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Original Research Article

PROPHYLACTIC AND THERAPEUTIC EFFECTS OF ALLICIN AGAINST GOATS EXPERIMENTALLY INFECTED WITH COLIBACILLOSIS

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Abstract: The current investigation was carried out to evaluate the prophylactic and therapeutic effects of allicin against goats experimentally infected with *Escherichia coli (E. coli).*Twelve native breed goats, one year old, belonging to the private farms of Faculty of Veterinary Medicine, Zagazig University, Egypt, were used in this study. The animals were divided into three groups (4 goats each); prophylactic group (G1) received allicin "2.7 mg/kg twice daily" then infected with *E. coli*, treatment group (G2) was experimentally infected with *E. coli*, then received the above mentioned allicin dose and lastly untreated control group (G3) were remained without any interference. All goats were exposed to complete and comprehensive clinical examination. Blood samples were collected from jugular veins of goats to determine blood pictures and some selective parameters. Clinical investigation of goats revealed a significant increase of mean rectal temperature, heart and respiration rates on the 7th day in G2 till the 28th day. Hematological analysis showed that oral administration of allicin had no significant effect on the hematological parameters, hemoglobin (Hb), red blood cells (RBCs) and packed cell volume (PCV)". A significant increase in white blood cells (WBCs) and neutrophils on the7th, 14th, 17th and 28th days in both treated groups was observed. Biochemical analysis showed significant increase of malondialdehyde (MDA) levels in both groups following *E. coli* inoculation then decreased significantly till retuned to the normal range on the 28th day. Total antioxidant capacity (TAC) was increased significantly on the 7th day in G1 but it significantly decreased in G2 during the same period then increased significantly on both groups till the 28th day. Immunological results showed marked increase in both research treated groups. Allicin administration increase the release of interleukin-6 (IL-6), interleukin- 12 (IL-12) and tumor necrosis factor-alpha (TNF-α) in both infected groups than in the untreated control one.

Key words: Allicin; antioxidant; colibacillosis; goats; immunoglobulins

Introduction

Small ruminant production, sheep and goats, plays an important role in livestock development, particularly in developing countries like Egypt. It was increased recently due to lower feeding costs and other management practices. Goats are able to produce milk, meat and hair even in harsh environments (1). Low milk and meat production from goats is due to poor genetic composition and poor nutritional status (2).

As possible alternatives to growth promoters, aromatic plants and their extracts have increased attention in recent years. Garlic (*Allium sativum*) has been used since ancient times in folk medicine (3). *Allium sativum* is a member of the family *Liliaceae*. Bioactive garlic ingredients include several sulfur-containing compounds such as alliin, diallyl sulphide (DAS) and allicin (4).

Allicin (diallylthiosulfinate) has many therapeutic properties, but it is probably the most commonly scientifically researched that it has anti-microbial activity against many pathogenic microbes as *Bacillus* spp., *Escherichia coli* (*E. coli*), *Mycobacterium* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Streptococcus* spp. (5). This anti- microbial

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effect of allicin is thought to be due to its oxidative interaction with important thiol-containing enzymes (6). In addition to its antimicrobial effect, garlic is a natural antioxidant and has antihypertensive properties (3). Furthermore, garlic extract treatment has been shown to enhance the activation role of T-lymphocytes, natural killer cells, interleukins and improving the immune responses (3).

Colibacillosis is one of the significant diseases caused by pathogenic serotypes of *E. coli* in farm animals (7). Two types of infections have been associated with *E. coli,* enteric and septicemic infections. The enteric type is distinguished by varying degrees of diarrhea, dehydration, and elevated mortality in untreated cases (8). The pathophysiology of colibacillosis is due to K99 + adhesin antigen and heat stable enterotoxin, the major virulence attributes of enteric strain of *E. coli* in farm ruminants (9). It is a significant cause of economic loss in raising sheep and goat and is one of the important zoonotic diseases (10). The cell wall of Gram-negative bacteria contains endotoxin; the major biologically active component of the endotoxin is lipopolysaccharide (LPS), which is a potent inducer of inflammation (11).

Plant extracts and essential oils were evaluated frequently against bacteria (12, 13). Garlic has long been considered the most effective plant for treating bacterial infections. Allicin is a garlic defense molecule that has broad antimicrobial activities against both Gram-positive and Gram-negative bacteria (14). Thus, the objective of this study was to investigate the effects of oral allicin administration as a prophylactic and treatment natural agent against the experimental infection of goats with *E. coli* by estimating the blood profile and some biochemical parameters, which reflect the oxidative stress and the immunological status of the animals.

Materials and methods

Ethical approval

The study was approved by the Institutional Animal Care and Use Committee (IACUC), Zagazig University (Approval No. ZU-IACUC/2/F/121/2021).

Bacterial strain

Non-verotoxigenic strain of *E. coli* O157 was kindly provided by Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University to be used for the experimental infection with a concentration of $2x10^9$ colony-forming units (CFU) / mL prior to inoculation (15).

Antibacterial agent

Allicin Max™ capsule (180 mg allicin powder/capsule) obtained from Allicin International Ltd, Rye, East Sussex, UK was administered orally to the animal.

Experimental design, animal management and feeding regime

The experiment was carried out on Baladi goats at Faculty of Veterinary Medicine Farm, Zagazig University, Egypt. The study lasted 70 days, from November 2020 to January 2021. Twelve clinically-normal, non-pregnant female native-breed goats of one-year age weighing 30± 2 kg were included in this study. The goats have been randomly divided into three groups (G1, G2 and G3); each consists of four animals. The animals were housed for 15 days to acclimatize and were monitored during this period. Each group (n = 4) was reared in the same pen during the whole experiment period.

Goats in G1 received 2.7 mg/kg of allicin twice a day at 12-h intervals for seven consecutive days after dissolving it in normal saline as a prophylactic dose (16). The animals were then experimentally infected with the above mentioned dose of *E. coli* O157 strain. After seven days, we evaluated animals` conditions and clinical symptoms. Group 2 was experimentally infected with *E. coli* and the animal state and clinical signs were observed for seven days. Thereafter, the animals were administered the allicin orally for seven consecutive days after dissolving in normal saline as treatment dose of 2.7 mg/kg twice daily with 12-h intervals (16). The remaining G3 was kept as a control without interference. During the experimental period, the animals were maintained on forage and concentrate supplements at a ratio of 70:30. The animals were fed twice a day with a commercial goat pellet concentrate. All animals were fed green fodder "Barseem" when was available.

Blood sampling

Two blood samples were collected from each selected animal via jugular vein puncture. For hematological studies, 2 mL of blood was taken into

Table 1: The effect of allicin supplement as prophylactic or treatment in goats experimentally infected with *E. coli* 0157 on vital signs

Parameter	Group 1	Group 2	Group 3	*P*-value
Temp (°C) / day				
0	39.00 ± 0.058	39.13 ± 0.120	39.10 ± 0.058	0.54
7th	39.27 ± 0.176b	41.23 ± 0.145a	39.33 ± 0.088b	< 0.001
14th	39.47 ± 0.145b	40.17 ± 0.203a	39.17 ± 0.145b	0.014
17th	39.43 ± 0.033a	39.53 ± 0.088a	39.13 ± 0.067b	0.013
28th	39.23 ± 0.033	39.13 ± 0.120	39.20 ± 0.153	0.824
42th	39.10 ± 0.058	39.23 ± 0.120	39.13 ± 0.067	0.555
56th	38.97 ± 0.033b	39.13 ± 0.088ab	39.23 ± 0.033a	0.045
HR/min / day				
0	85.00 ± 1.528	83.33 ± 2.028	84.00 ± 0.577	0.744
7th	84.33 ± 1.333b	104.0 ± 3.786a	85.33 ± 0.882b	0.002
14th	95.33 ± 0.882a	100.0 ± 2.887a	85.33 ± 1.453b	0.005
17th	93.33 ± 0.882a	98.00 ± 2.887a	84.67 ± 0.882b	0.006
28th	90.67 ± 0.667	92.00 ± 3.055	85.67 ± 1.333	0.132
42th	88.00 ± 0.577	89.67 ± 2.728	83.33 ± 0.882	0.087
56th	84.67 ± 0.333	87.33 ± 1.333	84.33 ± 0.333	0.077
Resp./min / day				
0	24.00 ± 0.577	24.67 ± 0.882	23.33 ± 1.667	0.723
7th	29.67 ± 0.333b	37.33 ± 1.453a	23.00 ± 0.577c	< 0.001
14th	28.00 ± 0.577b	34.67 ± 1.453a	24.00 ± 0.577c	0.001
17th	26.00 ± 0.577b	32.00 ± 1.155a	24.00 ± 0.577b	0.001
28th	24.00 ± 0.577	27.33 ± 1.202	24.00 ± 1.000	0.079
42th	24.67 ± 0.333	26.00 ± 0.577	24.33 ± 0.333	0.072
56th	24.00 ± 0.577	25.33 ± 0.882	25.00 ± 0.577	0.422

Values are represented as mean ± SEM. Means within the same row carrying different superscripts (a, b, and c) are significant at $p < 0.05$. Temperature (Temp), heart rate (HR), respiration (Resp)

potassium ethylene diamine tetra-acetic acid (k2 EDTA) tubes. The other blood sample of 10 mL was taken in plain tubes without anticoagulant, allowed to stand at room temperature for 2 h, then the serum was separated by centrifugation at 10000 rpm/10 minutes, as previously described (17). The blood samples were taken from the three groups before starting of the experimental design. Moreover, blood sampling was repeated twice on 7th and 14th day of the experiment. The fourth blood samples were taken after 72 h from end of experiment then on 28th, 42th and 56th days post experiment. Blood samples from the control group were included in each time interval.

Hematobiochemical screening

A complete blood picture was determined using an automated blood cell analyzer (Sysmex XT-2000iV, Kobe, Japan). Total proteins (TP) and albumin (Alb), were measured using commercial diagnostic kits obtained by Biomerieux, Spain. Serum globulins were calculated mathematically by subtracting albumin from total proteins (17).

Malondialdehyde (MDA) was assessed using Cusabio Biotech's ELISA Kits (Cusabio Biotech inc, Houston, Texas USA). Serum total antioxidant capacity (TAC) was determined using Bio diagnostic kits (Cusabio Biotech inc, Houston, Texas USA) (18). The determination of immunoglobulins (IgA, IgM, and IgG) was conducted according to Toma et al. (19). The concentrations of interleukin-6 (IL-6), interleukin-12 (IL-12) and tumor necrosis factor-alpha (TNFα) were determined by quantitative enzyme immunoassay (ELISA), using a commercial kit (MyBioSource, San Diego, CA, USA) according to previously issued papers (20-22).

Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA), using Statistical Package for Social Sciences software, version 16.0 (SPSS Inc., Chicago, IL). The level of significance between groups was determined by Duncan's post hoc test. The results are expressed as means ± standard error (SE). A *p*-value of less than 0.05 was considered statistically significant.

Results

There was no significant difference between the clinical observations of goats in G1 after allicin administration and those of control goats. However, goats in G2 represented variable clinical symptoms that appeared shortly after seven days of infection with *E. coli* as dull, depression, soft feces to mild diarrhea. The mean rectal temperature and heart and respiration rates of these animals were significantly increased on the 7th day (41.23 ± 0.14 °C, 104.0 ± 3.78/min and 37.33 ± 1.45/min, respectively) and then returned to normal on the 28th day (39.13 ± 0.12°C, 92.00 ± 3.05/min and 27.33 ± 1.2/min, respectively) (Table 1).

Allicin administration had no significant influence on the hematological parameters (Hb, PCV and RBCs) in prophylactic and treated groups in comparison with control group throughout different experiment periods ($P > 0.05$). There was a significant increase in WBCs and neutrophil count on the 7th, 14th, 28th and 42th days of the experiment in prophylactic and treated groups ($P < 0.05$) then they gradually decreased until returning to the normal range on the 56th day (WBCs, G1, 23.00 ± 0.57; G2, 24.00 ± 0.57 *vs.* control G3, 23.23 ± 0.39/10^3/mL; $P > 0.05$, while neutrophils; G1, 5.6 ± 0.3; G2, 6.2± 0.3 *vs.* control G3, 5.6± 0.1/10^3/mL; $P > 0.05$) (Table 2).

Total proteins, albumin and globulin were significantly increased in G1 after allicin administration on 7th day (TP, 7.4± 0.08 *vs.* control 6.2 ± 0.03; Alb, 3.7 ± 0.1 vs. control 3.2 ± 0.1 and finally globulin, 3.6±0.2 *vs.* control 3.0± 0.1) and this increase continued till 28th day of the study. While they significantly decreased in G2 after *E. coli* inoculation on 7th day and gradually increased on 14th and 17th day of study.

The level of theses parameters returned similar to the control on 28th day ($P > 0.05$). Malondialdehyde levels were higher in both groups following *E. coli* inoculation on the 14th day in G1 (10.6± 0.2 *vs.* G3, 6.1± 0.1) and on day 7th in G2 (10.4 ± 0.2 *vs.* control, 6.1 ± 0.1) then decreased significantly till it returned to the normal range on the 28th day ($P > 0.05$). Total antioxidant capacity was increased significantly on 7th day in the G1 but it fell significantly in G2 during the same time period then increased significantly on both groups till 28th day a and became within the normal range thereafter (Table 3).

In comparison to the control group, allicin administration elevated the immunological measures (IgG, IgM and IgA) in both research groups, and this elevation was starting to be elevated significantly on 7th day in G1 then highly significant increased on the 14th day in G1 after *E. coli* inoculation, which continued to be significantly elevated until the day 28 of the trial. While in the G2 the increase values of immunoglobulins started to be significant on 14th day after oral administration of allicin and continued until day 42th of study ($P < 0.05$) (Table 4).

The release of IL-6, IL-12, and TNF-α was considerably significantly higher in both infected groups than in the control one, from 7th day of the trial in G1 and continued to 28th day while start to be marked increase in 14th day in G2 and this increase continued till 42th day of the study then reduce the level of inflammatory markers to normal level as in control group (Table 4).

Discussion

Regarding the clinical symptoms, the increase in body temperature in goats following *E. coli* inoculation was consistent with a previous study in which certain endogenous pyrogens, collectively known as interleukins, are released from activated phagocytic cells and reticuloendothelial cells of the host in response to the inflammation (23). Colibacillosis causes an increase in respiration and heart rates due to elevated body temperature and hypotension induced by *E. coli* endotoxin. According to the obtained results, oral administration of allicin improves the clinical symptoms in goats in both treated groups.

Table 2: The effect of allicin supplement as prophylactic or treatment in goats experimentally infected with *E. coli* O157 on hematological parameter

Hematological parameter	Group 1	Group 2	Group 3	*P*-value
Hb (g/dL) / day				
0	12.12 ± 0.394	11.91 ± 0.045	11.85 ± 0.202	0.54
7th	11.66 ± 0.102	11.77 ± 0.145	11.40 ± 0.265	< 0.001
14th	11.83 ± 0.117	11.75 ± 0.176	11.57 ± 0.176	0.014
17th	11.79 ± 0.195	11.82 ± 0.199	11.66 ± 0.145	0.013
28th	11.99 ± 0.062	11.65 ± 0.105	11.73 ± 0.145	0.824
42th	12.06 ± 0.097	11.85 ± 0.152	11.80 ± 0.153	0.555
56th	11.79 ± 0.069	11.93 ± 0.328	12.03 ± 0.067	0.045
PCV(%) / day				
0	39.10 ± 0.321	38.90 ± 0.058	38.90 ± 0.115	0.729
7th	38.47 ± 0.305	38.60 ± 0.379	38.10 ± 0.379	0.612
14th	38.43 ± 0.233	38.47 ± 0.285	37.17 ± 1.167	0.391
17th	38.53 ± 0.318	38.69 ± 0.218	37.83 ± 0.167	0.097
28th	38.62 ± 0.154	38.67 ± 0.088	38.57 ± 0.233	0.918
42th	38.87 ± 0.161	39.00 ± 0.100	39.17 ± 0.120	0.343
56th	38.79 ± 0.066	39.25 ± 0.029	38.73 ± 0.328	0.198
RBCs10^6/mL / day				
0	7.013 ± 0.104	7.133 ± 0.088	7.067 ± 0.120	0.733
7th	7.100 ± 0.208	7.333 ± 0.203	6.967 ± 0.203	0.483
14th	6.907 ± 0.103	6.700 ± 0.208	6.567 ± 0.133	0.357
17th	7.160 ± 0.226	7.333 ± 0.176	6.967 ± 0.219	0.501
28th	7.007 ± 0.080	7.547 ± 0.195	7.267 ± 0.219	0.174
42th	6.873 ± 0.072	7.333 ± 0.219	7.033 ± 0.033	0.124
56th	7.040 ± 0.130	7.163 ± 0.148	7.180 ± 0.144	0.756
WBCs 10^3/mL / day				
0	22.93 ± 0.233	22.93 ± 0.088	22.93 ± 0.296	1
7th	29.00 ± 0.577^a	31.33 ± 1.453^a	22.83 ± 0.167^b	0.001
14th	34.00 ± 0.577^a	31.00 ± 0.577^b	23.07 ± 0.233^c	< 0.001
17th	34.00 ± 0.764^a	34.27 ± 0.371^a	23.00 ± 0.577^b	< 0.001
28th	31.33 ± 0.601^a	31.33 ± 0.333^a	23.20 ± 0.306^b	< 0.001
42th	27.17 ± 0.441^a	25.67 ± 0.667^{ab}	24.33 ± 0.441^b	0.025
56th	23.00 ± 0.577	24.00 ± 0.577	23.23 ± 0.393	0.422
Neutrophils 10^3/mL / day				
0	5.333 ± 0.333	5.333 ± 0.333	5.667 ± 0.333	0.729
7th	10.00 ± 0.577^a	10.67 ± 0.882^a	6.000 ± 0.577^b	0.006
14th	15.00 ± 1.155^a	11.00 ± 1.155^b	5.500 ± 0.289^c	0.001
17th	21.17 ± 1.014^a	17.00 ± 0.577^b	5.500 ± 0.289^c	< 0.001
28th	12.00 ± 0.577^a	13.67 ± 0.882^a	5.333 ± 0.240^b	< 0.001
42th	12.13 ± 0.570^a	7.000 ± 0.577^b	5.333 ± 0.240^b	< 0.001
56th	5.667 ± 0.333	6.233 ± 0.338	5.633 ± 0.120	0.312

Values are represented as mean ± SEM. Means within the same row carrying different superscripts (a, b, and c) are significant at $P < 0.05$. N.B RBCs expressed as (10^6/mL); Neutrophils (10^3/mL). Hemoglobin (Hb.) g/dL), packed cell volume (PCV) (%), red blood cells (RBCs), white blood cells (WBCs) (10^3/mL)

Table 3: The effect of allicin supplement as prophylactic or treatment in goats experimentally infected with *E. coli* O157 on total proteins, albumin, globulin, MDA and TAC

Parameter	Group 1	Group 2	Group 3	*P*-value
TP (g/dL) / day				
0	6.267 ± 0.067	6.210 ± 0.150	6.217 ± 0.044	0.906
7th	7.423 ± 0.087a	5.167 ± 0.088c	6.267 ± 0.033b	< 0.001
14th	7.467 ± 0.203a	6.300 ± 0.058b	6.233 ± 0.120b	0.001
17th	8.347 ± 0.222a	7.300 ± 0.153b	6.333 ± 0.067c	< 0.001
28th	7.207 ± 0.058a	7.567 ± 0.240a	6.267 ± 0.133b	0.003
42th	6.113 ± 0.054	5.910 ± 0.081	6.227 ± 0.166	0.205
56th	6.133 ± 0.086	6.363 ± 0.134	6.387 ± 0.156	0.369
Alb (g/dL) / day				
0	3.097 ± 0.078	3.140 ± 0.067	3.360 ± 0.204	0.379
7th	3.767 ± 0.145a	2.767 ± 0.145b	3.267 ± 0.145ab	0.008
14th	3.650 ± 0.104a	3.200 ± 0.115b	3.250 ± 0.087b	0.04
17th	4.233 ± 0.145a	3.900 ± 0.058a	3.163 ± 0.068b	0.001
28th	3.833 ± 0.088	3.760 ± 0.288	3.267 ± 0.035	0.119
42th	3.233 ± 0.083	3.367 ± 0.088	3.407 ± 0.159	0.57
56th	3.213 ± 0.024	3.280 ± 0.049	3.200 ± 0.115	0.726
Globulin(g/dL) / day				
0	3.170 ± 0.130	3.070 ± 0.153	2.857 ± 0.187	0.416
7th	3.657 ± 0.219a	2.400 ± 0.153b	3.000 ± 0.153b	0.007
14th	3.817 ± 0.192a	3.100 ± 0.173b	2.983 ± 0.044b	0.016
17th	4.113 ± 0.098a	3.400 ± 0.115b	3.170 ± 0.122b	0.002
28th	3.373 ± 0.116	3.807 ± 0.383	3.007 ± 0.134	0.145
42th	2.877 ± 0.041	2.543 ± 0.170	2.817 ± 0.017	0.12
56th	2.920 ± 0.066	3.083 ± 0.153	3.187 ± 0.271	0.612
MDA nmoL/mL / day				
0	6.910 ± 0.485	6.567 ± 0.088	6.493 ± 0.211	0.621
7th	6.720 ± 0.081b	10.40 ± 0.208a	6.133 ± 0.186c	< 0.001
14th	10.67 ± 0.240a	10.90 ± 0.557a	6.133 ± 0.176b	< 0.001
17th	7.900 ± 0.208a	8.233 ± 0.120a	6.100 ± 0.153b	< 0.001
28th	7.200 ± 0.208	7.300 ± 0.115	6.667 ± 0.240	0.123
42th	6.867 ± 0.186	7.267 ± 0.145	6.700 ± 0.289	0.239
56th	6.077 ± 0.253	6.500 ± 0.115	6.700 ± 0.115	0.106
TAC ng/mL / day				
0	13.67 ± 0.333	13.67 ± 0.353	13.67 ± 0.186	1
7th	15.10 ± 0.208a	11.83 ± 0.167c	13.10 ± 0.208b	< 0.001
14th	15.20 ± 0.200a	15.07 ± 0.133a	13.07 ± 0.067b	< 0.001
17th	15.13 ± 0.120a	15.07 ± 0.176a	12.80 ± 0.115b	< 0.001
28th	15.10 ± 0.208a	14.93 ± 0.067a	13.07 ± 0.176b	< 0.001
42th	13.37 ± 0.315	12.92 ± 0.186	13.03 ± 0.376	0.579
56th	13.17 ± 0.120	13.57 ± 0.257	13.10 ± 0.153	0.234

Values are represented as mean ± SEM. Means within the same row carrying different superscripts (a, b, c and d) are significant at $P < 0.05$. Total proteins (TP), albumin (ALB.), malondialdehyde (MDA), total antioxidant capacity (TAC)

Table 4: The effect of allicin supplement as prophylactic or treatment in goats experimentally infected with *E. coli* 0157 on some immunological and inflammatory parameters.

Parameters	Group 1	Group 2	Group 3	*P*-value
IgG (ng/mL) / day				
0	313.0 ± 13.53	308.7 ± 4.978	310.0 ± 5.774	0.941
7th	357.3 ± 15.07a	306.7 ± 2.404b	306.3 ± 4.667b	0.012
14th	436.7 ± 8.819a	376.7 ± 8.819b	317.0 ± 3.786c	< 0.001
17th	373.7 ± 11.70a	340.0 ± 5.774b	315.0 ± 3.606b	0.005
28th	372.0 ± 11.37a	341.7 ± 4.410b	310.0 ± 5.292c	0.004
42th	315.0 ± 2.887b	342.3 ± 3.930a	312.7 ± 3.180b	0.001
56th	308.7 ± 7.688	309.7 ± 3.480	312.3 ± 4.333	0.889
IgM (ng/mL) / day				
0	144.3 ± 0.882	143.3 ± 2.404	144.0 ± 2.082	0.932
7th	169.0 ± 6.658a	150.0 ± 1.155b	144.0 ± 1.000b	0.01
14th	194.3 ± 2.963a	168.3 ± 1.667b	143.3 ± 2.028c	< 0.001
17th	181.0 ± 2.082a	169.0 ± 2.082b	144.0 ± 0.577c	< 0.001
28th	179.7 ± 2.186a	171.3 ± 2.404b	148.0 ± 1.155c	< 0.001
42th	150.7 ± 1.764b	169.3 ± 0.667a	147.0 ± 1.528b	< 0.001
56th	149.0 ± 0.577	151.0 ± 3.786	146.7 ± 0.882	0.45
IgA (ng/mL) / day				
0	126.3 ± 1.202	126.0 ± 2.000	124.0 ± 0.577	0.482
7th	143.7 ± 4.410a	129.0 ± 1.528b	124.7 ± 0.333b	0.006
14th	147.3 ± 5.487a	150.0 ± 3.786a	126.0 ± 1.000b	0.009
17th	151.3 ± 2.603a	154.0 ± 2.082a	124.7 ± 0.333b	< 0.001
28th	154.7 ± 1.453a	157.3 ± 1.764a	125.0 ± 1.528b	< 0.001
42th	129.3 ± 3.180	126.3 ± 1.202	124.7 ± 0.882	0.326
56th	128.3 ± 2.848	126.7 ± 0.333	125.7 ± 1.453	0.615
IL-6 (pg/mL) / day				
0	82.33 ± 1.856	83.33 ± 0.882	79.33 ± 2.333	0.329
7th	92.00 ± 1.528a	82.67 ± 0.882b	82.33 ± 2.028b	0.007
14th	100.3 ± 0.882a	91.67 ± 0.882b	81.33 ± 0.882c	< 0.001
17th	92.33 ± 1.202b	113.3 ± 4.702a	83.33 ± 0.333b	0.001
28th	92.67 ± 1.856a	93.67 ± 0.882a	82.33 ± 1.453b	0.003
42th	82.33 ± 1.667b	93.33 ± 0.882a	82.00 ± 1.528b	0.002
56th	74.67 ± 2.028b	80.67 ± 1.202a	82.67 ± 0.333a	0.015
IL-12 (pg/mL) / day				
0	82.33 ± 2.404	79.00 ± 2.082	75.67 ± 1.453	0.144
7th	89.00 ± 0.577a	76.00 ± 0.577b	76.33 ± 1.333b	< 0.001
14th	102.0 ± 3.055a	89.00 ± 0.577b	76.00 ± 1.155c	< 0.001
17th	90.00 ± 1.155b	106.7 ± 3.528a	75.33 ± 1.453c	< 0.001
28th	89.00 ± 0.577b	91.00 ± 0.577a	76.33 ± 0.333c	< 0.001
42th	78.00 ± 1.000b	90.00 ± 1.155a	73.33 ± 0.882c	< 0.001
56th	75.50 ± 1.155	73.33 ± 0.882	74.00 ± 1.155	0.4
TNF-α (pg/mL) / day				
0	74.33 ± 1.202	72.33 ± 2.404	71.33 ± 2.906	0.659
7th	90.50 ± 1.607a	79.00 ± 0.577b	69.33 ± 2.963c	0.001
14th	108.0 ± 1.528a	101.3 ± 1.764a	70.67 ± 2.906b	< 0.001
17th	101.0 ± 2.082b	109.3 ± 1.764a	70.67 ± 1.764c	< 0.001
28th	96.67 ± 2.404a	96.33 ± 2.186a	69.67 ± 1.453b	< 0.001
42th	93.00 ± 1.528a	92.00 ± 2.082a	69.00 ± 0.577b	< 0.001
56th	71.00 ± 0.577	70.33 ± 0.882	68.67 ± 2.333	0.55

Values are represented as mean ± SEM. Means within the same row carrying different superscripts (a, b, c and d) are significant at $p < 0.05$. Immunoglobulin (Ig), interleukin (IL), tumor necrosis factor (TNF)

The essential hematological alterations in this study were the increase in total leukocytes and neutrophils in the treatment group (G2), which could be due to infection, immune system problems, or stress (24). The obtained leukocytosis represented by neutrophilia may be attributed to the induced stress during *E. coli* infection, causing the release of endogenous corticosteroids. In harmony with our results, garlic powder supplementation had no positive effect on Hb, PCV, and RBC in finishing pigs (25). Improved hematological parameters could be due to reducing bacterial infection or parasitic infestation or due to improved nutrient supply as a response to allicin supplementation (26, 27).

Higher serum total protein and albumin level were consistent with a previous study (28). A significant increase in globulin concentration may be attributed to tissue injury (29). The substantial increase in globulin in goats following *E. coli* inoculation could be attributed to the pathogenic agents stimulating the immune system (30). The significant increase of MDA on the 14^{th} day in G1 and on the 7^{th} day in G2 may be explained as infectious diseases causing inflammatory reactions associated with enhanced oxidative reactions and limiting antioxidant defense capabilities (31) Excessive lipid peroxidation in plasma and cells could explain these findings. Allicin acts as an antioxidant by trapping radicals when it reacts with free thiol-containing enzymes. Allicin has been proven to scavenge hydroxyl radicals, limit superoxide formation, and inhibit superoxide production (32). The decreased MDA values on the 17^{th} day in both groups and the significant increase of TAC in both groups after allicin administration may be attributed to the antioxidant nature of allicin (33). The results indicated that allicin treatment ameliorated oxidative stress.

Garlic has the ability to boost an animal's immune system. Our findings were consistent with those of Alam et al. (34), who reported that allicin administration has antioxidant, anti-inflammatory, and immunostimulant effects. Allicin induced IgG, IgM and IgA elevation provided evidence for allicin's immunostimulatory effect, which is consistent with its effect on calves (35). Additionally, the obtained results showed that allicin administration improved serum immunoglobulins as immunity markers, showing the highest improvement of immunity. Higher immunoglobulins could be related to increased cytokine production, which could imply an immunomodulatory effect of allicin (36). This demonstrates the ability of allicin to induce a humoral- and cellular-mediated immune response to decrease inflammatory reaction provoked by infection (36). So, the allicin's capacity to modulate the synthesis of these components may help to alleviate some of the symptoms of infection and to lower disease severity.

Interleukins are certain endogenous pyrogens released by the host's activated phagocytic cells and reticulo-endothelial cells in reaction to inflammation, tissue damage, bacterial toxins, and other illnesses. Cytokines have a crucial role in the inflammatory response and the progression of infection (37).Some cytokines (IL-6, IL-12, and TNF-α,) have been shown to increase during infections (38). Allicin stimulates anti-inflammatory effects by stimulating of pro-inflammatory cytokines (36). Garlic or garlic constituents have an immunomodulatory effect on cytokine production. The selected interleukins (IL-6, IL-12, and TNF-α) increased significantly after *E. coli* inoculation. Our finding strongly supported the previous results of Fernández et al. (39), who reported a significant interaction between the microorganisms and cytokine markers. Nevertheless, our results are inconsistent with a previous work (40) which found that allicin had a strong inhibitory effect on the three inflammatory factors, TNF-α, IL6 and IL12. Garlic extract has recently been shown to stimulate TNF- gene production in murine macrophages (41). The present study demonstrated that allicin administration could modulate the immune response in goats toward the bacterial infection, colibacillosis, therefore maintaining the body's defensive mechanisms within normal ranges. Thus, oral administration of allicin preparation may be a practical strategy to increase animal immunity, which benefits profitability in livestock production.

Conclusion

It was concluded that dietary supplementation of allicin with normal ration would be an additional management approach to improve the immunological and antioxidant status of goats, which

would increase their resistance to bacterial infection, particularly colibacillosis.

All of the authors were contributed in the study design and in the writing of the manuscript. The samples were collected by NEA and EMF, who also monitored the animals. The data was analyzed by YHB and MB, and the results were reviewed by AB. The final manuscript was read and approved by all authors.

The authors declare that they have no competing interests.

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