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Metabolic, Fermentation, and Hematological Parameters of Buck Goats Fed on Diets Containing Ziziphus spina-Christi Leaves

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Abstract

The objective of the study was to assess the effect of supplemented diets with Ziziphus spina-Christi (ZSC) leaf on the metabolic, fermentation, and hematological parameters of 12 buck goats. ZSC leaves were collected from (Sidr trees) scattered throughout Baghdad, Iraq.The study contained 3 groups that included four animals each. The First set was daily fed on 20 g/kg (T1 = 2%) of ZSC along with a basic diet. The second set was daily given a basic meal plus 40 g/kg (T2 = 4%) of ZSC. The third set was called (Control) which was only offered *ad libitum* daily.The samples of ruminal fluid were collected from 6 goats at the end of each month. Ruminal parameters were measured including pH values, total bacterial count (TBC), and volatile fatty acids (VFA), containing acetic, butyric, and propionic acids. The Hematological samples were collected to assess the blood picture, such as WBCs, RBCs, and platelets, which were recorded by using an auto-analyzer. The levels of VFAs were significantly increased in both T1 and T2 groups in comparison to the control, but no significant differences were observed in pH rumen among groups until the end of the trial where control and treated groups had significantly different ruminal pH. Similarly, treated groups were significantly distinguished from the control in terms of the total bacterial counts. In hematological parameters, buck goats recorded significant differences in WBCs in the T1 and T2 groups compared to the control. Also, there were significant differences in lymphocytes % and monocytes % were observed. Likewise, treated groups revealed that RBC, platelets, and Hb differed significantly ($P \leq 0.05$). In conclusion, the ZSC supplementation (20 g/kg and 40 g/kg) could improve the bacterial community, fermentation, and hematological parameters in the buck goat diet.

Keywords: Ziziphus spina-Christi, buck goats, Total bacterial count, pH

Introduction

Herbal medicine is referred to as a method that uses herbs, herbal materials, herbal preparations, and completed herbal medicines that have plant parts, other plant components, or mixtures as active agents. These herbs are made from plant parts such as seeds, leaves, stems, blossoms, and roots (18). The local Arab population uses every component of the plant to support a healthy lifestyle of life (31). In the Arabic language, they refer to Ziziphus spina-Christi as (Nabka or Sidir). In Iraq's central and southern region, identified as Nabag, the plant Zizyphus spina-Christa grows massively (30). Research explores herbal medications and plants to enhance animal performance without antibiotic resistance, replacing synthetic medications (19). Researchers are becoming increasingly interested in medicinal herbs due to their antioxidant properties, bioactive compounds, and lower levels of toxicity (23). Crop preparations, including seeds, sprouts, and seedlings, contain health-promoting phytochemicals like flavonoids and phenolic acids, potentially increasing their agri-food value for functional foods and nutraceuticals (45)

Sider leaves powder was employed, which is a significant multifunctional plant that has been used in Middle Eastern traditional medicine and is frequently used in the management of pain and inflammatory-related issues in the treatment of human and animal infections. In general, Ziziphus species are safe and non-toxic to people (38). On the other hand, there are plenty of inexpensive, easily available resources (41).

Small branches are ground up and fed to camels and goats as feed during the dry season, which is when leaves are used as food for animals. According to biological research (7), the Ziziphus plant has many significant pharmacological properties. Due to their usage in traditional medicine, Ziziphus leaves are regarded as one of the most important. Additionally, it contains a variety of biological compounds, including alkylphenols, pro-anthocyanidins, flavonoids, and flavones. These chemicals hold promise for enhancing both human and animal health (35). Sider, a safe and protective substance, is used in ethnomedicinal to treat various illnesses such as hypercholesterolemia, Alzheimer's disease, the flu virus, and sneezing (37).

Ziziphus spina-Christi (ZSC) is also an excellent source of natural antioxidants that can be utilized to lower levels of total phenolic compounds, reduce lipid peroxidation, and boost the activity of the endogenous antioxidant enzyme (49). Flavonoids, which have been widely employed as anticancer, antimicrobial, antiviral, and antibacterial agents, were present in the leaves of ZSC (50). This phenolic, flavonoid, and condensed tannins can be metabolized into intermediate metabolites in the rumen by boosting the protein, fiber, and fat content of feeds as well as by interacting with the rumen microbiome (1). ZSC contains vitamins like A and E. Vitamin A is essential for maintaining organ performance, while vitamin E, a fat-soluble vitamin, is crucial for growth and various biological processes (5). Additionally, it includes vitamins C, A, B1, B2, B5, and B12, as well as various minerals (10,6).

Thus, the study aimed to investigate the effects of ZSC leaves as a natural feed additive on functions of the digestive system such as ruminal fluid containing pH values, volatile fatty acids, and bacterial community in buck goats plus some blood parameters.

Material and Method:

Design, setting, and date of the experiment:

Twelve male goats, 12 to 15 months old, weighing 22 to 28 kg on average, were brought from the nearby market. Animals are kept on a farm at Baghdad University's veterinary college from December to February. These animals were permitted to graze freely on the college fields for a few hours each day while being fed a simple diet of roughage and concentrates. It was kept on the farm for two weeks to give it time to adjust. Groups T1 and T2 received daily rations of 20 g/kg or 40g/kg ZSC plus a simple diet. while Group C consumed a typical diet each day.

Preparation of study food:

The Gardens of Baghdad collected Ziziphus spina Christi leaves from the College of Veterinary Medicine. The samples were then sent to the Ministry of Science and Technology and Department of Environment and Water laboratories for determination of the total phenolic content. A 50.12 mg/g result was obtained. Before being ground in a blender to a thickness of 1 mm and baked for three hours at 70 ℃, the gathered leaves were dried in the shade. They were then split into two groups and given weights of 40 and 20 grams, respectively (22).

Rumen production trails

After two hours post-feeding, the samples (ruminal fluid) were collected at the end of each month. Ruminal parameters include pH, total bacterial count (TBC), and volatile fatty acids (VFA), which include acetic, butyric, and propionic acids, as described by (24) with some modifications to conduct further analysis. The samples were divided into three parts and put in a single-use tube for the next experiments.

Estimation of the Total Bacterial Count (TBC):

The rumen fluid was taken down and collected in a 60 ml specimen container. Then, after filtering, 1 ml of it was extracted. Each sample was prepared by diluting (10 folds) in a solution containing 0.1% (w/v) sterile peptone water. For each dilution, the dilution was then applied to nutrient agar plates. The number of colonies that have expanded is counted after the plates have been incubated (16)

Rational pH evaluation:

The pH samples were immediately measured using a digital pH Checker (Benchtop pH meter LBPM-A20) and balanced between four and nine standardized buffer solutions, as mentioned by (43).

Calculating the percentages of volatile fatty acids

The VFAs (Acetic, Butyric, and Propionic Acids) were identified through this test. 5 ml of the sample was taken, added to a bomb marked with a 50 ml capacity, and then left on the vibrator for 5 hours. The sample was subsequently filtered and kept in the refrigeration unit, and the examination was performed. Following that, additional experiments were conducted in the laboratories of the Ministry of Science and Technology's Department of Environment and Water using a Gas Chromatograph (model Shimadzu 2010/Japanese), a flame detector in place (FID), and capillary sodium (type 1-DE) in lengths of 30 meters, 0.25 micrometers, and 0.25 millimeters. The temperatures of the injection and capping areas were (330, and 280, respectively), while the temperature of the separating column gradually increased. The formula was used to establish the VFA percentage as described by (47) and (46)

Hematological examinations

The sample was put inside the EDTA anticoagulant tube, which also contained gel and a clot activator. The parameters of the blood picture, such as WBCs, RBCs, and platelets, were then obtained by using an auto-analyzer.

Result and discussion

Table (1) presents the results of determining the volatile fatty acid (VFA) levels in rumen fluid two hours after consumption of ZSC leaves. The levels of VFA interestingly, acetic, propionic, and butyric acids increased significantly ($P \leq 0.05$) in both T1 and T2 samples with higher concentrations of ZSC (T2) as a result of fermentation in comparison to the control. Longer fermentation times resulted in significantly higher total VFA values within groups with a p-value of less than 0.05, but no significant differences were found between the values of the control group and the other treated groups (T1 and T2).

Acetic, butyric, and propionic acid concentrations were measured in Group T1, which received 20g of ZSC. The results for the third period showed a statistically significant improvement (P 0.05), with VFA values of (52.02 0.73, 24.31 1.33, and 23.29 0.30, respectively). The first and second periods' results showed no significant increase. The increase was noticeable throughout the experiment in group T2, which received 40g of ZSC, with values of (49.150.53, 21.230.3, and 19.770.26). Propionic acid values for the control group throughout the experiment were 11.010.40, 13.921.31, and 13.180.18. In contrast, the control group showed no discernible increase during any of the periods.

Table (1): Effects of various ZSC concentrations on volatile fatty acids from ruminal fluid. (n = 6 ±SE)

	Acetic acid			Butyric acid			Propionic acid		
Group	1st period	2nd period	3rd period	1st period	2nd period	3rd period	1st period	2nd period	3rd period
Control	A41.01 ±0.94b	A40.85 ±0.26c	A44.25 ±0.79c	A12.91 ±0.38c	A14.18 ±0.29c	A13.95 ±0.35c	B11.01 ±0.40c	A13.92 ±1.31c	A13.18 ±0.18c
T1 (20g/kg)	B45.78 ±0.46a	B46.21 ±0.75a	A52.02 ±0.73a	C18.35 ±0.28a	B20.21 ±0.33a	A24.31 ±1.33a	B18.08 ±0.61a	B19.44 ±0.32a	A23.29 ±0.30a
T2 (40g/kg)	C41.34 ±0.89b	B43.92 ±0.74b	A49.15 ±0.53b	C16.37 ±0.53b	B18.17 ±0.30b	A21.23 ±0.34b	C14.91 ±0.59b	B16.43 ±0.34b	A19.77 ±0.26b
LSD	2.12			1.62			1.48		

Various capital letters in the row and other small letters in the column both result in significantly different ($P<0.05$).

In contrast to (48) findings that ZSC consumption did not affect total VFAs, acetate, or butyrate concentrations in cattle, the increased ZSC in the research led to a higher total VFA and a wider range of acetic acid generation. Nevertheless, (25) found that assuming less ZSC increased the overall VFA level while lowering the acetate ratio.

The aforementioned discrepancies may be caused by the different ways that ZSC and the compounds that follow can affect the bacteria that produce VFA. Bacteria, particularly those that produce cellulose and hemicellulose, are essential for the production of VFA from soluble carbohydrates (11,40). Rumen microbes use VFA as a primary energy source for ruminant animal needs (9).

The ZSC increased acetic acid concentrations but did not alter propionic or butyric acid levels. Additionally, a study on the effects of short-term elevated outside temperature on ruminal VFA dynamics revealed changes in rumen VFA fluxes; while total VFAs remained unchanged, the thermal environment effect changed relative proportions (12). The current findings may therefore be consistent with other plausible explanations, such as variations in rumen VFA fluxes in small livestock being related to higher ambient temperatures or environmental conditions, which can be lessened by the supplementing method described in this work (39).

Evaluation of pH values:

In the study, the pH levels of the rumen in the T1 and T2 groups were not significantly different until the third period. However, there was a significant difference in the ruminal pH levels between the control and treated groups. The study found that the pH levels of the rations were within the normal range of 6.4 to 7.0, which is consistent with previous research indicating that there are no negative effects of ZSC on ruminal pH levels. The pH levels in T1 were higher than the control group but within normal bounds. This is shown in Table (2).

Table (2): Effects of different concentrations of ZSC on pH values of rumen fluid. (n = 6 ±SE)

pH Groups	1st period	2nd period	3rd period
Control	B6.47±0.06a	A6.60±0.04a	A6.72±0.04b
T1 (20g/kg)	C6.52±0.06a	B6.70±0.04a	A6.97±0.07a
T2 (40g/kg)	B6.45±0.08a	A6.65±0.05a	A6.80±0.07ab
LSD	0.17		

Various capital letters in the row and other small letters in the column both result in significantly different ($P<0.05$).

By previous studies (48,25), this study found no differences in the ruminal pH value of rumen goats given ZSC, with ruminal pH values within the typical range (6.92 and 7.07). These results suggest that the microbial rumen appears to have a high adaptability to the suggested treatment, regardless of additions or modifications in the composition of plant second-generation compounds derived from ingesting ZSC. However, a recent study on ZSC's impact on rumen pH came up with conflicting results. Polyphenols reduce stress and maintain healthy rumen pH in animals (42)

Flavonoids are beneficial for animals under stress and in mild acidosis cases (36,32). Due to structural similarities and the presence of tannins, cinnamic acid, and other phenolics (39), flavonoids have a significant balancing effect on the pH of the rumen.

Total bacterial count

In all three periods, the treated groups distinguished significantly from the control in terms of the total bacterial counts of rumen fluid. In comparison within groups demonstrated significant changes in the total bacterial counts in both T1 and T2 of ZSC. While there were no significant differences found within the control groups, the groups had significantly different measurements from the control groups in T1 and T2 (P 0.05).) as illustrated in Table (3).

Table (3): Effects of different concentrations of ZSC on total bacterial count of rumen fluid. (n = 6 ±SE)

Groups / Log^{10}	1st period	2nd period	3rd period
Control	A10.78±0.01a	A10.80±0.01a	A10.78±0.01a
T1(20g/kg)	A10.71±0.01b	B10.66±0.02b	B10.63±0.01b
T2 (40g/kg)	A10.66±0.02c	A10.63±0.01b	B10.56±0.01c
LSD	0.03		

Various capital letters in the row and other small letters in the column both result in significantly different (*P<0.05*).

According to (13), the bioactive chemicals in ZSC may interfere with the semi-permeability of rumen membranes and inhibit their function, particularly in carbohydrate degradation. Furthermore, ZSC appears to encourage nutrient fermentation rather than acting as an antibacterial. According to research by (17) the ZSC has been shown to influence microbial activity to improve food digestion in the rumen and increase the ruminal VFA content.

By affecting the cytoplasmic membrane's function, the development of the bacterial cell wall, or the synthesis of nucleic acids, flavonoids prevent the growth of Gram-positive bacteria. By increasing propionate synthesis in comparison to acetate, flavonoids have been suggested for inclusion in ruminant diets to increase productivity (26). Additionally, polyphenols affect bacteria with an antibacterial effect (27).

Following ZSC administration, the proportion of ruminal microorganisms including protozoa, total bacteria, and fungal zoospores changed. When ZSC was added, the overall bacterial population remained unchanged, supporting a recent finding by (7) that ZSC could not

increase bacterial activity, particularly that of cellulolytic bacteria. Contrarily, a rise in ZSC was connected to a fall in methanogenic bacteria. Once ZSC has engulfed this membrane, bacteria are no longer able to control ion gradients, electron mobilization, phosphorylation cascades, protein translocation, and other enzymatic processes using chemiosmotic control. High-phenolic plants increase goat biotransformation enzyme production (33). Against Gram-negative and Gram-positive bacteria, ZSC powder exhibits promising antibacterial properties (21).

Hematological parameters

According to Table (4), buck goats recorded significant differences (P≤0.05) in the number of white blood cells (WBCs) of T1 and T2 groups compared to the control during the trial. Also, there were significant differences (P≤0.05) in lymphocytes (LYM%) and monocytes (MON%) were observed in two periods with different ZSC concentrations, especially in T1 and T2 groups. However, T2 showed a greater decrease with high ZSC concentration compared to T1 with low ZSC concentration (10.74±0.25, 9.67±0.22); (55.50±1.51, 51.01±1.71) and (9.82±0.25, 8.26±0.12) respectively. No significant difference (P≥0.05) was observed in WBC% in the control group during the same period, but significant differences were observed in LYM% and MON%.

Table (4): Effects of different concentrations of ZSC on lymphocytes and monocytes. (n = 6 ±SE)

	WBC%		LYM%		MON%	
Group	1st period	2nd period	1st period	2nd period	1st period	2nd period
Control	A11.57 ±0.37a	A11.33 ±0.34a	A48.50 ±1.34c	B43.57 ±0.78b	A11.13 ±0.29a	B10.14 ±0.17a
T1 (20g/kg)	A10.68 ±0.43a	B9.23 ±0.34b	A59.78 ±0.18a	B50.41 ±1.46a	A10.75 ±0.17a	B9.92 ±0.26ab

T2 (40g/kg)	A10.74 ±0.25a	B9.67 ±0.22b	A55.50 ±1.51b	B51.01 ±1.71a	A9.82 ±0.25b	B8.26 ±0.12b
LSD	0.99		3.80		0.66	

Various capital letters in the row and other small letters in the column both result in significantly different (*P<0.05*).

Blood tests are useful in research because they provide quantitative indicators of physiological adjustments made in response to outside influences, such as toxic stress in test animals (1). The results of (4) are consistent with the hypothesis that the lower WBC count in the two experimental groups may have been caused by the higher concentration of ZSC (40 g/kg) administered orally to the group receiving treatment.

An essential component of the host's defense system, white blood cells (WBCs) are in charge of protecting cells from pathogenic organisms like bacteria, fungi, parasites, and viruses. Inflammation and abnormalities of the bone marrow are indicated by higher WBC levels, whereas leukopenia or the use of anti-infective medications are indicated by lower WBC levels (44). Additionally, the fact that the polymorphonuclear leukocyte levels are unaffected suggests that the ZSC may not have triggered an inflammatory response, although these levels are typically high during inflammation. Unlike ROS like O2, OH, LO, and LOO, flavonoids interact with these radicals and neutralize them by transferring a hydrogen atom or an electron to form a stable quinine molecule (4). One of the most substantial natural antioxidants that fight oxidative stress is vitamin E which is found in ZSC (22). In (8) Vitamins are organic substances without energetic value but necessary for the metabolism of animals or human organisms (20). conducted a study on the effects of sub-acute administration of ZSC. The study found that there were no significant changes in hematological parameters, except for some minor differences. The researchers examined different doses of ZSC leaves in a control group and discovered no significant alterations in white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and basophils across the treatment groups.

Hematological cells (Hb, RBCs, PLT):

Table (5) reveals that different groups' average red blood cell RBC, platelets PLT, and hemoglobin Hb levels differed significantly (P≤0.05). Significant differences (P≤0.05) were found between the groups from the beginning to the end of the second period. T1 had a registered RBC value of in the first period 12.10±0.24, which climbed to 13.27±0.24 in the second period. T2 on the other hand had RBC values of 13.12±0.25 and 14.50±0.21 in the first and second periods, respectively, similar to how T2 performed in Hb and PLT in the second

period with a high concentration of ZSC. When the concentration was raised, the outcomes improved.

Table (5): Effects of different concentrations of ZSC on hematological cells. (n = 6 ±SE)

Cells groups	RBC		Hb		PLT	
	1st period	2nd period	1st period	2nd period	1st period	2nd period
Control	B10.23 ±0.24c	A11.30 ±0.10c	A8.42 ±0.19b	A8.82 ±0.1b	341.25 ±3.94	389.00 ±3.77
T1 (20g/kg)	B12.10 ±0.24b	A13.27 ±0.24b	B8.30 ±0.32b	A9.42 ±0.2b	350.75 ±5.02	395.00 ±2.55
T2 (40g/kg)	B13.12 ±0.25a	A14.50 ±0.21a	B9.37 ±0.18a	A11.62 ±0.21a	372.25 ±3.72	463.25 ±3.35
LSD	0.66		0.64		11.83	

Various capital letters in the row and other small letters in the column both result in significantly different ($P<0.05$).

Hematological markers are essential in these investigations because they are sensitive indicators of physiological changes brought on by outside factors, particularly stress brought on by toxic substances in test animals. Minerals like calcium (1,270 mg%), iron (7.2 mg%), and magnesium (169 mg%) are abundant in ZSC leaves. Iron is essential for treating anemia and helps transport oxygen and carbon (IV) oxide between cells as well as produce blood (38). The bone marrow can be regulated during this process by the hormone EPO, which is unique to RBCs. Although translational and post-transcriptional mechanisms are also involved in the regulation of EPO gene expression, the transcriptional phase is the main control point. According to experimental data, erythropoiesis which is mediated by EPO may be responsible for the beneficial effects of ZSC in treating blood deficiencies (14). Thus, significant increases in Hb, RBC, and PLT were found to be the only hematological parameters that the subacute treatment with the extract significantly affected. As seen in Table 5, there were no appreciable differences between the treatment groups and the control group when comparing Hb, red blood cells, white

blood cells, neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Sider contains flavonoid compounds, which are powerful antioxidants that shield a variety of body cells from damage. For example, red blood cells are protected by flavonoid compounds because they help to keep antioxidant enzymes active and eliminate free radicals. This prevents fats from oxidizing in the membranes of various body cells (2). ZSC's positive effects include recycling heme iron during erythrophagocytosis, controlling immune response bidirectionally, and regulating erythropoiesis through hypoxia-inducible factor-induced erythropoietin activation (29)

The analysis of hematology parameters in animal studies has a high relevance and predictive value for humans because hematological alterations like anemia are typically accompanied by bone marrow toxicity (14). Additionally, since polymorphonuclear leukocyte levels are typically elevated during inflammations, the lack of effect on these levels suggests that the ZSC may not have induced an inflammatory process. The platelets are crucial to the coagulation process, and this study found that the tested samples had significantly elevated platelet counts, which indicated hemostatic activity Platelets are important in the coagulation pathway (15). This study found that the only changes in hematological indicators resulting from ZSC therapy were significantly higher platelet levels in T2, with a P-value of (463.25±3.35). Sider's active ingredient, vitamins C and E, exhibits antioxidant properties, which can either stop or reduce oxidative stress in various plants (3). Flavonoids aid in iron deficiency anemia growth, improving hematological parameters and iron in hepatocellular tissue and ferroprotein representation (34).

Conclusion:

The goal of the current study was to identify the findings that would support the possibility that using ZSC leaves (20 g/kg and 40 g/kg) could enhance the bacterial community, fermentation, and hematological parameters of buck goats. Nevertheless, through the conclusion of the study, when the control and treated groups had considerably different ruminal pH, there were certainly no significant changes in the levels of VFAs between the two treated groups and the control group. As far as the total bacterial counts, the treatment was substantially different from the control. Buck goats in both treatments showed substantial variations in WBCs as opposed to the control in hematological parameters. Significant variations in lymphocyte percentage and monocyte percentage were also seen. Additionally, treated groups showed a significant difference in RBC, platelets, and Hb. Consequently, using this plant should be considered when applying industrial materials is not interesting. Future studies are needed to confirm the potential benefits of ZSC found in this research.

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