml/kg of 6% ACV solution (600 mg ACV), every day, for 40 days).

Blood samples were taken from the jugular vein of all lambs before and after the study.

Laboratory measurement

Red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb), fibrinogen, total protein (TP), white blood cell (WBC) count, and absolute count of neutrophils, band neutrophils, lymphocytes, eosinophils, and monocytes were determined by routine hematology tests.

Total immunoglobulin G (IgG) concentration was assayed by turbidimetric immunoassay method as previously described [25, 26].

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Table 2.

Hematological parameters and IgG in lambs after administration of Vitamin A and ACV compared to control group

Parameter	Case group under vitamin A injection			P value	Control group			P value	Case group under oral administration of ACV		
	Min	Max	Mean ±SE		Min	Max	Mean ±SE		Min	Max	Mean ±SE
RBC(104/µl)	1125	1155	1142.7 ± 9.06	0.053	1187	1274	1220.4 ± 27.09	0.440	982	1342	1143± 74.9
PCV (%)	33	34	33.4 ± 0.33	0.065	35	38	36 ± 1	0.872	29	40	35.5 ±2.39
Hb (g/dl)	11	12	11.44 ± 0.29	0.186	11.66	12.66	12.1 ± 0.29	0.560	9.6	13.33	11.51± 0.77
TP (g/dl)	6.4	7.1	6.67 ± 0.21	0.413	6.8	7.3	6.8 ± 0.06	0.383	6.8	7.4	6.52 ±0.29
Fibrinogen (mg/dl)	100	400	266.7 ± 88.19	0.742	200	400	233.4 ± 33.4	0.057	100	100	100 ± 0
WBC/µl	8511	14151	10917.7 ± 1679.3	0.070	5484	7400	6561 ± 565.8	0.808	1270	9320	6011.5 ± 1772
Neutrophil/µl	4514	5382	4898.7 ± 255.3	0.002*	2557	3040	2765.7 ± 143.2	0.815	910	4810	3005 ± 813.1
Band neutrophil/µl	112	207	143.7 ± 31.7	0.051	40	70	53.4 ± 8.8	0.080	60	100	80 ±8.1
Lymphocyte/µl	3519	7320	5113 ± 1139.2	0.204	2500	3750	3283 ± 394.05	0.041*	4250	6900	5337.5 ± 571.3
Eosinophil/µl	244	724	449.4 ± 142.8	0.298	232	340	274 ± 33.4	0.601	130	400	232.5 ± 58.3
Monocyte /µl	122	517	313 ± 114.2	0.329	155	2	185 ± 15	0.548	108	260	156.5 ± 35.8
IgG (g/l)	1.34	1.40	1.36 ± 0.018	0.022*	1.25	1.29	1.24 ± 0.026	0.004*	1.55	1.90	1.73± 0.07

*$p <$ 0.05, RBC: red blood cell, PCV: packed cell volume, Hb: hemoglobin, TP: total protein, WBC: white blood cell, IgG: immunoglobulin G, min: minimum, max: maximum, SE: standard error.

Statistical analysis

The data obtained before and after drug administration were analyzed by paired T-Test in SPSS software. Also, the data of different groups were analyzed using the independent-sample t-test. $p < 0.05$ was considered significant.

Conclusion

Administering vitamin A and ACV in sheep is safe. They also improve the immune system. Vitamin A and ACV increase total IgG, and also vitamin A increases neutrophils and ACV increases lymphocytes. In general, they can be used to improve the immune system in sheep.

Authors' Contributions

Conceived and designed the experiments: M.T., and E.F. Performed the experiments: M.T., and M.A.. Wrote the paper: A .Sh.

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Competing Interests

The authors declare that there is no conflict of interest.

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