MENOUFIA JOURNAL OF ANIMAL, POULTRY AND FISH PRODUCTION

https://mjapfp.journals.ekb.eg/

AZOLLA (*AZOLLA PINNATA*) LEAVES EXTRACT SUPPLEMENTATION: EFFECT ON DIGESTIBILITY, BLOOD CONSTITUENTS, AND IMMUNITY STATUS OF OSSIMI SHEEP

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Received: April 20, 2025 Accepted: June. 1, 2025

ABSTRACT: The present study was carried out to investigate the effect of ethanolic extract of Azolla (*Azolla pinnata*) leaves supplementation (AE) on the performance and immunity status of Ossimi sheep. Twelve Ossimi rams, 20 months old with an average body weight of 44.22 ± 2.45 kg were divided into three comparable groups (four rams /group) according to their live body weight. Animals in the control group were fed clover hay and a concentrate feed mixture (CFM) at a 40:60% roughage concentrate ratio without Azolla extract (AE0). The treated groups received the control diet supplemented with Azolla extract either 200 mg (AE1) or 400 mg (AE2) per kg of dry matter (DM). Results indicated that Azolla extract at both levels as a feed additive significantly enhanced (P<0.01) DM digestibility values compared to the non-additive diet (AE0), with values of 57.02, 56.09, and 54.45 for AE2, AE1, and AE0, respectively. Animals on the AE2 diet recorded the highest (P<0.01) CP, CF, and NFE digestibility values being 59.68, 57.68, and 68.43%, followed by AE1; 58.75, 56.98, and 67.83. However, the AE0 group showed the lowest (P<0.01) values for corresponding values; 57.01, 54.88 and 67.00% respectively, a similar trend was observed with nutritive value, where the AE2 diet showed the highest (P<0.01) value of TDN (61.75%) and AE0 recorded the lowest one (58.72%), AE1 was intermediate (61.04%). Digestive crude protein (DCP %) followed a similar pattern to TDN. Azolla extract additives in both levels; 200 and 400 mg/ kg DM, improved (P<0.01) nitrogen balance by 7.64 and 14.96 % above the control group, respectively. Total VFA in the rumen was significant in response to Azolla extract at two hours. post feeding, the AE2 group recorded the highest (P<0.01) values (16.70 meq/dl) compared to the control group (AE0) which recorded the lowest (P<0.01) value (15.14 meq/dl), while AE1 was intermediate (15.57 meq/dl). Rumen NH3-N concentration followed a similar pattern to rumen VFA. Azolla extract additives at level 400 mg/kg DM (AE2) recorded the highest (P<0.01) values of serum total protein and albumin (6.44 and 3.80 g/dl respectively) being higher (P<0.01) than AE1 group (6.14 and 3.50 g/dl), which also was significantly higher (P<0.01) than AE0 group (5.72 and 2.97 g/dl respectively). Glucose concentration was significantly higher (P<0.01) with AE1 and AE2 groups (66.30, and 66.03 mg/dl respectively) compared to the control group (64.98 mg/dl). Liver and kidney functions, as well as hematological parameters were insignificant as affected by Azolla extract. Immunoglobulin A (IgA) values were improved (P < 0.01) with Azolla extract groups; AE1 and AE2 (35.65 and 35.63 mg/dl, respectively) compared to the control group (32.90 mg/dl). Immunoglobulin G (IgG) values followed a similar trend. Interleukin 2 improved (P < 0.01) with the AE2 group (61.43 Pg/ml) compared to AE0 and AE1 groups (58.90 and 59.70 Pg/ml, respectively). All blood criteria were within the average values of the blood characteristics of sheep, indicating that Azolla extract did not have any adverse effect on animals' health and hygiene.

Keywords: Azolla, extract, sheep, digestibility, immunity.

INTRODUCTION

The global market for feed additives was assessed at USD 37.92 billion in 2024, with expectations of growth to USD 39.80 billion in 2025 and reaching USD 53.66 billion by 2032. A notable segment within this market is the increasing utilization of plant extracts as feed additives, which is gaining momentum due to a rising preference for natural alternatives over synthetic options. Between 2020 and 2025, this sector is anticipated to grow at a compound annual growth rate (CAGR) of around 7.5%,

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potentially achieving a valuation of USD 1.5 billion by 2025 according to Markets and Markets report. This upward trend is largely driven by heightened awareness regarding the advantages of phytogenic feed additives, which promote animal health and productivity and contribute to decreased dependence on antibiotics (Franz *et al*., 2020; Alem, 2024).

The interest in utilizing and promoting Azolla as a feed source for livestock has been on the rise, primarily due to its superior protein content, which ranges from 19% to 30%, surpassing that of most green forage crops and aquatic macrophytes. Additionally, Azolla possesses a favorable profile of essential amino acids, particularly lysine, making it an advantageous nutritional option for various animal species, including ruminants, poultry, pigs, and fish (Hasan and Chakrabarti, 2009). This aquatic fern can serve as a feed substitute for animals such as sheep, goats, pigs, and rabbits. However, the existing literature regarding the nutritional benefits of Azolla specifically for ruminants remains limited (Kumari *et al*., 2021).

Feed additives play a crucial role in animal nutrition by enhancing the quality of feed and the safety and nutritional value of animal-derived products, while also promoting the overall health and performance of livestock (Nayel et al., 2019). Among these additives, plant extracts are recognized for their herbal origins and are incorporated into animal diets to boost both productivity and the quality of the resulting products; they enhance the growth performance of animals and improve nutrient digestibility, primarily due to the favorable influence of plant secondary metabolites (PSM) on the activity of ruminal microorganisms (Xu *et al*., 2010; Jiménez-Peralta *et al*., 2011; Nayel, 2021). Additionally, these extracts may facilitate an increased flow of amino acids to the duodenum (Harvey, 2008; Mapiye *et al*., 2010). Certain rumen bacterial species possess the ability to metabolize phenolic compounds (Salem *et al*., 2010a), potentially serving as catalysts for fiber degradation by enhancing the accessibility of fibrolytic bacteria to dietary cell wall polysaccharides. Consequently, PSM as natural feed additives optimize the efficiency of rumen fermentation, improve protein metabolism, reduce methane emissions from the rumen, alleviate nutritional stressors such as bloat, and ultimately enhance overall animal health and productivity (Patra *et al*., 2006; Alem 2024).

The prevalent application of antibiotic feed additives can be attributed to their positive impacts on health, performance, and the efficiency of nutrient and energy utilization. However, the growing inclination towards more natural methods of animal production has fostered a more discerning perspective among consumers regarding the use of antimicrobial agents in feed. All plant extracts derived from natural sources are inherently safe due to their organic nature. Nevertheless, the potential for natural plant products to serve as productivity enhancers present a more cost-effective, safer, sustainable, and consumer-friendly alternative to synthetic additives (Tekeli *et al*., 2007).

Abou El-Fadel *et al*. (2020) demonstrated that the inclusion of Azolla in the diets of crossbred Osimi lambs led to an enhancement in the digestion coefficients of crude fiber and ether extract. Conversely, the digestibility of dry matter (DM), organic matter (OM), crude protein (CP), and nitrogen-free extract (NFE), along with the feeding values represented as total digestible nutrients (TDN) and digestible crude protein (DCP), experienced a decline. In contrast, Bhatt et al. (2021) found that increasing levels of Azolla positively influenced the digestibility of DM, OM, CP, EE, neutral detergent fiber (NDF), acid detergent fiber (ADF), and TDN in Sahiwal calves.

The antimicrobial properties of plant extracts can be linked to various secondary metabolites, such as saponins, terpenoids, and phenylpropanoids, which are found in the essential oil components of numerous plant species. However, information is scarce regarding the influence of plant extracts on microbial fermentation within the rumen. Earlier studies utilizing in-vitro continuous culture techniques with diverse plant extracts and their secondary metabolites have indicated that certain extracts possess the ability to alter rumen

microbial fermentation processes (Cardozo *et al*., 2004; Busquet *et al*., 2005).

Numerous studies have indicated that phytochemical-rich plants and their extracts present viable alternatives to traditional antibiotics and chemical additives for use as rumen modifiers. These botanical products have the potential to enhance fermentation efficiency, reduce methane emissions, and possibly boost animal productivity. However, a significant limitation of much of the existing research is the insufficient focus on the antibacterial properties and cytotoxic effects of the plant extracts examined in these studies (Patra and Saxena 2009; Akanmu *et al*., 2020).

Alagan *et al*. (2020) reported an increase in hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC), and white blood cells (WBC) at a 5% inclusion level of Azolla. Conversely, Singh *et al.* (2021) found no significant differences in hematological parameters, such as PCV, Hb, RBC, and WBC, across the various experimental groups. However, they observed an increase in total protein and albumin levels, alongside a decrease in blood urea nitrogen levels in Decani ram lambs that were supplemented with Azolla pinnata. Similarly, El-Deeb *et al*. (2021) indicated that the majority of hematological traits in rabbits were unaffected by the incorporation of Azolla into their diets.

Effects on the immune system: The impact of saponins-based adjuvants as PSM on the immune system is noteworthy, particularly in their interaction with rumen bacteria. Research indicates that saponins may stimulate the synthesis of cytokines, including interleukins and interferon, which could play a role in mediating their immuno-stimulatory effects (Francis *et al*., 2002). Furthermore, evidence suggests that saponins might bolster the immune response by facilitating the absorption of antigens from the gastrointestinal tract and other biological membranes (Oda *et al*., 2000; Salem *et al*., 2012).

The present study was carried out to investigate the effect of Azolla (Azolla pinnata) leaf extract on digestibility, nutritive value, nitrogen balance, rumen fermentation, biochemical and hematological parameters, and immunity status of Ossimi sheep.

MATERIAL AND METHODS

The study was conducted in compliance with the Scientific Research Ethics and Animal Use Committee (SRE & AUC) Faculty of Agriculture, Menoufia University (Reference No. SREC -MUAGR-01-2025).

Azolla Preparation, Extraction, and GC-MS chromatogram analysis

Fresh samples of the plant were obtained from a commercial farm situated in Tala town, Menoufia Governorate, Egypt. The entire plant underwent multiple washes with distilled water to eliminate any dust particles, followed by shadow drying at ambient temperature for a duration of 3 to 4 days. The leaves of *Azolla pinnata* were then ground using a 0.25 mm screen to produce a fine powder. A total of 100 grams of this Azolla leaf powder was subjected to extraction in a 70% hydro-ethanolic solution (100 g/L) at a temperature of 40 °C for 72 hours (El-Desoky *et al*., 2017). The resulting ethanolic extract of the Azolla leaves was filtered using Whatman No. 1 filter paper (Little Chalfont, Buckinghamshire, HP7 9NA, UK). The supernatant obtained from this process was subsequently evaporated at 45 °C to eliminate ethanol, achieving complete dryness, after which it was stored at −20 °C (Hashem *et al*., 2019).

The chemical composition of Azolla leaf extract was analyzed utilizing a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) equipped with a direct capillary column TG–5MS (30 m x 0.25 mm x 0.25 µm film thickness). Through GC-MS analysis, a total of fourteen bioactive phytochemical compounds were identified, encompassing a diverse range of active phytochemicals including alkenes, alkanes, esters, ethers, and carboxylic acids. These compounds exhibit significant potential for interaction with other substances, and their chemical profiles were thoroughly characterized, detailing parameters such as Retention Time (RT), chemical composition, molecular formula, molecular weight, and percentage of peak area, as presented in Table 1 and Figure 1.

Table 1: The major bioactive compounds of the Azolla extract using GCMS*

No.	Compound	RT [min]**	Peak area	Molecular Weight	Molecular formula
1	Pentane, 3-methyl-	5.13	15.53	86	C_6H_{14}
2	2-Aminohexanoic acid	5.99	22.5	131	$C_6H_{13}NO_2$
4	1,3-Propanediol,2-ethyl-2-(hydroxymethyl)-	7.10	6.53	134	$C_6H_{14}O_3$
5	Octanoic acid, methyl ester	9.53	8.45	158	C_9H18O_2
6	1-Pentanol, 5-[(tetrahydro-2h-pyran-2-yl) oxy]-	9.73	4.44	188	$C_{10}H_{20}O_3$
7	Oleic Acid	18.62	10.03	282	$C_{18}H_{34}O_2$
8	Alanine,3-(benzyloxy)-, l-o-benzylserine	19.07	1.13	195	$C_{10}H_{13}NO_3$
9	Vitamin A aldehyde	20.68	6.64	284	$C_{20}H_{28}O$
10	12,15-Octadecadiynoic acid, methyl ester	20.91	4.72	290	$C_{19}H_{30}O_2$
11	9-Hexadecenoic acid	21.55	5.77	254	$C_{16}H_{30}O_2$
12	9-Octadecenoic acid	22.29	4.63	282	$C_{18}H_{34}O_2$
13	12,15-Octadecadiynoic acid, methyl ester	22.87	5.93	290	$C_{19}H_{30}O_2$
14	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester.	23.55	2.00	444	$C_{28}H_{44}O_4$

*GCMS: Trace GC Ultra-ISQ mass spectrometer.
** RT: retention time (min).

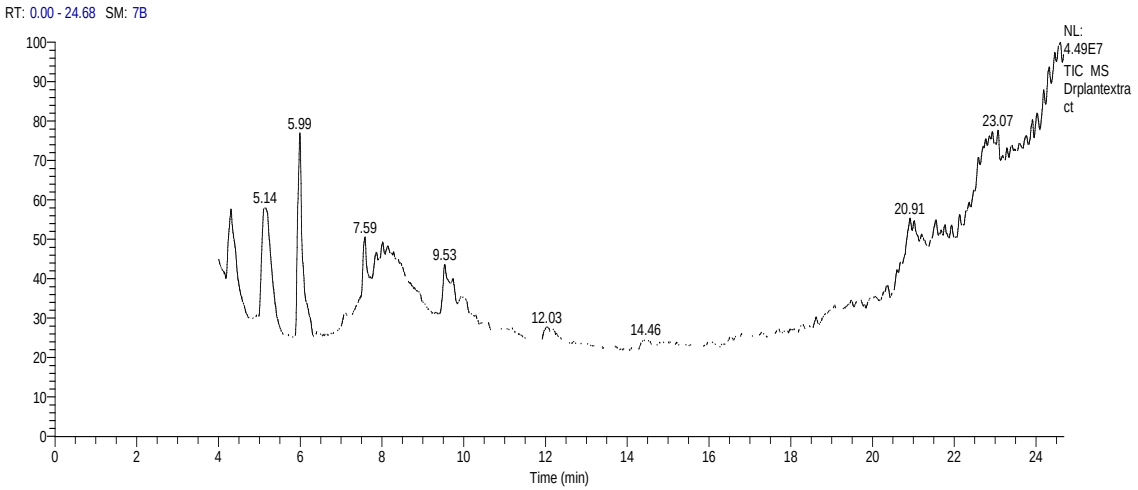

Fig.1. GC-MS chromatogram of Azolla leaves extract

Animal Management and Feeding Procedure

Twelve Ossimi rams, 20 months old and with an average body weight of 44.22 ± 2.45 kg, were allocated into three comparable groups, four sheep per group, based on their live body weight. The animals in the control group fed clover hay and concentrate feed mixture (CFM) in a ratio of 40:60, without the addition of Azolla extract (AE0). The treated groups were provided with the same control diet but with the supplementation of Azolla extract at 200 mg (AE1) or 400 mg (AE2) /kg DM. Animals were fed twice daily at 9:00 and 16:00 h., water was always available. Sheep were fed to meet their requirements of DM according to (NRC, 1985). The chemical composition of the experimental feeds and diets is detailed in Table 2, and the analysis of the experimental ingredients, diets, and fecal samples was conducted following the protocols established by the Association of Official Analytical Chemists (AOAC, 2000).

Digestibility, nitrogen balance and rumen fermentation

Four rams from each group participated in this study, which involved individual feeding in digestion metabolic cages as outlined by Maynard *et al.* (1979) to facilitate the separate collection of feces and urine. The rations were administered bi-daily at 8:00 AM and 4:00 PM, with water provided ad libitum throughout the experimental duration. The apparent digestion coefficients of nutrients were assessed through the total fecal collection method, which included a preliminary period of 21 days followed by an additional 5 days dedicated to the quantitative collection of feces and urine. The daily fecal output was measured, and samples representing 10% of this output were dried at 70°C for 48 hours until a constant weight was achieved, after which they were ground. Fecal samples from the 5-day collection period were combined and stored in polyethylene bags for subsequent analysis. To evaluate nitrogen balance, urine was collected in containers holding 100 ml of 10% HCl to maintain a pH below 2.00, thereby preventing nitrogen loss due to ammonia volatilization and inhibiting bacterial growth. Daily urine output was gathered from each ram, with 20 ml aliquots taken and combined for the 5-day collection period, then refrigerated until total nitrogen analysis. Rumen liquor samples were collected at 0, 2, and 4 hours post-feeding using a rubber stomach tube inserted into the rumen via the esophagus. These samples were filtered through four layers of cheesecloth, homogenized, and the pH was measured immediately. A preservative was added to stabilize ammonia nitrogen levels, and some of the liquor was stored at -20°C for chemical analysis in dried glass bottles, with 0.5 ml of toluene and 1 ml of paraffin oil added to each sample. The preservation of rumen liquor samples for ammonia nitrogen (NH3-N) determination followed the method established by Ahmed (1976), while the analysis of volatile fatty acids (VFA) was conducted using steam distillation techniques (Eadie *et al*., 1967).

Blood sampling and measurements

Blood samples were obtained from all rams after each experiment, specifically two hours after feeding, through a jugular vein puncture into two separate tubes. The first tube contained ethylene diamine tetra acetic acid (EDTA) to inhibit coagulation, allowing for the analysis of hematological parameters by facilitating the separation of blood plasma. The second tube, which lacked any anticoagulant, was utilized for serum separation and underwent centrifugation at 4000 rpm for 15 minutes. The evaluation of hematological and biochemical parameters was conducted using standard kits supplied by Spectrum, Germany. Furthermore, the quantification of immunoglobulin A (IgA), immunoglobulin G (IgG), and interleukin-2 (IL-2) was performed utilizing the enzyme-linked immunosorbent assay (Thomas, 1998).

Statistical analysis

Data analysis was conducted utilizing the Statistical Analytical System (SAS, 2002), Version 9.3.1, following the General Linear

Model represented as follows: Yij = μ + Ti + eij. In this equation, Yij denotes the parameters being analyzed, μ signifies the overall mean, Ti represents the treatment effect (i = 1 …and 3), and eij indicates the random error associated with the observations. Duncan's multiple-range test was employed to assess the differences among the means (Duncan, 1955).

RESULTS AND DISCUSSION

Impact of Azolla Extract on Nutrient Digestibility

The influence of Azolla plant extract on the digestion coefficients of the experimental diets is detailed in Table 3. This table indicates that the inclusion of Azolla extract at both tested levels as a feed additive significantly enhanced the digestibility of dry matter (DM) compared to the non-additive diet (AE0), with values recorded at 57.02, 56.09, and 54.45% for AE2, AE1, and AE0, respectively, demonstrating a statistically significant difference ($P<0.01$). Notably, the DM digestibility values between the higher (AE2) and lower (AE1) levels of Azolla extract did not show significant variation. The highest digestibility values for crude protein (CP), crude fiber (CF), and nitrogen-free extract (NFE) were observed in the AE2 group, with percentages of 59.68, 57.68, and 68.43%, respectively, followed closely by the AE1 group at 58.75, 56.98, and 67.83%. In contrast, the AE0 group exhibited the lowest digestibility values for these components, recorded at 57.01, 54.88, and 67.00%, with significant differences noted among all experimental diets regarding these parameters influenced by the Azolla extract. The digestibility of ether extract (EE) remained largely unaffected by the experimental additive, with values ranging from 61.49 to 62.67%. These findings may be attributed to the rich composition of plant secondary metabolites in Azolla, which are known to enhance nutrient intake and improve digestibility by positively influencing ruminal microbial activity (Xu *et al*., 2010; Jiménez-Peralta *et al*., 2011; Salem *et al*., 2012) or by increasing the flow of amino acids to the duodenum (Harvey, 2008). Numerous studies have explored the potential impacts of plant secondary metabolites as natural feed additives to enhance the efficiency of rumen fermentation, with evidence suggesting that they can improve protein metabolism (Patra *et al.*, 2006). Additionally, the high fermentation of carbohydrates from *Azolla pinnata* has been associated with decreased pH levels due to increased production of total volatile fatty acids (TVFA) and enhanced organic matter digestibility (Odetokun, 2000; El-Ashry *et al*., 2003; Hassanein *et al*., 2023; Nayel *et al*., 2024).

The anti-helmintic properties of PSM enhance nutrient digestibility, ruminal fermentation processes, and overall animal health (Salem *et al*., 2010b). A marked increase in dry matter (DM) digestibility was observed in diets incorporating Azolla compared to control groups. Previous studies have similarly indicated that rations containing 6% Azolla meal yielded the highest DM digestibility (Ganai *et al*., 2016; Reddy *et al*., 2009), while a 20% inclusion of *Azolla pinnata* resulted in significantly elevated digestibility of dry matter and organic matter, including crude protein, crude fiber, and ether extract (Al-Suwaiegh, 2023). Conversely, research by Ghodake *et al*. (2012) and Kumar *et al*. (2012) reported a decline in the digestibility of DM, crude protein, crude fiber, ether extract, and nitrogen-free extract with increased Azolla meal incorporation in the diets of Osmanabadi kids. Furthermore, the chemical composition and biomass productivity of Azolla, characterized by high lignin and polyphenol content alongside low energy levels, may restrict its digestibility (Brouwer *et al*., 2018; Brouwer *et al*., 2019). Carotenoids and other bioactive compounds present in Azolla can be effectively absorbed by the body, thereby enhancing animal performance. Additionally, the consumption of green *Azolla pinnata* supplies essential amino acids, particularly lysine, as well as pro-vitamins (Hossiny *et al*., 2008), vitamin B12 (Leterme *et al*., 2010), and vital minerals such as calcium, potassium, phosphorus, iron, magnesium, and copper (El-Naggar and El-Mesery, 2022). Recent findings by Nayel *et al*. (2024) revealed that the

digestion coefficients for sun-dried Azolla (*Azolla pinnata*) were significantly higher (P<0.05) than those for clover hay in terms of DM and CP digestibility, although the digestibility of NFE was notably lower than that of clover hay, with no significant difference observed for crude fiber.

Impact of Azolla Extract on Nitrogen Balance

The findings regarding nitrogen utilization, which include nitrogen intake, fecal nitrogen, urinary nitrogen, and nitrogen balance, are presented in Table 3. There were no significant differences (P<0.05) in nitrogen intake or urinary nitrogen excretion among the animals that received experimental diets with varying amounts of Azolla extract. However, a significant reduction (P<0.01) in fecal nitrogen excretion was observed in animals that were fed diets supplemented with Azolla extract at concentrations of 200 and 400 mg/kg DM, with values of 11.45 and 11.23 g/d, respectively, in comparison to the control diet, which had a fecal nitrogen excretion of 11.96.

The low (P<0.05) loss N in feces for supplemented groups, but not observed in UN, emphasis that both levels of Azolla extract were more effective for digesting N than absorption pathway. Azolla extract additive in both levels (200 and 400 mg/ kg DM), improved N balance by 7.64 and 14.96 % above the control group, respectively. Animals on AE1 and AE2 groups recorded higher (P<0.01) NB values (6.62 and 7.07, respectively) compared to those in the control group (6.15 g/d), with significant differences among all experimental diets.

The effect of Azolla extract on biological value is illustrated in Table 3. The higher level of Azolla extract additive (AE2) showed the highest (P<0.05) biological value (42.50%), compared to the control group (38.77%), While AE1 was intermediate (40.62%). This may be due to the inclusion of plant extracts like Azolla that may enhance microbial protein synthesis in the rumen, which may mitigate nitrogen losses in feces, and positively influence nitrogen balance (Adesogan *et al*., 2019). Azolla enhanced the protein intake of the animals but also contributed to a more sustainable agricultural model. The economic advantages for farmers are highlighted, indicating that the integration of Azolla could result in lower feed expenses and enhanced productivity in livestock (Brouwer *et al*., 2018). The use of Azolla recognized for its substantial protein content and capacity to fix atmospheric nitrogen, may improve nitrogen availability in the rumen, thereby optimizing the nitrogen balance in ruminants (Pormohammad *et al*., 2020). Although nitrogen intake remained consistent across all experimental groups, the excretion of nitrogen via feces and nitrogen retention as a percentage of intake were notably elevated at a 50% Azolla feeding level (Das *et al*., 2017). Conversely, substituting a concentrate mixture with sundried Azolla did not influence nitrogen intake; however, nitrogen excretion in feces was significantly greater at a 20% Azolla inclusion. Additionally, the nitrogen ingested and nitrogen retention per day, as well as the percentage of nitrogen retention, was significantly reduced with the addition of 20% Azolla in the concentrate mixture for goats (Sihag *et al*., 2018). Plant secondary metabolites, including phenolic compounds (such as carotenoids, alkaloids, phenolic acids, flavonoids, and tannins), saponins, and essential oils, are well-documented for their role in enhancing protein utilization and animal productivity (Kim *et al*., 2015; Demirtaş *et al*., 2018; Shaaban *et al*., 2020). Recent findings by Nayel *et al*. (2024) proved that nitrogen balance mirrored the nutritive value, being significantly higher (P< 0.05) at 19.14 g N/d for rams fed sun-dried Azolla compared to those receiving clover hay, which yielded only 7.73 g N/d.

Impact of Azolla Extract on Nutritive Values

The nutritive values of experimental diets are presented in Table 3. Concerning the influence of Azolla extract on nutritional values, it appears that the improvement in nutrient digestibility is

reflected in the total digestible nutrient percentage (TDN %) and digestible crude protein percentage (DCP %). A consistent trend was noted, with the AE2 diet exhibiting the highest (P<0.01) TDN value of 61.75%, while the AE0 diet recorded the lowest at 58.72%, and the AE1 diet fell in between at 61.04%. Similarly, DCP% also demonstrated a comparable pattern, with AE2 achieving the highest (P<0.01) value, followed by AE1 at 10.19, and AE0 presenting the lowest significant value of 9.91. Generally increasing Azolla extract levels showed higher (P<0.01) nutritive values among all experimental diets. Concerning feeding value, Bhatt *et al.* (2021) reported that TDN was increased. Likewise, Kumari *et al*. (2021) illustrated that TDN and DCP were increased with adding Azolla. However, Abou El-Fadel *et al*. (2020) noted that nutritive value as TDN and DCP were decreased by increasing Azolla level. The nutritive value was found to be significantly higher (P < 0.05) in the group receiving 20% *Azolla pinnata* when compared to both the control group and the group with 10% *Azolla pinnata* (Al-Suwaiegh, 2023). This notable enhancement in nutritive values can be linked to the elevated levels of protein, beta-carotene, and essential minerals present in *Azolla pinnata*. Furthermore, the incorporation of green *Azolla pinnata* into the diet contributes essential amino acids, particularly lysine, along with pro-vitamins (Hossiny *et al*., 2008), vitamin B12 (Leterme *et al*., 2010), and various minerals such as calcium, potassium, phosphorus, iron, magnesium, and copper (El-Naggar and El-Mesery, 2022). In a related study, Nayel *et al*. (2024) observed that the increased nutrient digestibility positively influenced the overall nutritive value, as indicated by the digestible crude protein (DCP) and total digestible nutrients (TDN%) metrics. Specifically, the DCP and TDN% values for sun-dried Azolla were significantly (P < 0.05) greater than those for clover hay, with increases of 58.36% and 10.36%, respectively.

Table 3: Nutrient digestibility, nitrogen balance, and nutritive value as affected by Azolla extract

Item	Treatments			SEM	*P*- value
	AE0	AE1	AE2		
Dry matter, DM%	54.45^{b}	56.09^{a}	57.02^{a}	0.411	0.005
Crude protein, CP%	57.01^{c}	58.75^{b}	59.68^{a}	0.399	<0.001
Crude fiber, CF%	54.88^{c}	56.98^{b}	57.68^{a}	0.421	<0.001
Ether extract, EE%	61.49	62.67	62.08	0.237	0.116
Nitrogen free extract, NFE%	67.00^{c}	67.83^{b}	68.43^{a}	0.210	<0.001
Feed-N (g/d)	27.83	27.76	27.86	0.050	0.754
Feces-N (g/d)	11.96^{a}	11.45^{b}	11.23^{c}	0.111	<0.001
Urine-N (g/d)	9.71	9.69	9.56	0.082	0.779
Nitrogen balance (g/d)	6.15^{c}	6.62^{b}	7.07^{a}	0.141	0.002
Digested crude protein, DCP%	9.91^{c}	10.19^{b}	10.40^{a}	0.074	0.002
Total digestible nutrients, TDN%	58.72^{c}	61.04^{b}	61.75^{a}	0.459	<0.001
Biological value, BV %	38.77^{b}	40.62^{ab}	42.50^{a}	0.648	0.030

AE0: The control group was fed clover hay and concentrate feed mixture (CFM) at a 40:60 % roughage concentrate ratio.AE1:control diet supplemented with 200 mg Azolla extract /kg DM. AE2:control diet supplemented with 400 mg Azolla extract/kg DM.. BV, biological value = ((NI- (FN+UN)/(NI-FN)) X 100. SEM: standard error of means. P-value: Probability value.

^{a,b,c} means within each row with different superscript differ significantly

Impact of Azolla Extract on Rumen Fermentation

Table 4 revealed that rumen pH values at zero time (before feeding) were insignificant as affected by Azolla extract additive to sheep diets with two levels of 200 and 400 mg/kg DM, being 6.85, 6.89, and 6.85 for AE0, AE1, and AE2 respectively. At Two hours post feeding pH values were significantly (P<0.01) decreased by an increase in Azolla extract additive, being 6.19 and 5.85 for AE1, and AE2 respectively compared to the control group (6.53), differences between all experimental diets were significant (P<0.01). Rumen pH values at four hours post feeding as affected by Azolla extract additive were insignificant between all groups. Our finding agrees with Sharma *et al.* (2022) who reported that rumen pH was decreased by adding *Azolla pinnata* at different levels (0, 150, 250, and 350g) in Sirohi kids. However, the average rumen pH in the Azolla-fed group; 6.62 was higher than the control; 6.56 (Kumar *et al.*, 2015). The effect of different levels of Azolla (0, 10, and 20%) on rumen pH at zero time was insignificant, but increasing Azolla levels increased rumen pH from 5.38 and 6.3 to 5.84 and 6.79 in 3 and 6 hours, respectively (Abou El-Fadel *et al.,* 2020). Additionally, high fermentation of *Azolla pinnata* carbohydrates has been shown to decrease pH values due to increases in TVFA production and higher digestibility of organic matter (Odetokun, 2000; El-Ashry *et al.,* 2003; Hassanein *et al.,* 2023). The exploration of plant extracts as alternatives to antibiotics in managing sub-acute ruminal acidosis underscores the potential of these extracts to stabilize ruminal pH and enhance microbial populations. This is particularly relevant in the context of increasing dietary ferment ability in ruminants, which can lead to acidosis. This emphasizes the need for feed additives that can stabilize ruminal pH and enhance microbial populations (Ahmed *et al.*, 2022).

Ruminal total VFA concentrations as affected by experimental diets supplemented with Azolla extract are shown in Table 4. At zero time (just before morning feeding) all animals had the lowest ruminal VFA concentrations being 14.61, 14.54 and 14.64 meq/dl for control (AE0), lower level of Azolla extract (AE1) and higher level of Azolla extract (AE2), respectively, total VFA values were insignificant as affected by Azolla extract. The experimental groups started to show significant effects in response to increased Azolla extract levels at two hours post feeding for VFA concentrations, higher levels of Azolla extract (AE2) recorded the highest (P<0.01) values (16.70 meq/dl), while the control group (AE0) recorded the lowest (P<0.01) value (15.14 meq/dl), lower level of Azolla extract (AE1) was intermediate (15.57 meq/dl). The changes in total volatile fatty acids (VFA) concentration continue to increase to reach a peak at four hours post-feeding with no significant differences in response to Azolla extract additive. The results were compatible with Sharma *et al.* (2022) who reported that TVFA of Sirohi kids increased by increasing Azolla supplementation, this may be attributed to the high fermentation of *Azolla pinnata* carbohydrates which increases TVFA production, consequently improving organic matter digestibility (Odetokun, 2000; El-Ashry *et al.,* 2003; Hassanein *et al.,* 2023). Additionally, high flavonoid content has improved ruminal fermentation by increasing the production of total volatile fatty acids (Broudiscou *et al.,* 2000; Demirtaş and Pişkin, 2015). Otherwise, the results of the current study disagree with Kumar *et al.* (2015), who found that the average total volatile fatty acid (TVFA) concentration in the Azolla-fed group (54.42) was lower than the control.

Plant extracts containing phenolic compounds have been shown to enhance the fermentative activity of rumen microorganisms, leading to increased production of volatile fatty acids (VFA) and improved dry matter digestibility (Demirtaş and Pişkin, 2020). This

enhancement may be attributed to the hydrolysis of phenolic compounds into more bioactive forms by rumen bacteria, which can subsequently promote the synthesis of aromatic amino acids and boost the enzymatic activity of certain bacterial groups (Aura, 2008; Broudiscou *et al*., 2002). Consequently, phenolic compounds can exert both positive and negative influences on rumen microorganisms. It is also important to recognize that the observed effects may be influenced by other plant metabolites present in the extracts, even in minimal amounts (Broudiscou *et al*., 2002). Furthermore, recent research by Jahani-Azizabadi *et al*. (2022) demonstrated that calves administered a daily dose of 4 mL of a phytobiotic-rich herbal extract mixture exhibited a higher abundance of total bacteria, with an increase in the molar proportions of acetate noted in calves receiving 12 mL/d of the herbal extract compared to those on a control diet.

The impact of Azolla extract additive on rumen ammonia concentrations is presented in Table 4. Rumen NH_3-N values at zero time were insignificant between all experimental diets. Otherwise, rumen NH3-N values started to be significant ($P<0.01$) among studied diets at 2h post-feeding as a response to increasing Azolla extract level; the higher level of Azolla extract (AE2) recorded the highest ($P<0.01$) value of rumen NH3-N (29.42 mg/dl) at 2 h. post feeding compared to the control group (AE0) which recorded the lowest (28.00 mg/dl) value at the same time. The AE1 group was intermediate (28.83 mg/dl). The concentrations of NH3-N at 4 hr. were 27.33, 27.35, and 27.62 mg/dl for AE0, AE1, and AE2, respectively with no significant differences between all experimental diets. In this regard, ammonia-nitrogen in the rumen liquor of lambs in (0 and 3h.) was increased with increasing Azolla level from (7.19 – 11.7) mg/100ml to (27.82 – 40.35) mg/100ml in (0 and 3h), respectively. However, Ammonia-nitrogen in rumen liquor not different after 6h. (Abou El-Fadel *et al.,* 2020). Plants and products derived from them present a viable alternative for rumen management (Wallace *et al*., 2010). In vivo investigations have indicated that tannins sourced from various plants can lead to a reduction in ruminal ammonia (NH3) levels while simultaneously promoting the flow of non-NH3 nitrogen to the duodenum in sheep. This phenomenon has been linked to subtle trends indicating lower nitrogen excretion in urine (specifically with tannins) and reduced ammonia emissions from manure. Additionally, saponins and plants containing saponins have been shown to enhance protein flow from the rumen, primarily by inhibiting ciliate protozoa (Wina *et al*., 2005). Research by Kumar *et al.* (2015) revealed that the ammonia nitrogen concentration in groups fed azolla (11.31) was lower than that of the control group (11.59). It is important to note that the observed effects may also arise from other plant metabolites, even in minimal concentrations within the extracts (Broudiscou *et al*., 2002). The influence of saponins on nitrogen metabolism in the rumen is largely attributed to their detrimental effects on protozoa, which play a significant role in nitrogen retention due to their proteolytic activity on both dietary and microbial proteins (Patra and Saxena, 2009). Nevertheless, some studies have reported no change in protozoa counts (Polyorach *et al*., 2016) or even an increase (Ramírez-Restrepo *et al*., 2016) in the presence of saponins. Long-term studies suggest that the efficacy of saponins may vary and even diminish over time (Jouany and Morgavi, 2007), likely due to microbial adaptation (Patra, 2012). More recently, Hassanein *et al*. (2023) found that the concentration of ammonia nitrogen in rumen fluid increased with higher levels of Azolla (0, 10, and 20%) in the diets of Zaraibi dairy goats.

Table 4: Rumen parameters as affected by Azolla extract

Incubation time	Experimental diets			SEM	*P*- value
	AE0	AE1	AE2		
Rumen liquor pH					
0h.	6.85	6.89	6.85	0.094	0.983
2h.	6.53^{a}	6.19^{b}	5.85^{c}	0.084	<0.001
4h.	6.42	6.40	6.33	0.060	0.851
TVFA, mg/dl					
0h.	14.61	14.54	14.64	0.053	0.741
2h.	15.14^{c}	15.57^{b}	16.70^{a}	0.20	<0.001
4h.	16.06	16.19	16.24	0.061	0.536
NH3-N, meq/dl					
0h.	26.01	26.11	26.12	0.031	0.296
2h.	28.00^{c}	28.83^{b}	29.42^{a}	0.181	<0.001
4h.	27.33	27.35	27.62	0.080	0.272

AE0: The control group was fed clover hay and concentrate feed mixture (CFM) at a 40:60 % roughage concentrate ratio. AE1: control diet supplemented with 200 mg Azolla extract /kg DM. AE2: control diet supplemented with 400 mg Azolla extract/kg DM. SEM: standard error of means. P-value: probability value. $^{a, b, c}$ means within each raw with different superscripts differ significantly.

Impact of Azolla Extracts on Blood Biochemical Parameters

Blood biochemistry of sheep as affected by Azolla extract additives are presented in Table 5. Generally, all values were within the normal values of the blood characteristics of sheep. Data in Table 5 illustrates the effect of feeding Ossimi sheep on diets supplemented with Azolla extract on blood protein parameters (total protein (TP), albumin (A), and globulin (G). It is evident that data showed significant (P<0.05) differences among the experimental groups for serum total protein (TP), and albumin (Alb), thus may indicate more availability of nitrogen in tissue (Singh *et al.,* 2021). Globulin (Glu) concentrations were insignificant. Azolla extract additive at level 400 mg/kg DM (AE2) recorded the highest (P<0.01) values for total protein and albumin (6.44 and 3.80 g/dl, respectively) being significantly higher (P<0.01) than the AE1 group (6.14 and 3.50 g/dl), which was also significantly higher (P<0.01) than AE0 group (5.72 and 2.97 g/dl). Differences were significant among all experimental groups. The average total protein ranged from 5.72 to 6.44 g/dl versus 2.97 to 3.80 g/dl for albumin and 2.64 to 2.75 g/dl for globulin. It was obvious that total protein (TP), and albumin (A) increased by increasing Azolla extract levels. Glucose concentration was significantly higher (P<0.01) in AE1 and AE2 groups (66.03, and 66.30 mg/dl respectively) compared to the control group, AE0 (64.98 mg/dl), with no significant differences among AE1 and AE2 groups. Blood protein data complied with Kamel and Hamed (2021) observed that albumin and total protein were increased in the dietary inclusion of dried Azolla. In the same context, serum albumin levels were higher in Azolla additive to Nellore sheep diets. However total protein and blood glucose were not different between diets and systems (Reddy *et al.,* 2009; Vahedi *et al.,* 2021). On the other hand, total, protein, and globulin were not affected by adding Azolla except serum albumin

was decreased by 30% Azolla level in rabbit diets (Abdelatty *et al.,* 2021). Meanwhile, total protein and plasma albumin did not change in all treatments as affected by increasing Azolla levels in Hariana heifers diets (Roy *et al.,* 2016). In contrast, plasma total protein decreased, with increased Azolla. However, albumin was not affected by adding Azolla (Abou El-Fadel *et al.*, 2020). In the context of the effect of plant extract, serum albumin and total serum protein concentrations increased ($P<0.05$) linearly in dairy calves with increasing the mixture of phytobiotic-rich herbal extract (Immunofin, IMPE) supplementation compared to control group (Jahani-Azizabadi *et al.,* 2022). Other studies emphasized that total protein and albumin were increased by adding Azolla. However, blood glucose was lowest in 16% Azolla level (Sherif *et al*., 2022). Also, *Azolla pinnata* caused a significant increase in plasma metabolites; total protein, and globulin (Al-Suwaiegh, 2023). It can be concluded that *Azolla pinnata* contain high protein compounds and other components that might be helpful in modulating plasma metabolites.

Elevated concentrations of AST, ALT, and ALP, exceeding 140 U/L, 245 U/L, and 464 U/L respectively, may indicate potential hepatotoxicity, as these enzymes are indicative of liver function (Lepherd *et al.,* 2009; Oh *et al.,* 2017). Values of serum ALT, and AST (Table 5), were insignificant as affected by Azolla plant extract. Serum ALT and AST values were slightly increased by increasing the Azolla extract level, ranging from 49.33 to 51.15 U/l for ALT versus 117.97 to 118.78 U/l for AST. The finding was in agreement with Roy *et al.* (2016) who observed that ALT and AST concentrations were higher at 5% Azolla level in Hariana heifers' diets. Likewise, AST and ALT were increased with increased Azolla (Abou El-Fadel *et al.,* 2020). In the same context, ALT and AST in Zaraibi goat's blood were higher in 20% Azolla level than 0% and 10% levels (Hassanein *et al.*, 2023). Otherwise, AST and ALT were decreased in the dietary inclusion of dried Azolla (Kamel and Hamed, 2021). However, Sherif *et al.* (2022) reported that AST and ALT were not affected by adding Azolla. On the other hand, the supplementation of a phytobiotic-rich herbal extract, known as Immunofin (IMPE), did not influence the blood levels of aspartate aminotransferase and alanine aminotransferase in dairy calves (Jahani-Azizabadi *et al.,* 2022).

Data of serum urea and creatinine (Table 5) were insignificant as affected by Azolla plant extract. Blood urea ranged from 31.90 to 32.53 mg/dl versus 1.02 to 1.08 mg/dl for creatinine. Generally, the respective values were within the normal range, indicating that the addition of Azolla extract in the present study did not have any adverse effect on kidney functions. Blood urea nitrogen levels serve as an important indicator of renal function, as urea, a metabolic waste product, is typically eliminated through glomerular filtration. However, in certain circumstances, urea can be recycled in the rumen through the urea cycle (Devappa *et al.,* 2010). Data on kidney functions complied with Abdelatty *et al.* (2021) who noted that blood parameters like urea and creatinine were not affected by adding Azolla at 30% Azolla level in rabbit diets. However, blood urea and creatinine were decreased as affected by adding Azolla (Kamel and Hamed, 2021). On the contrary, urea and creatinine in Zaraibi goat's blood were higher in 20% Azolla level than 0% and 10% levels (Hassanein *et al.,* 2023). Also, Al-Suwaiegh (2023) reported a significant increase in plasma urea caused by *Azolla pinnata* inclusion. In the context of extract effect, urea nitrogen of dairy calves was not affected by a mixture of phytobiotic-rich herbal extract (Immunofin, IMPE) supplementation (Jahani-Azizabadi *et al.,* 2022)

In a recent study, Ahmed *et al.* (2023) demonstrated that the influence of *Azolla pinnata* on blood parameters was not statistically significant ($P>0.05$) across all experimental groups. Since the results of blood biochemistry fell within the normal range, it indicates that there is no adverse effect on liver and kidney functions. Consequently, the inclusion of *Azolla pinnata* extract is considered safe for maintaining liver and kidney health, with no associated negative effects.

Table 5: Blood biochemistry as affected by Azolla extract

Item	Treatments			*SEM*	*P*- value
	AE0	AE1	AE2		
Total protein (g/dl)	5.72[c]	6.14[b]	6.44[a]	0.092	<0.001
Albumin (g/dl)	2.97[c]	3.50[b]	3.80[a]	0.101	<0.001
Globulin (g/dl)	2.75	2.64	2.64	0.027	0.319
Glucose (mg/dl)	64.98[b]	66.03[a]	66.30[a]	0.180	<0.001
ALT(U/l)	49.33	49.90	51.15	0.90	0.708
AST(U/l)	117.97	118.21	118.78	0.90	0.934
Urea(mg/dl)	31.90	32.41	32.53	0.80	0.940
Creatinine(mg/dl)	1.08	1.05	1.02	0.032	0.800

AE0: The control group was fed clover hay and concentrate feed mixture (CFM) at a 40:60 % roughage concentrate ratio. AE1: control diet supplemented with 200 mg Azolla extract /kg DM. AE2: control diet supplemented with 400 mg Azolla extract/kg DM. ALT, alanine transaminase; AST, aspartate aminotransferase; SEM: standard error of means. P-value: probability value.

[a, b, c] means within each row with different superscripts differ significantly.

Impact of Azolla Extract on Hematology and Immunity Status

The data presented in Table 6 showed that the hematological parameters of the experimental groups remained largely unchanged following the administration of Azolla extract. Specifically, the blood Hemoglobin (Hb) levels were observed to range between 11.75 and 12.75 g/dl, while red blood cell (RBC) counts varied from 3.97 to 4.18, and white blood cell (WBC) counts were recorded between 8.54 and 8.65. These values, along with other hematological metrics, fell within the normal physiological range, suggesting that Azolla extract did not adversely affect the health of the animals. This observation aligns with the findings of Anitha *et al*. (2016), who reported that various hematological parameters, including PCV, Hb, RBC, WBC, MCV, MCH, MCHC, and DLC, were not significantly influenced by the incorporation of Azolla into the diet of rabbits. Furthermore, El-Deeb *et al*. (2021) corroborated that the inclusion of Azolla did not impact most hematological traits; also, Singh *et al*. (2021) noted no significant differences in PCV, Hb, RBC, and WBC due to the addition of *Azolla pinnata*. Conversely, Alagan *et al.* (2020) found elevated levels of Hb, PCV, RBC, and WBC at a 5% Azolla inclusion rate. Overall, the findings from these hematological assessments suggest that the addition of Azolla extract does not significantly alter the hematological parameters in Ossimi sheep.

The immune response plays a crucial role in the overall health of animals (Ingvartsen and Moyes, 2013). Furthermore, Angulo and Angulo, (2023) have indicated that the concept of trained immunity can serve as an effective strategy to enhance the immune response against a diverse array of pathogens. One of the prevalent methods for assessing immunity involves measuring serum immunoglobulin concentrations. The findings presented in Table 6 illustrate the effects of Azolla extract on various immune parameters (IgA, IgG, and IL-2). Notably, the IgA levels were significantly elevated ($P < 0.01$) in the groups treated with Azolla extract, AE1 and AE2, recording values of 35.65 and 35.63 mg/dl, respectively, compared to the control group (AE0), which had a value of 32.90 mg/dl; no significant differences were observed between the AE1 and AE2 groups. Similarly, the IgG concentrations exhibited a comparable pattern, with values of 5.90, 8.50, and 8.72 mg/dl for the AE0, AE1, and AE2 groups, respectively. Additionally, the interleukin-2 levels were significantly higher ($P < 0.01$) in the AE2 group, which recorded a value of 61.43 Pg/ml, compared to the AE0 and AE1 groups, which had values of 58.90 and 59.70 Pg/ml, respectively. These results suggest that Azolla extract positively influences the immune status

of Ossimi sheep, with all measured immune parameters falling within the normal range for sheep blood characteristics.

B cells play a crucial role in the synthesis of specific immunoglobulins, notably IgA and IgG (Gelsinger and Heinrichs, 2017). Research has demonstrated that calves experiencing severe diarrhea exhibit lower levels of IgG compared to their healthy counterparts (Villarroel *et al*., 2013). Furthermore, Wang *et al*. (2015) indicated a negative correlation between serum IgG levels and the severity of diarrhea in dairy calves. However, the findings of the current study contrast with those of Jahani-Azizabadi *et al*. (2022), who found no significant increase in serum IgG levels following supplementation with a phytobiotic-rich herbal extract at doses of 4, 8, and 12 mL/d when compared to a control group. This variation in immune response may be attributed to the different concentrations of the extracts used. Nonetheless, flavonoids are recognized for their capacity to enhance animal immunity through their anti-pathogenic, antioxidant, and anti-inflammatory properties (Linville *et al*., 2018). In addition, saponins uniquely stimulate the cell-mediated immune response and promote antibody production, requiring only minimal doses to exert adjuvant effects. Saponins have been shown to induce the production of cytokines, including interleukins and interferon, which may contribute to their immuno-stimulatory properties (Francis *et al*., 2002). Evidence suggests that saponins can enhance immune responses by facilitating the uptake of antigens from the gastrointestinal tract and other membranes (Oda *et al*., 2000). Overall, plant extracts significantly bolster animal health by enhancing their immune systems, thereby achieving optimal breeding standards that mitigate disease and improve both animal performance and the quality of animal products (Yin and Yang, 2020).

Table 6: Blood hematology and immunity status as affected by Azolla extract

Item	Treatments			*SEM*	*P*- value
	AE0	AE1	AE2		
Hb (gm/dl)	12.63	12.75	11.75	0.212	0.101
RBCs (× 10^6/µl)	4.18	4.17	3.97	0.049	0.143
WBCs (× 10^3/µl)	8.54	8.65	8.59	0.086	0.889
PLT (× 10^3/µl)	168.88	171.03	161.03	2.235	0.158
MPV (fl)	8.68	8.05	8.15	0.185	0.366
MCV(fl)	92.08	91.50	91.25	0.167	0.110
MCH (pg)	29.98	30.18	28.73	0.298	0.085
MCHC (%)	34.00	34.40	33.98	0.130	0.357
LYM (× 10^3/µl)	2.30	2.38	2.38	0.050	0.780
MID (× 10^3/µl)	1.68	1.53	1.65	0.039	0.254
GRA (× 10^3/µl)	5.60	5.75	5.78	0.070	0.591
Neutrophil. (× 10^3/µl)	6.51	6.30	6.13	0.077	0.133
IgA, mg/dl	32.90b	35.65a	35.63a	0.410	<0.001
IgG, mg/dl	5.90b	8.50a	8.72a	0.40	<0.001
Interleukin 2 (Pg/ml)	58.90b	59.70b	61.43a	0.40	0.001

AE0: The control group was fed clover hay and concentrate feed mixture (CFM) at a 40:60 % roughage concentrate ratio.AE1:control diet supplemented with 200 mg Azolla extract /kg DM. AE2:control diet supplemented with 400 mg Azolla extract/kg DM. Hb, Hemoglobin; RBC, Red blood cell; WBC, White blood cell; PLT, Platelets; MPV, Mean Platelets volume; MCV, Mean cell volume; MCH, Mean Corpuscular Volume; MCHC, Mean Corpuscular Hemoglobin Concentration; LMY, Lymphocytes; MID, Mid-range Absolute Count; GRA, Granulocytes. SEM: standard error of means. P-value: probability value.

[a, b, c] means within each row with different superscripts differ significantly.

Recently, Piao *et al*. (2023) highlighted the distinctive benefits of phytogenic extracts, noting their natural origin and broad availability, as well as their low residue levels and environmentally friendly, renewable nature. These extracts have the potential to enhance animal immunity and optimize microbial composition within the gastrointestinal tract. As a result, phytogenic extracts are increasingly recognized as a preferred choice for promoting animal health.

Generally, all immunity status values were within the average values of the blood characteristics of sheep. Azolla extract additive had no adverse effect on animal blood criteria, animal hygiene, and immunity status.

CONCLUSION

Incorporating Azolla extract into Ossimi sheep diets can enhance the digestibility of DM, CP, CF, and NFE. The addition of Azolla extract at levels, 200 and 400 mg/kg DM resulted in significant enhancements in nitrogen balance, with increases of 7.64% and 14.96% over the control group, respectively. Furthermore, serum total protein and albumin levels were notably elevated with higher concentrations of Azolla extract; glucose levels were also significantly greater in the Azolla extract groups. Additionally, Azolla extract positively influenced immunity status.

Further researches are needed to establish the optimal dosage, and assess nutrients bioavailability of Azolla extract. Consequently, there remains significant potential for the advancement of plant extracts in animal nutrition in the future.

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تأثير إضافة المستخلص الإيثانولى لأوراق نبات الأزولا على معاملات الهضم ، قياسات الدم و الحالة المناعية للأغنام الأوسيمي

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[٢] قسم تغذية الحيوان والدواجن- مركز بحوث الصحراء- مصر

الملخص العربى

أجريت التجربة الحالية لدراسة تأثير إضافة المستخلص الإيثانولي لأوراق نبات الأزولا (*Azolla pinnata*) على أداء الأغنام الأوسيمي. تم إستخدام ١٢ رأس من ذكور الأغنام الأوسيمي عمرها حوالى ٢٠ شهراً ومتوسط أوزانها ٤٤,٢٢ ± ٢,٤٥ كجم , قسمت الحيوانات إلى ثلاث مجموعات وزنية مماثلة على ثلاثة علائق تجريبية; المجموعة الاولي(العليقة الضابطة) :AE0(40%دريس برسيم + ٦٠% مخلوط علف مركز بدون اضافة مستخلص الأزولا . المجموعة الثانية: AE1: (العليقة الضابطة مع اضافة ٢٠٠ مجم مستخلص أزولا/ كجم مادة جافة. المجموعة الثالثة : AE2 (العليقة الضابطة مع اضافة ٤٠٠ مجم مستخلص أزولا/ كجم مادة جافة ،أظهرت النتائج أن إضافة مستخلص الأزولا بكلا المستويين أدى إلى تحسن (P<0.01) معاملات هضم المادة الجافة ، حيث بلغت قيم معاملات هضم المادة الجافة: ٥٧,٠٢، ٥٦,٠٩، و ٥٤,٤٥ % لمجموعات AE2، AE1، و AE0 على الترتيب. سجلت الحيوانات المغذاه على المستوى العالى من مستخلص الأزولا (AE2) القيم الأعلي (P<0.01) لمعاملات هضم البروتين الخام والألياف الخام والكربوهيدرات الذائبة (٥٩,٦٨، ٥٧,٦٨ و٦٨,٤٣% على الترتيب) ، تلتها مجموعة مستوى الإضافة الأقل (AE1) حيث بلغت ٥٨,٧٥، ٥٦,٩٨، و ٦٧,٨٣% لنفس الترتيب , بينما سجلت المجموعة الضابطة (AE0) أقل القيم لنفس معاملات هضم المركبات الغذائية السابقة. تحسن (P< 0.01) مجموع المركبات الغذائية المهضومة (TDN%) لمجموعة AE2 (61.75%) مقارنة بمجموعة AE0 التى سجلت القيمة الأقل (٥٨,٧٢%)، فى حين سجلت مجموعة AE1 القيمة المتوسطة (٦١,٠٤%). إتخذت قيم البروتين الخام المهضوم (DCP%) نمطاً مماثلا ل TDN.إضافة مستخلص الأزولا في كلا المستويين؛٢٠٠ و ٤٠٠ مجم/كجم مادة جافة أدى إلى تحسن ميزان النيتروجين (P<0.01) بنسبة ٧,٦٤ و ١٤,٩٦% على الترتيب مقارنة بالمجموعة الضابطة. بدأ تركيز الأحماض الدهنية الطيارة بالكرش فى تسجيل زيادة معنوية (P<0.01) إستجابة لإضافة مستخلص الأزولا بعد ساعتين من التغذية، حيث سجلت مجموعة AE2 أعلى القيم (١٦,٧٠ ملمكافئ/ ديسيلتر)، وسجلت المجموعة الضابطة (AE0) القيم الأقل (١٥,١٤ ملمكافئ/ ديسيلتر)، في حين اتخذت AE1 قيما متوسطة (١٥,٥٧ ملمكافئ/ ديسيلتر). إتخذت أمونيا الكرش NH3-N نمطًا مماثلا للأحماض الدهنية الطيارة. أدت إضافة مستخلص الأزولا عند مستوى ٤٠٠ ملجم/كجم مادة جافة (AE2) إلى تحسن(P<0.01) قيم البروتين الكلي والألبيومين في الدم (٦,٤٤ و ٣,٨٠ جم/ديسيلتر على الترتيب) مقارنة بمجموعة AE1 ذات مستوى الاضافة الأقل (٦,١٤ و ٣,٥٠ جم/ديسيلتر) والمجموعة الضابطة (٥,٧٢ و ٢,٩٧ جم/ديسيلتر على التوالي). سجل جلوكوز الدم قيما أعلى معنويا (P<0.01) مع مجموعتي إضافة المستخلص; AE1 و AE2(٤٤,٩٨ و٤٦,٠٣ مجم/ديسيلترعلى التوالي) مقارنة بالمجموعة الضابطة (٤٦,٣٠ مجم/ديسيلتر). لم تتأثر قيم وظائف الكبد و الكلى و صورة الدم معنويا باضافة مستخلص الأزولا, تحسن (P <0.01) الجلوبيولين المناعي IgA مع مجموعات إضافة مستخلص الأزولا; AE1 وAE2 (٣٥,٦٥و٣٥,٦٣ مجم/ديسيلتر، على التوالي) مقارنة بالمجموعة الضابطة؛ AE0 (٣٢,٩٠ ملجم/ديسيلتر). إتبعت قيم الجلوبيولين المناعي IgG إتجاهًا مشابهًا لـ IgA . تحسن الإنترلوكين ٢ (P <0.01) مع مجموعة AE2 (٦١,٤٣ بيكوجرام/مل) مقارنة بمجموعتي AE0 و AE1 (٥٨,٩٠. و٥٩,٧٠ بيكوغرام/مل، على التوالي)، مما يشير إلى أن إضافة مستخلص الأزولا أدت إلى تحسن الحالة المناعية للأغنام الأوسيمي. جميع قياسات الدم كانت ضمن القيم الطبيعية لصفات الدم فى الأغنام، مما يدل على أن إضافة مستخلص الأزولا إلى علائق الأغنام الأوسيمى لم يكن له أي تأثير سلبي على الحالة الصحية للأغنام.