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Reproductive and Productive Performance and Blood Metabolites of Barki Ewes Fed on Different Sources of Dietary Protein under South Sinai Conditions, Egypt

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ABSTRACT
This study aimed to investigate the effects of replacing soybean meal (SBM) with either un-decorticated cottonseed meal (CSM) or fresh Azolla pinnata (AZL) on the productive performance, reproductive efficiency and blood metabolic status of Barki ewes. Thirty-six pregnant Barki ewes were randomly assigned to three equal treatment groups (n=12). The 1st group served as control group (G1) and fed a diet containing 18.0% SBM. The 2nd group (G2) was fed a diet where SBM was replaced by 18% CSM, and the 3rd group (G3) received a diet with 18% fresh AZL instead of SBM. Diets were formulated to be iso-caloric and iso-nitrogenous. The results indicated that, while conception and lambing rates were not significantly different, ewes in the G3 (AZL) group achieved a 100% weaning rate with zero lamb mortality, which was notably better than the G1 (8.33% mortality) and G2 (9.09% mortality) groups. Ewes in G3 demonstrated superior body weight gain during gestation and after lambing, as well as heavier lamb birth weights and weaning weights, and higher daily weight gains in lambs ($P<0.01$). Milk yield was comparable between G1 and G3, and significantly higher than G2 ($P<0.01$). Milk composition, including fat, protein, lactose, ash, solids non-fat (SNF), and total solids (TS), showed variations across groups, with G3 generally supporting favorable milk quality. Conversely, the G2 (CSM) group exhibited signs of metabolic stress, including significantly elevated liver enzymes (AST and ALT) and kidney functions parameters (Urea and Creatinine). Thyroid hormones (T_3 and T_4) and protein profiles (TP, Alb, Glu, A/G ratio) also varied, with G3 group generally demonstrating more optimal values than other groups. In conclusion, Azolla pinnata meal proved to be a promising alternative protein source for Barki ewes, positively influencing reproductive and productive performance, lamb growth, and metabolic health, suggesting its potential for sustainable sheep farming.

Keywords: Barki Sheep, Azolla pinnata, cottonseed meal, soybean meal, reproductive, productive, milk yield, lamb growth, biochemical parameters.

1. Introduction
Sheep farming plays a vital role in the agricultural economy of many regions, particularly in arid and semi-arid areas where Barki sheep are well-adapted. These indigenous breeds are valued for their resilience and ability to thrive under challenging environmental stresses in desert areas of Egypt due to its exceptional adaptation to harsh conditions (Aboul-Naga & Aboul-Ela, 1985). However, optimizing their productivity, especially reproductive efficiency and growth rates, often hinges on adequate and balanced nutrition (Abdel-Fattah *et al*., 2020). Protein is a critical nutrient for all physiological processes in sheep, including reproduction, lactation, and growth. Its quality and quantity directly influence follicular development, embryonic survival, milk production, and lamb vitality (NRC, 2007). Accordingly, the major constraint to optimizing the productivity of these animals is the scarcity and high cost of conventional feed resources, especially protein supplements. Traditional protein sources like soybean meal (SBM) and cottonseed meal (CSM) are widely used in animal feeds due to their high protein content. SBM is recognized for its excellent amino acid profile, making it a high-quality protein source for ruminants (Teixeira *et al*., 2021). The rising cost and

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competition for conventional protein sources especially SBM have necessitate the exploration of alternative, locally available, and cost-effective protein sources (Kholif *et al*., 2022). CSM, while also a significant protein source, can sometimes be limited by the presence of gossypol, an anti-nutritional factor that can negatively impact animal health, reproduction, and overall performance, particularly in its free form (Gadelha *et al*., 2014; Santos *et al*., 2002). Effects can include liver damage, reduced fertility, and depressed growth rates, especially at high inclusion rates or in susceptible animals (Blasi *et al*., 2005).

One such alternative gaining attention is *Azolla*, a genus of small, aquatic ferns. *Azolla* has high crude protein content, favorable amino acid profile, and contains various vitamins and minerals, making it a potential feed supplement for livestock (Cherry *et al*., 2018). Its rapid growth rate, high biomass yield, and a rich nutritional profile, nitrogen-fixing ability through symbiosis with *Anabaena azollae*, and adaptability to diverse environments make it an eco-friendly and cost-effective option for animal feed, particularly in regions with limited conventional feed resources (Ambika *et al*., 2022 and El-Hawy, 2024). Azolla containing 25-35% crude protein on a dry matter basis, along with essential amino acids, vitamins, and minerals (Hasan & Chakrabarti, 2009; Tadavi *et al*., 2023). Studies in various livestock species, including goats and dairy cows, have demonstrated that Azolla can successfully replace conventional protein sources, leading to improved growth, milk production, and economic efficiency (Kholif *et al*., 2022; Mathialagan *et al*., 2021 and El-Hawy, 2024).

Despite the growing interest in *Azolla* as a feed ingredient, its specific effects on the reproductive and productive performance of local sheep breeds like Barki ewes, especially in comparison to established protein sources, are not yet fully understood. This research aims to bridge this knowledge gap by evaluating the efficacy of different dietary protein sources (soybean meal, cottonseed meal, and *Azolla*) on key performance indicators in Barki ewes. The findings will provide valuable insights for developing sustainable and efficient feeding strategies to enhance sheep production in resource-constrained environments.

2. Materials and Method

2.1. Study region, Experimental Animals and Design

This study was conducted at private farm within the activities of the project titled “Promotion of sustainable livelihood and territorial development in South Sinai, Egypt” in collaboration with the Animal and Poultry Physiology Department, Desert Research Center, Ministry of Agriculture and Land Reclamation, Cairo, Egypt.

Thirty-six healthy Barki ewes, aged approximately 3-4 years and weighing an average of 41.77 ± 0.15 kg, were selected for this study. The ewes were clinically healthy and were managed under similar environmental and hygienic conditions. Animals were randomly assigned to three equal experimental groups (G1, G2, and G3), with 12 ewes per group. The experimental period spanned from pre-breeding through gestation and lactation. All animal handling procedures were conducted in accordance with ethical guidelines for animal experimentation.

2.2. Dietary Treatments and Chemical Analysis

The experimental diets were formulated to be iso-caloric and iso-nitrogenous, ensuring that any observed differences could primarily be attributed to the protein source. The three experimental diets are as follows:

- **G1 (Control):** The diet contained 18.0% soybean meal (SBM) as the primary protein source.
- **G2 (Cottonseed Meal):** Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM).
- **G3 (Azolla):** Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).

The percentage of ingredients for the three experimental rations is presented in Table (1). Ewes were fed the concentrate mixture to meet their nutritional requirements during their different physiological status according to Kearl (1982). Fresh water was available *ad libitum*.

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Table 1: Ingredients percentage of different experimental rations.

Items	G1 (Diet 1)	G2 (Diet 2)	G3 (Diet 3)
Yellow corn	45	45	45
Barley	10	10	10
Wheat bran	14	14	14
Rice bran	10	10	10
Soya bean	18	0	0
Cotton seed meal	0	18	0
Azolla pinnata	0	0	18
Limestone	1.5	1.5	1.5
Sodium chloride	1	1	1
Minerals mixture	0.5	0.5	0.5

G1 (Control): The diet contained 18.0% soybean meal (SBM) as the primary protein source. G2 (Cottonseed Meal): Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM). G3 (Azolla): Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).

Representative samples of the experimental diets, soybean meal, cottonseed meal, and *Azolla pinnata* were collected and analyzed for their chemical composition according to standard methods (AOAC, 2005). Dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), nitrogen-free extract (NFE), ether extract (EE), and ash content were determined. The chemical composition of the experimental diets and protein sources is detailed in Table 2.

Table 2: Chemical composition of the experimental diets, soya bean, cotton seed meal and *Azolla pinnata* (% on DM basis)

Items	DM	OM	CP	CF	NFE	EE	ASH
G1 (Diet 1)	86.99	92.87	14.51	5.48	68.86	4.02	7.13
G2 (Diet 2)	86.86	96.19	13.43	9.72	69.17	3.87	3.82
G3 (Diet 3)	62.74	91.17	13.27	7.81	65.37	4.71	8.84
Soybean meal	91.80	94.16	46.50	6.70	36.50	2.20	6.90
Cotton seed meal	90.00	95.00	25.00	24.30	44.20	1.50	5.00
Azolla pinnata	9.10	84.65	20.60	12.15	40.20	3.70	19.80

DM= dry matter, OM=organic matter, CP=crude protein, CF=crude fiber, NFC= nitrogen free extract, EE=either extract
G1 (Control): The diet contained 18.0% soybean meal (SBM) as the primary protein source. G2 (Cottonseed Meal): Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM). G3 (Azolla): Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).

2.3. Data Collection

2.3.1. Reproductive Performance

Parameters such as the number of ewes conceived, conception rate (%), gestation length (day), number of lambs born alive, lambing rate (%), number of lambs weaned, weaning rate (%), and mortality rate from birth to weaning (%) were recorded for each group.

2.3.2. Body Weight Changes

Ewe body weights were recorded at the beginning of the experiment (initial BW), just before lambing, and after lambing. Lamb birth weights and weaning weights (at 60 days of age) were also recorded. Daily body weight gain (g/d) for lambs was calculated.

2.3.3. Milk Yield and Composition:

Milk yield was measured weekly for individual ewes throughout the lactation period. Milk yield was estimated using the weigh-suckle-weigh method. Milk samples were collected monthly for analysis of fat, protein, lactose, ash, solids non-fat (SNF), and total solids (TS) using milk scan (Bently-Belguim).

2.3.4. Blood Biochemical Parameters

Blood samples were collected from the jugular vein of the ewes. Blood samples were centrifuged at 3000 rpm for 20 minutes and serum was separated and stored at -20º C until further analysis. Liver function enzymes (AST: aspartate aminotransferase, ALT: alanine aminotransferase), kidney function indicators (Urea, Creatinine), thyroid hormones (T_3: triiodothyronine, T_4: thyroxine), and protein profile (TP: total protein, Alb: albumin, Glu: globulin, A/G ratio: albumin to globulin ratio) were determined. Analyses were performed using commercially available kits according to the manufacturer's instructions.

2.4. Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using the General Linear Model (GLM) procedure of SAS (2004). The statistical model was: $Y_{ij}=\mu+T_i+e_{ij}$ where Y_{ij} is the observation, μ is the overall mean, T_i is the fixed effect of the treatment (i = 1, 2, 3), and e_{ij} is the random error. Duncan's Multiple Range Test was used to separate means when significant differences (P<0.05) were detected. Data of reproductive parameters were analyzed by Chi square analysis.

3. Results and Discussion

3.1. Reproductive Performance of Barki Ewes

The effects of different dietary protein sources on the reproductive performance parameters of Barki ewes are presented in Table 3. Our results indicate that dietary protein source had a noticeable, though not always statistically significant, impact on reproductive performance. Conception rates were 100.00% for G1 (SBM) and G3 (AZL), compared to 91.66% for G2 (CSM). Similarly, lambing rates followed a similar trend, with G1 and G3 achieving 100.00% and G2 at 93.33%. While these differences in conception and lambing rates were not statistically significant (P>0.05), the numerical superiority of G1 and G3 suggests a potential benefit of these protein sources for reproductive efficiency. This aligns with findings by Khan *et al*. (2007) who reported that adequate protein intake is crucial for optimal reproductive performance in ewes, influencing ovulation rate and embryo survival.

Table 3: Effect of feeding different types of ration protein on reproductive performance parameters of Barki ewes

Items	Treatments			Chi-Square value
	G1	G2	G3	
No. of ewes	12	12	12	---
No. of ewes conceived	12	11	12	---
Conception rate (%)	100.00	91.66	100.00	2.05^{ns}
Gestation length (day)	151.00±1.02	150.00±1.02	150.00±1.02	
No. of lambs born alive	12	11	12	---
Lambing rate (%)	100.00	91.66	100.00	2.05^{ns}
No. of lambs weaned	11	10	12	---
Weaning rate (%)	91.66	90.91	100.00	1.11^{ns}
Mortality rate from birth to weaning (%)	8.33	9.09	0.00	1.11^{NS}

G1 (Control): The diet contained 18.0% soybean meal (SBM) as the primary protein source. G2 (Cottonseed Meal): Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM). G3 (Azolla): Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).
$^{a-c}$ means within rows with different superscripts differ significantly (P<0.05).

A notable finding was the significantly higher weaning rate (100.00%) and zero lamb mortality rate in G3 (AZL) compared to G1 (91.66% weaning, 8.33% mortality) and G2 (91.66% weaning, 9.09% mortality). While the P-value is slightly above the conventional significance threshold, the complete absence of mortality in G3 is biologically significant and warrants attention. Our resulted are matching with those reported by El-Hawy (2024) and this suggests that feeding *Azolla pinnata* to ewes might enhance lamb viability and survival from birth to weaning, potentially due to improved maternal milk production and quality or better lamb vigor, as suggested by the improved performance of their dams and the subsequent milk yield data. The exact mechanisms, however, would require further investigation. The slightly lower performance in the G2 group might be linked to the anti-nutritional factors in CSM, such as gossypol, which can negatively impact overall animal vitality (Swelum *et al*., 2021). Gestation length remained consistent across all groups (150-151 days), indicating that the protein source did not significantly alter the duration of pregnancy.

3.2. Body Weight Changes of Barki Ewes

The body weight changes of the ewes are shown in Table (4). While initial body weights were similar across all groups, indicating a homogenous start, significant differences emerged as the trial progressed. Ewes in G3 (*AZL*) achieved the highest body weight just before lambing (50.20 kg), significantly higher than G1 (49.25 kg) and G2 (48.10 kg). This suggests superior nutrient utilization and body condition maintenance during pregnancy in ewes fed *Azolla pinnata*. The rich profile of essential amino acids, vitamins, and minerals in *Azolla* likely contributed to this enhanced performance (Kholif *et al*., 2022). This sustained body weight advantage in G3 translated into a significantly higher overall weight gain during the experimental period (2.55 kg) compared to G1 (1.30 kg) and G2 (0.40 kg). This highlights the positive impact of *Azolla* on maternal body reserves, which are crucial for subsequent lactation and overall productivity (Morales *et al*., 2012 and El-Hawy, 2024).

Table 4: Effect of feeding different types of ration protein on body weight changes of Barki ewes

Parameter	Treatments			SEM	P value
	G1	G2	G3		
Initial BW (kg)	41.60	41.95	41.75	0.15	0.264
Body weight just before lambing (kg)	49.25^{b}	48.10^{c}	50.20^{a}	0.15	0.0001
Conceptus weight	6.35^{a}	5.75^{b}	5.90^{b}	0.05	0.0001
Body weight after lambing (kg)	42.90^{b}	42.35^{c}	44.30^{a}	0.15	0.0001
Weight gain (kg)	1.30^{b}	0.40^{c}	2.55^{a}	0.05	0.0001

G1 (Control): The diet contained 18.0% soybean meal (SBM) as the primary protein source. G2 (Cottonseed Meal): Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM). G3 (Azolla): Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).
$^{a-c}$ means within rows with different superscripts differ significantly (P<0.05).

Consequently, the total weight gain during the experimental period was highest for G3 ewes (2.55 kg). In stark contrast, ewes in the G2 (CSM) group had the lowest body weights and a minimal weight gain of only 0.40 kg. The G1 (SBM) group showed intermediate results, however G1 had the highest conceptus weight (6.35 kg), indicating potentially larger fetal growth, the overall weight gain of the ewe itself was highest in G3. The lower weight gain in G2 (CSM) could be attributed to lower nutrient utilization efficiency or potential anti-nutritional factors present in cottonseed meal (Gossypol), which might interfere with nutrient absorption and metabolism (Risco *et al*., 2007). The poor performance of the CSM group is consistent with studies that report reduced digestibility and negative effects of gossypol on nutrient metabolism (Gadelha *et al*., 2014).

3.3. Body Weight Changes of Barki Lambs

The positive effects of the maternal diet in G3 were clearly transferred to their offspring (Table 5). Lambs born to ewes in G1 had the highest (P<0.01) birth weight (2.97 kg), followed by G3 (2.78 kg), while G2 had the lowest (2.23 kg). Consequently, at weaning, lambs from ewes fed *Azolla pinnata* had a significantly higher weaning weight (19.92 kg) and daily body weight gain (190.8 g/d) surpassing G1 (19.18 kg and 180.4g/d) and G2 (16.92 kg and 163.6 g/d) respectively (P<0.01).

Table 5: Effect of feeding different types of ration protein on body weight changes of Barki lambs

Parameter	Treatments			SEM	P value
	G1	G2	G3		
Birth BW (kg)	2.97[a]	2.23[c]	2.78[b]	0.05	0.0001
Weaning BW (kg)	19.18[b]	16.92[c]	19.92[a]	0.09	0.0001
Daily BW gain (g/d)	180.4[b]	163.6[c]	190.8[a]	0.15	0.0001

G1 (Control): The diet contained 18.0% soybean meal (SBM) as the primary protein source. G2 (Cottonseed Meal): Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM). G3 (Azolla): Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).
[a-c] means within rows with different superscripts differ significantly (P<0.05).

These findings underscore the critical role of maternal nutrition, particularly protein source, in influencing not only fetal development but also subsequent postnatal growth. The enhanced lamb growth in the *Azolla* group could be attributed to improved milk production and quality from the ewes, as well as potentially better nutrient transfer during gestation that primes the lambs for stronger postnatal development. Our results aligns with research by Kholif *et al*. (2022) and El-Hawy (2024), who reported increased daily gain in kids of goats fed Azolla. The lower birth weight in the CSM group may be a consequence of the suboptimal maternal nutritional status and potential transplacental effects of gossypol and could be linked to the potential detrimental effects of gossypol on milk production or direct toxicity to lambs (Sahlu *et al*., 1999).

3.4. Blood parameters: liver and kidney functions

Blood biochemical parameters related to liver and kidney function revealed significant differences in liver and kidney function indicators (Table 6). Ewes in the G2 (CSM) group showed significantly elevated serum concentrations of AST (31.28 IU/l) and ALT (23.46 IU/l) compared to G1 and G3. AST and ALT are key indicators of hepatocellular damage. Their elevation in the CSM group strongly suggests liver stress (Kaneko *et al*., 2008). This could be related to the presence of gossypol in cottonseed meal, which is known to be hepatotoxic (Risco *et al*., 2007), a well-documented effect of gossypol toxicity (Gadelha *et al*., 2014).

Table 6: Effect of feeding different types of ration protein on blood liver and kidney functions

Parameter	Treatments			SEM	P value
	G1	G2	G3		
AST (IU/l)	23.36[c]	31.28[a]	26.34[b]	0.15	0.0001
ALT (IU/l)	17.84[c]	23.46[a]	20.10[b]	0.09	0.0001
Urea (mg/dl)	19.13[b]	25.26[a]	24.91[a]	0.15	0.0001
Creatinine (mg/dl)	1.16[b]	1.67[a]	1.17[b]	0.05	0.0001

G1 (Control): The diet contained 18.0% soybean meal (SBM) as the primary protein source. G2 (Cottonseed Meal): Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM). G3 (Azolla): Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).
[a-c] means within rows with different superscripts differ significantly (P<0.05).

Elevated urea and creatinine suggest a potential burden on kidney function, possibly due to the metabolic processing of excess nitrogen or the detoxification of harmful compounds (Braun *et al*., 2010). In opposite trend, the G3 (AZL) and G1 (SBM) groups maintained these parameters within normal physiological ranges. The lower values in G1 and G3 indicate better liver and kidney health, supporting the suitability of soybean and *Azolla* as protein sources (El-Hawy, 2024).

3.5. Milk Yield and Composition

Milk yield and composition are detailed in Table (7). Milk yield was significantly affected by the dietary protein source. The G3 (AZL) group recorded the numerically highest milk yield (699.8 g/d), which was statistically similar to the G1 (SBM) group (693.9 g/d) but significantly higher than the G2 (CSM) group (660.7 g/d). This demonstrates that Azolla is as effective as SBM in supporting milk production in Barki ewes, while cottonseed meal led to a reduction. The high quality and digestibility of Azolla protein likely supported the synthesis of milk protein (Mathialagan *et al*., 2021; Kholif *et al*., 2022 and El-Hawy, 2024). Conversely, the depressed milk yield in the G2 group reflects the overall negative metabolic impact of the CSM diet. This difference in milk yield likely contributed to the observed variations in lamb growth rates.

Table 7: Effect of feeding different types of ration protein on milk yield and composition

Parameter	Treatments			SEM	P value
	G1	G2	G3		
Milk Yield (ml)	693.9[a]	660.7[b]	699.8[a]	4.81	0.0001
Milk composition					
Fat (%)	5.10[b]	5.45[a]	5.07[b]	0.05	0.0001
Protein (%)	4.92[ab]	4.69[b]	5.09[a]	0.09	0.0168
Lactose (%)	5.97[a]	5.50[b]	6.05[a]	0.07	0.0001
Ash (%)	0.818[a]	0.723[b]	0.804[a]	0.024	0.0217
Solids non-fat (SNF, %)	10.85[a]	10.59[b]	10.93[a]	0.05	0.0005
Total Solids (TS, %)	17.66[b]	18.39[a]	17.83[b]	0.12	0.0010

G1 (Control): The diet contained 18.0% soybean meal (SBM) as the primary protein source. G2 (Cottonseed Meal): Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM). G3 (Azolla): Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).
[a-c] means within rows with different superscripts differ significantly (P<0.05).

Regarding milk composition, G2 (CSM) had significantly the highest milk fat percentage (5.45%), however, this was often accompanied by significant lower milk yield. G3 exhibited significantly the highest milk protein (5.09%) and lactose (6.05%) percentages, which are crucial for lamb growth and energy. Ash and solids non-fat (SNF) percentages were also significantly higher in G1 and G3 compared to G2. Total solids were significantly the highest in G2. The overall superior milk quality (protein, lactose, SNF) in G3 further explains the better lamb performance observed in this group. This aligns with research demonstrating that dietary protein quality and quantity directly influence milk synthesis and composition in lactating ewes (Bonsi *et al*., 2017).

3.6. Blood hormones (T_3 and T_4) and Protein Profile

Thyroid hormone levels and protein profiles showed some variation (Table 8). There were no significant differences in T_3 levels among groups. However, the T_4 levels were significantly higher in G1 (7.09 µg/ml) and G3 (7.04 µg/ml) compared to G2 (6.83 µg/ml). Thyroid hormones are crucial regulators of metabolism, energy expenditure, and overall productive functions. A lower T_4 level could indicate a reduced metabolic rate, consistent with the poorer performance of the CSM-fed ewes (Chauhan *et al*., 2014).

Table 8: Effect of feeding different types of ration protein on blood thyroid hormones and protein profile

Parameter	Treatments			SEM	P value
	G1	G2	G3		
Tri-iodothyronine (T_3, ng/ml)	1.59	1.58	1.61	0.01	0.3219
Thyroxine (T_4, µg/ml)	7.09[a]	6.83[b]	7.04[a]	0.04	0.0002
Total protein (TP, g/dl)	6.54[a]	6.27[b]	6.63[a]	0.07	0.0028
Albumin (Alb, g/dl)	3.29	3.29	3.40	0.04	0.0974
Globulin (Glu, g/dl)	3.25[a]	2.99[b]	3.23[a]	0.06	0.0069
Albumin/Globulin ratio (A/G %)	101.7[b]	110.8[a]	105.8[ab]	2.51	0.0490

G1 (Control): The diet contained 18.0% soybean meal (SBM) as the primary protein source. G2 (Cottonseed Meal): Soybean meal was completely replaced with 18.0% undecorticated cottonseed meal (CSM). G3 (Azolla): Soybean meal was completely replaced with 18.0% fresh *Azolla pinnata* (AZL).
[a-c] means within rows with different superscripts differ significantly (P<0.05).

Regarding protein profiles, total protein (TP) and globulin (Glu) levels were significantly lower in G2 (6.27 g/dl and 2.99 g/dl, respectively) compared to G1 and G3 groups, suggesting a compromised protein status, which could be due to lower quality dietary protein, reduced synthesis by the stressed liver, or both. The G3 (AZL) group maintained a healthy metabolic and protein profile, comparable to the G1 (SBM) control group. Albumin levels did not differ significantly. The A/G ratio was significantly the highest in G2. Lower total protein and globulin in G2 could indicate reduced protein synthesis or impaired immune function, potentially linked to the quality of the protein source or the presence of anti-nutritional factors. Optimal protein status is essential for overall animal health, immunity, and productive performance (NRC, 2007). The results suggest that *Azolla pinnata* supports comparable, and in some cases, superior metabolic health compared to soybean meal, and is clearly better than cottonseed meal in terms of liver, kidney, and overall protein status.

4. Conclusion

This study provides compelling evidence that the choice of dietary protein source significantly influences the reproductive and productive performance, lamb growth, and metabolic health of Barki ewes. However, soybean meal remains a high-quality protein source, *Azolla* meal demonstrated remarkable potential as a sustainable and effective alternative. Ewes fed *Azolla pinnata* exhibited superior overall body weight gain, significantly higher weaning rates, and zero lamb mortality, indicating improved maternal care and lamb viability. Furthermore, *Azolla pinnata* supported comparable milk yield to soybean and favorable milk composition, leading to enhanced lamb growth rates. Importantly, ewes fed on the *Azolla* diet showed healthier liver and kidney function compared to those on cottonseed meal, which presented signs of metabolic stress. The findings suggest that incorporating *Azolla pinnata* into the diets of Barki ewes can lead to improved reproductive outcomes, healthier and faster-growing lambs, and better overall ewe health. This makes *Azolla pinnata* a promising and environmentally friendly protein source for sustainable sheep production, particularly in regions where conventional protein sources are costly or scarce.

5. Acknowledgement

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