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REPLACING SOYBEAN MEAL WITH *Azolla pinnata* AND ITS EFFECT ON SOME RUMEN CHARACTERISTICS AND BLOOD PARAMETER

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ABSTRACT

This study was conducted at the College of Agricultural Engineering Sciences at the University of Baghdad. It lasted from November 15, 2022, until January 15, 2023. to determine the effect of replacing soybean meal with *Azolla plant* in the concentrated diet at a rate of 0%, 4%, 8%, and 12% on the properties of rumen fluid, the number of bacteria and protozoa, the coefficient of digestion of nutrients, and some blood properties for Awassi lambs. Twenty Awassi lambs were used, their weights ranged between 18 and 19 kg and their ages ranged from 3 to 4 months. They were housed in individual cages and fed a concentrated feed at 2.5% of body weight in the morning meal while roughage (barley straw) was provided freely.

The results showed that there were significant differences in the pH value of treatment T4 at the time of zero withdrawal of rumen fluid, as it was recorded at 7.74, while there were no significant differences between the treatments for the concentration of Ammonia Nitrogen (NH3-N) and *Volatile Fatty Acids* (*VFA*). The results showed a significant decrease (P≤0.05) in the numbers of bacteria and protozoa in the rumen fluid of the T2 treatment compared to the control treatment.

Keywords: Azolla plant, a wassi lambs, rumen fermentation, digestibility, blood parameters.

استبدال كسبة فول الصويا بنبات الازولا وتاثيرها في بعض خصائص الكرش ومعايير الدم

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الخلاصة

أجريت هذه الدراسة في كلية علوم الهندسة الزراعية في جامعة بغداد. واستمرت من 15 نوفمبر 2022 حتى 15 يناير 2023. لمعرفة تأثير استبدال كسبة فول الصويا بنبات الأزولا في العليقة المركزة بنسبة 0%، 4%، 8%، 12% على خواص سائل الكرش، وعدد البكتيريا، والبروتوزوا، ومعامل هضم العناصر الغذائية، وبعض خصائص الدم للحملان العواسي. تم استخدام 20 حمل عواسي تراوحت أوزانها ما بين 18 و 19 كغم وبأعمار من 3 إلى 4 أشهر. تم إيواؤهم في أقفاص فردية وتم تغذيتهم بأعلاف مركزة بمعدل 2.5% من وزن الجسم في وجبة الصباح بينما تم توفير العلف الخشن (قش الشعير) بشكل حر.أظهرت النتائج وجود فروق معنوية في قيمة الاس الهيدروجيني PH للمعاملة T4 في وقت السحب صفر لسائل الكرش حيث سجلت 7.74، بينما لم تكن هناك فروق معنوية بين المعاملات لتركيز نيتروجين الأمونيا NH_3-N والأحماض الدهنية الطيارة VFA. وبينت النتائج انخفاض معنوي (P≤0.05) في أعداد البكتيريا والبروتوزوا في سائل الكرش للمعاملة T2 بالمقارنة مع معاملة السيطرة.

الكلمات المفتاحية: نبات الازولا، حملان عواسي، تخمرات الكرش، هضم، صفات الدم.

INTRODUCTION

Ruminant nutrition is typically influenced by the environment and heredity **(Abdullah, 2023; Mahmud & Mohammed,2019)** and as a result of the high cost of fodder used to feed livestock, additionally, it is challenging to find pure fodder outside of a forest **(EL-Delfi & Safi, 2023; Sahar.,2023)**. Researchers have started to concentrate on additions for fodder, such as yeast **(Hassan & Mohammed, 2017; Hassan & Mohammed, 2016; Areej *et al*.,2016)**, **(Sharma *et al*., 2021)**, vitamins, and hormones **(Alsamraee *et al*., 2014; Hassan & Mahmood, 2017)**. Additionally, due to the high cost of soybean meal in particular, they resorted to replacing it with other unconventional feeds and non-protein nitrogenous substances like urea. mainly due to the high cost of soybean meal. (**Hassan & Tawffek, 2009**) or by swapping corn gluten **(Hassan & Mohammed, 2017 17)** or urea with or without nitrogen carboxyl glutamine **(Alshefea & Hassan, 2021; Jassim& Mohammed., 2023)**, or by switching soybean meal for sesame meal **(Younis, 2021)** or sunflower meal **(Dawood, 2020)** to enhance the performance of ruminants. The Azolla plant is considered one of the alternative ingredients used in the production of animal feed because it provides 25 to 30 percent protein, as well as vitamins, minerals, and antioxidants **(Mathur *et al*., 2013).** Feeding green Azolla to Sirohi male kids increased the average daily gain and feed conversion ratio in a study by **Sharma *et al.* (2021).** According to some studies, switching from sunflower meal to Azolla had no effect on the performance of the animals**. (El-Fadel *et al*, 2020).** The purpose of the current study is to look into how the dry Azolla plant affects some blood parameters, bacterial count, and rumen characteristics in lambs.

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MATERIALS AND METHODS

The lambs were randomly distributed and divided into four equal groups (5 lambs/group). All animals were subjected to the same medical care and environmental conditions throughout the duration of the experiment. The raw materials for the components of the concentrated diet were provided from local markets and then mixed according to the required proportions to obtain four experimental diets. Table (1). Control treatment T1 (0% Azolla, 6% soybeans), T2 (4% Azolla, 4% soybeans), T3 (8% Azolla, 2% soybean), and T4 (12% Azolla, 0% soybean). Concentrated feed was provided at a rate of 2.5% of body weight, while coarse feed was provided freely. In the morning, the weights of the remaining feed from the previously provided meal are recorded. The lambs were weighed every two weeks during the experiment period. All chemical analyses were conducted according to what was stated in (AOAC 2010).

Table (1): Components of concentrate feed for treatments (%).

Ingredient	T1	T2	T3	T4
Barley grain	46	46	46	46
Wheat bran	46	44	42	40
Soybean meal	6	4	2	0
Azolla pinnata	0	4	8	12
Salts and lime	2	2	2	2

RUMEN FERMENTATION CHARACTERISTICS

As soon as the rumen liquid was collected, its pH was measured using an electronic pH meter calibrated against a standard buffer solution**.**

NH3-N measurement: Ammonia nitrogen (N-NH3) was measured according to (AOAC, 2010).

NH3-N (mg/100 ml) = [ml. HCl titration × normal HCl × 0.014/ volume of rumen fluid (1 ml)] × 100

Total volatile fatty acids: According to the steam distillation method, total volatile fatty acids (TVFAs) in rumen fluid were calculated **(Warner, 1964)**. The following equation was used to determine TVFAs:

TVFA (mmol/L) = (ml titration NaOH × normality of NaOH/ volume of rumen fluid (1 ml) × 1000) **(Warner, 1964)**

The total number of bacteria: according to **(Norden & Kass, 1968)** To count the number of bacteria, use the pour plate method. After the agar has solidified, the plate is incubated with 37C0 for a period of 24 to 48 hours. According to the protozoa count **(Warner, 1964)** the method of taking 1ml from the filtered rumen fluid and diluting it to 9 ml of the physiological solution normal slain, a drop of 0.3 ml of the diluted solution was drawn on the cells, counting the cells, (Hemocytometer) and eating with a slide cover, evenly distributed within squares and placed under the microscope lens (magnification power 400). The number of ciliates is obtained in Small squares **(Sujatha & Jeyakumar, 2009)** The following equation is applied after the number of protozoa in the squares was determined:

Number of cells/ml = number of counted cells/ number of squares x 10^6 x4

BLOOD PARAMETERS

During the last week of the experiment and within one day. Blood samples (10 ml) were withdrawn from via jugular vein puncture into vacationer tubes, which were immediately placed in refrigerator, before morning feeding to determine Serum glucose (SG), Serum total protein (STP) and Serum urea nitrogen (SUN), concentration Serum triglyceride and cholesterol. The blood samples were examined by an electrical device (BIOSYSTEMS BTS 350 Spain and BIOSENSOR SD Lipido Care Korea) that automatically put the sample after signaling the necessary examination.

INVITRO DIGESTIBILITY

The digestibility coefficient of dry matter and organic matter in the feed was measured laboratoryly using the method of **Tilly & Terry (1963)** in two successive stages because the diet contains concentrated feed and coarse feed (microbial digestion and enzymatic digestion). In the first stage, 0.5 g of the experimental diet was weighed and 40 ml was added to it. Artificial saliva and 10 ml of filtered rumen fluid were placed in laboratory digestion tubes with the addition of carbon dioxide gas twice daily. The samples were placed in a water bath at a temperature of 39°C for 48 hours, with the tubes stirred twice daily. In the second stage, pepsin (2) was added. ml) into the digestion tubes and left for another 48 hours for enzymatic digestion, after which the samples are filtered and the undigested feed remains are placed in ceramic lids and then dried using an electric oven at a temperature of 105°C for a full day.

STATISTICAL ANALYSIS

Data were statically analyzed as a 2*2 factorial experiment using a statistical program **(SAS, 2012)** and data were tested according to Duncan's multiple range test **(Duncan, 1955)** following: the mathematical equation given below: Yij= μ + Ti + eij Where: Yij = the observation of ij, μ=overall mean of Yij, eij = the experimental random error Ti = Effect of i (treatments)

RESULTS AND DISCUSSION

Rumen fermentation characteristic

The results showed a significant decrease in the pH value of the rumen fluid for treatment T3 at the time of withdrawal zero, as it recorded 7.53 compared to the control treatment, which recorded 7.56 while there was no significant difference between the treatments after 3 h and 6 h of morning feeding. Perhaps The reason for the decrease in the pH of rumen fluid after morning feeding is due to the stability of the rumen environment and the increase in the percentage of salts produced by saliva during the rumination process**. (Galvani *et al.*, 2014)**. **(Kumar *et al.*,2015)** reported that adding sun-dried Azolla to concentrated feed for buffalo bulls resulted in an increase in the pH value of rumen fluid after feeding. **Sharma *et al.* (2021)** pointed out the highly significant (P<0.01) effect of green Azolla (*Azolla pinnata*) supplementation in concentrate diets 0,150,250 and 350 gm, on rumen parameters (rumen pH and total volatile fatty acids) in Sirohi goat kids. It was concluded that green Azolla (*Azolla pinnata*) improved the rumen parameters in Sirohi kids when fed 250gm with a concentrated diet. The effect of replacing soybean meal with Azolla pinnata in the ruminant diet revealed no significant effect of level on Ammonia Nitrogen (NH3-N) and *Total Volatile Fatty Acids* (TVFA) values at 0,3 and 6 h from withdrawn in rumen fluid between treatments Table (2) **Rimi Hamidan *et al.*. (2022)** showed that mixing *A. filiculoides* plants with a Napier Silage formulation at a rate of 0%, 6%, 10%, 16%, and 23% led to an increase in the *Volatile Fatty Acids* (*VFA*) of treatment T4 by 120.0 mM/L compared to the control treatment of 87.9 mM/L

Table (2): The effect of replacing soybean meal with Azolla plant on some rumen characteristics of Awassi lambs (Mean ± SE).

TRT	0 hr.	3hr	6hr	0 hr.	3hr	6hr	0hr	3hr	6hr
T1	0.03 ± 7.56 ab	±0.156. 90	0.17 ± 6.80	5.83±0.58	7.58±0.58	8.76±0.00	6.33±0.22	9.08±0.91	6.83±0.50
T2	0.06 ± 7.63 ab	0.57 ± 6.80	0.06 ± 6.73	7.00±0.00	7.58±0.58	8.76±0.00	5.91±0.22	7.75±0.94	6.66±0.72
T3	0.06 ± 7.53 b	6.66 ± 0.12	0.6 ± 6.66	6.41±0.58	7.58±0.58	8.17±0.58	5.91±0.41	9.08±0.68	7.83±1.04
T4	0.06 ± 07.7 a	±0.066.53	6.83±0.14	5.83±0.58	8.17±0.58	8.17±0.58	6.60±0.14	9.00±0.28	7.91±0.36
Signiant	*	NS	NS	NS	NS	NS	NS	NS	NS

Means in the same column are NS :non-significantly, *Significant, (P≤0.05) T1 (0)% Azolla Pinnata, T2 (4)% Azolla Pinnata, T3 (8)% Azolla Pinnata, T4(12)% Azolla Pinnata

Bacteria, fungi and protozoa contribute to creating a distinct ecosystem. Protozoa are an evolving group that relies on interactions with other microbes. Table (3) shows the effect of replacing soybean meal with Azolla on the total number of bacteria and protozoa in the rumen fluid of Awassi lambs. The table also indicated a significant decrease ($P < 0.05$) in the number of bacteria for the T2 treatment before feeding, which amounted to 114 colonies/ml compared to the control treatment, which amounted to 124 colonies/ml. Treatment T1 recorded the highest number of bacteria after 3 and 6 hours of feeding, reaching 134 and 133 CFU/mL$\times 10^4$. The table showed that T2 and T4 increased the number of primitives. It reached 6.82 and 6.61 cell/ml $\times 10^5$ before morning feeding, respectively, and there were no differences between the treatments 6 hours after feeding. Ruminal protozoa have a lower flux in ruminant rennet than bacteria because they are conserved in feed particles **(Hook *et al.*, 2012)**. The increase in the number of microorganisms may be due to the increased benefit from the nutritional value of the Azolla plant, given that it contains mineral elements, amino acids, and a good percentage of protein that is used in the manufacture of microbial protein. **Sujatha & Jayakumar (2009)**. explained that the mineral elements found in the Azolla plant, such as potassium, iron, magnesium, vitamin A, vitamin B, and amino acids, led to an increase in the activity and number of microorganisms, including protozoa, in the rumen compared to the control treatment.

Table (3): Effect of replacing soybean meal with *Azolla plant* on Total count of Bacteria CFU /mL$\times 10^4$ and Count of protozoa cell/ml $\times 10^5$ in rumen fluid of Awassi lambs Mean ±SE.

Factor	Count of Bacteria CFU /mL$\times 10^4$			Total count of protozoa cell/ml ×105 in rumen fluid		
Treatments	0 hr.	3hr	6hr	0 hr.	3hr	6hr
T1	124±4.25 a	134 a ±5.13 a	133±2.18 a	5.81±0.23 c	7.30 ±0.19 c	5.97±0.05
T2	114±2.18 b b	114± 2.02c c	114±1.45 b	6.82±0.14 a	8.85±0.10 a	6.02±0.05
T3	124±0.88 a	117±4.37 bc	122±1.85 c	6.50±0.19 b	8.64±0.09b c	5.86±1.14
T4	126±0.88a a	128±0.57 ab	129±0.66a a	6.61±0.10 a	8.37±0.10 b	5.76±0.09
Signiant	*	*	*	*	*	NS

Means in the same column are NS :non-significantly, *Significant, (P≤0.05) T1 (0)% Azolla Pinnata, T2 (4)% Azolla Pinnata, T3 (8)% Azolla Pinnata, T4(12)% Azolla Pinnata

BLOOD PARAMETERS

Table (4) shows that there were no significant differences between the treatments when feeding on a concentrate diet in which soybean meal was replaced with *Azolla plants* 0, 4, 8, and 12% of blood samples at 0, 3, and 6 hours before and after the morning feeding. This could be a result of the Azolla plant's non-harmful effects on lambs' performance, as all outcomes were comparable with the control treatment for all examined attributes. Some studies have indicated that normal levels of glucose and urea between 44-80 and 10-40 mg/ 100 ml, respectively **(Kim *et al.*, 2012; Kohn *et al.*, 2005)**. Energy levels and dietary protein are the

key determinants of blood characteristics, and the animal itself is primarily responsible for the creation of blood sugar and its representation **(Singh *et al*., 2021)**. **(El-Fadel *et al*.,2020)** indicated that there were no significant differences in the concentration of total protein, urea and cholesterol when replacing sunflower oil with Azolla. **Roy *et al*. (2016)** showed that feeding calves on a concentrated diet in which the Azolla plant was replaced by 5% led to a decrease in blood urea. In another study, **Hassanein *et al.* (2023)** reported that there were no significant differences in urea and a significant increase in total blood protein when replacing sunflower meal in the concentrated diet by 0, 25, and 50% with Azolla plants, by 0, 10, and 20% in goat diet.

Table (4): Effect of replacing soybean meal with Azolla plant on blood parameters Glucose, Total protein, Urea, Triglycerides and Cholesterol mg/100ml of Awassi lambs Mean ±SE.

Factor TRT	Hr	Glucose	Total protein	Urea	Triglycerides	Cholesterol
T1	0	55.66±0.88	5.50±0.03	44.66±0.88	22.33± 0.33	42.33±0.88
	3	61.66±0.88	5.56±0.02	49.33±1.20	19.00±0.57	47.33±0.88
	6	65.66±0.33	5.68±0.10	47.66±0.33	26.33±1.20	45.66±0.33
T2	0	62.00±3.51	5.68±0.10	41.33±0.88	22.00± 0.57	43.33±0.88
	3	67.00±3.78	5.67±0.01	43.00±1.73	19.33± 0.88	48.33±0.88
	6	69.33±3.17	5.58±0.00	42.00±0.57	24.33± 0.33	46.33±0.33
T3	0	60.33±0.88	5.53±0.00	42.00±1.15	21.66± 0.33	44.00±0.57
	3	67.00±0.57	5.57±0.01	48.00±1.15	18.66± 0.66	48.33±0.66
	6	68.00±2.08	5.60±0.00	44.00±2.08	25.00±1.00	45.33±1.20
T4	0	58.00±3.05	5.53±0.00	43.00±1.73	21.66± 0.33	45.00±0.57
	3	63.66±3.71	5.56±0.00	48.00±2.08	18.33± 0.33	49.00±0.57
	6	69.00±3.05	5.58±0.01	44.33±2.40	23.66± 0.33	46.33±1.20
Significant		Ns	Ns	Ns	Ns	Ns

Means in the same column are NS :non-significantly,T1 (0)% Azolla Pinnata, T2 (4)% Azolla Pinnata, T3 (8)%, Azolla Pinnata T4(12)% Azolla Pinnata

IN VITRO DIGESTIBILITY

The results Table (5) indicated that there was no a significant in IVDMD AND IVOMD between treatments. This result was agreed with **Kumar *et al*. (2015)** who showed no differences in the digestibility coefficient of dry matter (DM) and organic matter (OM) between treatments when incorporating sun-dried Azolla meal in the concentrate mixture of about 25 % to buffalo bulls. Other study showed by **Parashuramulu *et al*. (2013)** increases in vitro digestibility of dry matter and organic matter (79.5and 63) 8%. The digestibility coefficient of dry matter and organic matter was similar to the control group when replaced

25% of the concentrated mixture with sun-dried azolla which fed to goats, and it reached (52.41, 55.46 and 57.89, 55.46) respectively **(Kumar *et al*., 2015)**. (**Roy *et al*., 2016)** found no differences in nutrient digestibility when heifers were fed a 5 percent *Azolla pinata* which was replaced in the treatment group. Whereas, **Rimi Hamidan *et al*. (2022)** found an increase in in vitro digestibility coefficient of dry matter and organic matter, when used Azolla filiculoides meal to replace the proportion of soybeans by (0, 6, 10, 16 and 23%) respectively. (**Hassanein *et al*., 2023)** found that by replacing sunflower meal at rates of 0, 25, and 50 percent with Azolla plants at rates of 0, 10, and 20 percent when feeding goats, there was a significant increase ($P \leq 0.05$) in the digestibility coefficient of dry matter at a replacement rate of 10 and 20 percent, which amounted to 67.57 and 64.96—sequentially compared to the control treatment 61.73, while there were no significant differences in the organic matter digestibility coefficient.

Table (5): Effect of replacing soybean meal with Azolla plant on in invitro digestibility**.**

Factors	Mean ±SE	
TRT	IVDMD	IVOMD
T1	53.57±2.09	60.59±2.37
T2	61.97±1.40	68.46±0.29
T3	59.14±2.68	72.96±8.16
T4	63.03±8.10	70.92±7.67
Significant	NS	NS

Means in the same column are NS :non-significantly,T1 (0)% Azolla Pinnata, T2 (4)% Azolla Pinnata, T3 (8)%, Azolla Pinnata T4(12)% Azolla Pinnata

CONCLUSION

When the concentrate mixture in Awassi lambs was substituted with azolla pinnata at a 0 , 4 , 8 and 12% level, Receptively , At this degree of replacement, and rumen fermentation metrics were not negatively impacted, suggesting that this might be employed as an alternative feed resource in farming systems.

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