IJAS Iranian Journal of Applied Animal Science

Effect of Hydroponic Barley Seedlings Substituting for Different Ratios of Basal Diets on Serum Biochemical Indexes and Rumen Fluid Microbial Diversity in Lactating Ewes

Research Article

Y. Ma[1], T. Guo[1,2*], Z. Zhang[1,2], G. Amat[1,2], Y. Jing[1], Y. Tuo[1] and L. Hou[1]

[1] Institute of Feed Research, Xinjiang Academy of Animal Science, Urumqi, China
[2] Key Laboratory, Xinjiang Feed Biotechnology, Urumqi, China

Received on: 27 Jun 2023
Revised on: 18 Oct 2023
Accepted on: 6 Nov 2023
Online Published on: Dec 2023

*Correspondence E-mail: guotaoxj@126.com

Online version is available on: www.ijas.ir

ABSTRACT

The purpose of this study was to investigate the effects of hydroponic barley seedlings on serum biochemical indices and rumen microbial diversity of lactating ewes instead of different proportion of basal diet. It provides theoretical basis for the health and scientific feeding and management of hydroponic barley seedlings in ruminants. Forty-eight lactation sheep with 2-3 years old, second and third parity, 45 ±5 kg weight, 6 ±2 lambing days and two lambing number, respectively were randomly divided into 6 groups of 8 repeats in each group and the experimental period was 36 days, including the pre-feeding period of 6 days and the formal period of 30 days. (1) Growth performance, the average daily gain of ewes was increased as the proportion of hydroponic barley seedlings increased and reached the highest in group C. The average daily gain of ewes in group B was the lowest, which may be caused by the low dry matter intake of large-sized sheep. (2) Regarding blood biochemical indexes, the content of total superoxide dismutase (T-SOD) in groups A and B was significantly higher than in groups CK1, CK2, A, B, C and D ($P<0.05$). The content of malondialdehyde (MDA) in group A, C was significantly lower than in groups CK1 and CK2 ($P>0.05$). The content of triglyceride (TG) in group D was significantly higher than in groups CK1 and CK2 ($P<0.05$). The content of total protein (TP) in groups A, B, C and D was significantly higher than in group CK1 ($P<0.05$), and groups C and D was significantly higher than in groups CK2 ($P<0.05$). The content of glucose (GLU) in group D was significantly higher thanin groups CK1 and CK2 ($P<0.05$). The content of interleukin-1β (IL-1β) in groups C and D was significantly higher than in groups CK1 and CK2 ($P<0.05$). The content of interleukin-2β (IL-2β) in groups A and D was significantly higher than in groups CK1 and CK2 ($P<0.05$). The content of interferon gamma (IFN-γ) in groups A and D was significantly higher than in groups CK1 and CK2 ($P<0.05$). The content of CD_4 in groups A, B and C was significantly higher than in group CK2 ($P<0.05$), and group D was significantly higher thanin group CK1 ($P<0.05$). (3) Microbial diversity in rumen fluid: there was no significant effect of different barley seedling ratios on in rumen fluid of ewes, and the coverage of each group was more than 0.99. The results showed that the microbial diversity data of each group acted accurately on the composition of rumen fluid microflora in lactating ewes. Different ratios of hydroponic barley seedlings could increase the relative abundance of *Firmicutes*, *Actinobacteriota*, *Prevotella*, *Selenomonas*, unclassified_ *Prevotella* and unclassified_ *Selenomonas* in the rumen fluid of ewes. Hydroponic barley seedlings can be used as supplementary green feed and can not only replace part of the basic diet. Mostly it can also improve the immune performance of female animals and alleviate oxidative stress. It can be concluded that in the ewes' diet 10%-15% inclusion of Hydroponic barley seedlings is helpful to improve the overall performances of the animal.

KEY WORDS hydroponic barley seedlings, lactating ewes, microbial diversity and biochemical indexes.

INTRODUCTION

Hydroponic barley seedlings have the advantages of good palatability, easy digestion, rich vitamins and other antioxidant substances, much fertilizer saving and high yield, stable output, short growth cycle, and so on. At present, hydroponic barley seedlings have been used as livestock and poultry feed locally and seasonally when there is a lack of green forage at home and abroad (Badran *et al*. 2017).

Raeisi *et al*. (2018) reported that hydroponic barley seedlings replaced part of barley grains with 7%, 14% and 21% fresh weight) in sheep fresh weight diet. With increased hydroponic barley seedling replacement, sheep's dietary dry matter intake, nitrogen intake, nutrient retention and digestibility were significantly increased. Hydroponic barley seedlings have higher vitamin content when they grow to about 8 cm tall, which can help herbivorous livestock to milk. Hydroponic barley seedlings at harvest period can provide vitamin E for herbivorous livestock, which plays a positive role in improving cellular antioxidation, protecting liver function and promoting reproductive performance (Morales *et al*. 2009). As a kind of green feed, the recommended additive amount of hydroponic barley seedlings in the ruminant diet was rarely reported, and it is still unable to guide the efficient production and utilization of livestock and poultry. This study is to explore the effects of hydroponic barley seedlings substituting for different proportions of basal diets causally on serum biochemical indexes and rumen fluid microbial diversity of lactating ewes and to provide a reference for rational and safe use of hydroponic barley seedlings in ruminant production and further research.

MATERIALS AND METHODS

Material test animals and materials

This experiment takes Hu sheep as the experimental animal, which is provided by the Hu sheep breeding base of Xinjiang Maiteng Animal Husbandry Science and Technology Development Co., Ltd. Feeding hydroponic barley seedlings, were purchased from Xinjiang Luchuangfeng Agricultural Development Co., Ltd.

Experimental design

In total, Forty-eight lactation sheep with 2-3 years old, second and third parity, 45 ±5 kg weight, 6 ±2 lambing days and two lambing number, respectively were randomly divided into 6 groups of 8 repeats in each group and the experimental period was 36 days, including the pre-feeding period of 6 days and the formal period of 30 days. According to the nutritional requirements of NRC (2007) 45 kg ewes during lactation, the control groups (CK1 and CK2) diet was prepared based on the principle of equivalent energy or nitrogen, and the treatment group was fed with hydroponic barley seedlings substituting for 5% (group A), 10% (group B), 15% (group C) and 20% (group D), respectively. This study was conducted in accordance with the standards of the animal ethic Sreview committee (protocol permit number: 2022024; 20 March 2022).

Feeding management

Before the beginning of the test, the sheep pens were sterilized and disinfected, and all the test lake sheep were orally dewormed with Ivermectin before the test, and ear numbers were marked. The whole animal feeding test was carried out in the same environment by house feeding, and the diets of each group were accurately weighed and evenly mixed according to the proportion of the formula, and then fed regularly at 9:00 and 17:00 every day, and the test period was 36 days, of which the pre-feeding period was 6 days, and the official period was 30 days. Table 1 shows the details of the dietary composition and nutritional concentrations.

Sample collection and testing

Sample collection

Before feeding (at 08:00 hours) on day 31, the blood was drawn from the jugular vein by using a heparin sodium anticoagulant tube. Blood samples were centrifuged at 600g for 15 min at room temperature; the supernatant was pipetted into 1.5-mL centrifuge tubes and frozen at -20 ˚C; Rumen fluid was collected immediately after slaughter on day 32 of the formal test. The filtered rumen fluid was divided into three 10 mL centrifugal tubes and frozen with liquid nitrogen for the changes of rumen microflora.

Determination of plasma biochemical indexes

On day 31 of the experiment, the samples were submitted to Nanjing Jianjian Bioengineering Institute of Biotechnology for determining the concentrations of urea, blood ammonia total antioxidant capacity (T-AOC), malondialdehyde (MDA), activities of superoxide dismutase (SOD), and glutathione peroxidase (GSH); glucose (GLU), creatine kinase (CK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), azelaic transaminase (AST), cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), total protein (TP), urea nitrogen (BUN); interleukin-1β (IL-1β), interleukin-2β (IL-2β), interferon gamma (IFN-γ), CD4-T lymphocytes (CD4) of ewes by using colorimetry.

Genomic DNA of rumen fluid sample was extracted by Hexadecyltrimethy ammonium bromide (CTAB) method, purity and concentration was detected by 0.8% agarose gel electrophoresis, diluted to 1 ng/μL for use.

Table 1 Experimental diet composition and nutritional level (in dry matter basis)

Items	Groups					
	CK1	CK2	A	B	C	D
Composition of raw materials (%)						
Corn silage	15.00	-	-	-	-	-
Hydroponic barley seedlings	0.00	0.00	5.00	10.00	15.00	20.00
Halm	21.60	26.20	24.89	23.58	22.27	20.80
Alfalfa 17% CP	10.10	15.00	14.25	13.50	12.75	12.00
Mixed concentrate	52.80	58.30	55.36	52.42	49.48	46.70
Premix[1]	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00
Measured nutritional level						
ME (MJ/kg)	10.09	10.09	10.13	10.17	10.21	10.25
CP (g/kg)	128.45	128.40	122.93	117.45	111.98	106.50
NDF (%)	39.93	33.26	33.02	32.77	32.53	32.29
Ca (g/kg)	4.68	4.56	4.36	4.15	3.95	3.75
P (g/kg)	3.14	2.90	2.79	2.69	2.58	2.48

[1] The premix provides per kg of concentrate supplement: vitamin A: 4200 IU; vitamin B_1: 0.4 mg; vitamin B_2: 2 mg; vitamin B_6: 1.2 mg; vitamin C: 20 mg; vitamin D_3: 880 IU; vitamin E: 500 IU; Pantothenic acid: 10 mg; Niacinamide: 100 mg; Copper: 25 mg; Iron: 107 mg; Manganese: 81 mg; Zinc: 74 mg; Iodine: 6 mg; Selenium: 14 mg; Cobalt: 3 mg and Choline Chloride: 120 mg.
CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.

Using the diluted genomic DNA as template, PCR amplification was performed using the V3 region specific primers of 16S rDNA. DNA extraction, PCR amplification, Illumina HiSeq sequencing and result analysis were all assisted by Beijing Baimike Bioinformation Technology Co., LTD.

Determination of plasma biochemical indexes

Data are expressed as means ± standard errors of the mean. Data were preliminarily sorted using MS-Excel 2010, and SPSS 20.0 (SPSS, 2011) was used for performing one-way ANOVA. If the difference between treatments was significant, Duncan' multiple-range test was used for comparisons, and orthogonal polynomials were used to evaluate the linear and secondary effects of changes in the linear and quadratic effects of serum biochemical indexes were examined. We used $0.05 \leq P < 0.10$, $P < 0.05$, and $P < 0.01$ to indicate a significant trend, significant difference, highly significant difference respectively. The rumen microbial data were analyzed by metastats using R software and performed an inter-group permutation test under various classification levels (phylum, class, order, family, genus, species), and P-values were obtained. Then Benjamini and Hochberg False Discovery Rate are used to correct the P-value. The species with significant differences between groups were analyzed using R software for inter-group Tukey test.

Ethical approval

All procedures in this study were approved by the Animal Experiment Ethics Committee of Feed Research Institute, Xinjiang Academy of Animal Science (permit number: 20 March 2022).

RESULTS AND DISCUSSION

According to Table 2 that the average daily gain of ewes in group C was significantly higher than that in groups CK1, CK2, B and D ($P<0.05$). Dry matter intake in group A was significantly higher than that in groups CK1, CK2, B, C and D. Dry matter intake in groups CK1 and B was significantly higher than that in groups CK2, B, C and D. Dry matter intake in groups C and D was significantly higher than that in group B. During the formal experiment, with the increased proportion of hydroponic barley seedlings in the replacement group, the average daily gain of ewes reached to highest in group C. The average daily gain of group B was the lowest, which may be caused by the low dry matter intake of large-size sheep.

According to Table 3, the content of SOD in groups A and B was significantly higher than CK1 and CK2, and that of groups A, B, CK1 and CK2 increased by 18.99%, 17.71% and 15.59%, 16.85% ($P<0.01$), respectively. There was no significant difference in the content of GSH-px among groups, but the content of GSH-px in groups A, B, C and D was higher than that in groups CK1 and CK2, and the content of MAD in groups A, B, C and D was significantly lower than that in groups CK1 and CK2.

Table 2 Effect of the ratio of wheat to wheat on the growth performance of ewes with different barley seedling ratios (n=8)

Items	Groups						SEM	P-value		
	CK1	CK2	A	B	C	D		Total	Liner	Twice
Initial weight (kg)	51.51	51.26	51.50	51.66	51.44	51.33	0.990	1.000	0.991	0.962
Final body weight (kg)	50.30	49.95	50.78	48.16	56.56	51.48	8.507	0.498	0.367	0.813
Average daily gain (g/d)	-40.42^{b}	-43.54^{b}	-24.17^{b}	-116.45^{b}	170.83^{a}	5.00^{ab}	14.516	0.004	0.056	0.554
Dry matter intake (kg/d)	2.13^{B}	2.30^{A}	2.14^{B}	1.76^{D}	1.92^{C}	1.97^{C}	0.045	< 0.001	< 0.001	0.017

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 Effects of different proportions of barley seedling on serum antioxidant capacity of ewe (n=8)

Items	Groups						SEM	P-value		
	CK1	CK2	A	B	C	D		Total	Liner	Twice
SOD U/mL	145.32^{B}	146.91^{B}	172.93^{A}	169.81^{A}	163.55^{AB}	162.97^{AB}	2.442	< 0.001	0.002	0.003
GSH-px U/mL	24.53	28.28	34.79	28.90	25.97	30.66	0.948	0.024	0.318	0.098
MAD nmol/mL	7.32^{a}	6.20^{ab}	4.50^{c}	5.56^{bc}	4.81^{c}	5.79^{bc}	0.228	0.001	0.006	0.001

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
SOD: superoxide dismutase; GSH: glutathione peroxidase and MDA: malondialdehyde.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

According to Table 4, there was no significant difference in TC, HDL-c, and LDL-c content between the groups (P>0.05); TG content in group D was extremely significantly higher than groups CK1 and CK2, increased by 88.46% and 53.13% (P<0.01), respectively.

According to Table 5, the TP content in groups A, B, C and D was significantly higher than CK1 group, increased by 11.13%, 9.50%, 13.28%, and 14.56% (P<0.05) respectively, and significantly higher in groups C and D than CK2, increased by 9.51%, 10.75% (P<0.05) respectively; there was no significant difference in BUN content between the groups (P>0.05).

According to Table 6, IL-1β content in groups C and D was extremely more significant than that in groups CK1 and CK2, increased by 22.64% and 37.59% (P<0.01), respectively; IL-2 β content in groups A and D was more significant than groups CK1 and CK2, increased by18.87%, 20.35%, 28.36% and 29.95% (P<0.05), respectively; IFN-γ content in groups A and D was extremely more significant than groups CK1 and CK2, increased by 22.38%, 30.40%, 34.36% and 43.17% (P<0.01), respectively; CD_4 content in groups A, B and C was extremely significantly higher than group CK2, increased by31.64%, 28.12% and 37.75% (P<0.01), respectively, and group D was extremely significantly higher than group CK1, increased by35.75% (P<0.01).

According to Table 7, Glu content in groups C and D was significantly higher than groups CK1 and CK2, increased by 36.34%, 31.83%, 39.44%, and 34.83% (P<0.01), respectively; CK content did not change significantly between the groups (P>0.05); LDH activity in group D was significantly higher than that in group CK2, increased by27.75% (P<0.05); aspartate aminotransferase content did not change significantly between the groups (P>0.05); ALT content in groups A and C was significantly higher than group B, increased by 49.78%, 46.26% (P<0.05), respectively.

According to Table 8, the results of alpha ewe rumen fluid diversity detection showed that there was no significant effect of different barley seedling ratios on ewe rumen fluid alpha diversity (P>0.05), but the coverage of each group exceeded 0.99, indicating that the data of each group can accurately reflect the composition of fecal microflora of lactating donkey foals.

According to Figure 2, the Venn diagram shows the number of OTUs shared among the six groups and the number of OTUs unique to each group. 388 OTUs were shared among the six groups, 1431 OTUs were unique to group CK1, 1684 OTUs were unique to group CK2, 1611 OTUs were unique to group A, 1630 OTUs were unique to group B, 2626 OTUs were unique to group C, and 1733 OTUs were unique to group D.

The number of OTUs unique to groups A, B, C, and D was higher than that of group CK1, and the number of OTUs unique to groups C and D was higher than that of group C.

Table 4 Effects of different proportions of barley seedlings on serum lipid related indexes of ewe (n=8)

Items	Groups						SEM	P-value		
	CK1	CK2	A	B	C	D		Total	Liner	Twice
TC mmol/L	1.62	1.41	1.59	1.60	1.69	1.89	0.039	0.015	0.004	0.049
HDL-c mmol/L	0.73	0.67	0.71	0.72	0.79	0.80	0.172	0.196	0.040	0.202
LDL-c mmol/L	0.56	0.42	0.55	0.64	0.51	0.55	0.183	0.021	0.342	0.700
TG mmol/L	0.26^{C}	0.32^{BC}	0.38^{B}	0.33^{ABC}	0.42^{AB}	0.49^{A}	0.146	< 0.001	< 0.001	0.609

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
TC: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein and TG: triglyceride.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 Effects of different barley seedling ratios on serum glucose and enzyme-related indexes of ewe (n=8)

Items	Groups						SEM	P-value		
	CK1	CK2	A	B	C	D		Total	Liner	Twice
TP g/L	70.26^{c}	72.68^{bc}	78.08^{ab}	76.94^{ab}	79.59^{a}	80.49^{a}	0.955	0.004	< 0.001	0.328
BUN mmol/L	6.85	7.94	9.39	7.83	8.08	8.58	0.149	0.008	0.003	0.214

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
TP: total protein and BUN: urea nitrogen.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of different proportions of barley seedlings on serum immune-related indexes of ewes (N=8)

Items	Group						SEM	P-value		
	CK1	CK2	A	B	C	D		Total	Liner	Twice
IL-1β ng/L	18.86^{C}	18.86^{C}	22.07^{BC}	19.90^{BC}	23.13^{AB}	25.95^{A}	0.571	< 0.001	< 0.001	0.180
IL-2β ng/L	26.87^{b}	26.54^{b}	31.94^{a}	26.35^{b}	29.96^{ab}	34.49^{a}	0.733	0.001	0.002	0.227
IFN-γ ng/mL	60.28^{C}	56.57^{C}	73.77^{B}	62.06^{C}	72.64^{B}	80.99^{A}	1.493	< 0.001	< 0.001	0.147
CD_4 ng/mL	180.59^{BC}	156.31^{C}	205.76^{B}	200.27^{B}	215.32^{AB}	245.15^{A}	6.047	< 0.001	< 0.001	0.225

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
IL: interleukin and IFN: interferon.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 Effects of different barley seedling ratios on serum glucose and enzyme-related indexes of ewe (n=8)

Items	Groups						SE	P-value		
	CK1	CK2	A	B	C	D		Total	Liner	Twice
Glu mmol/L	3.22^{b}	3.33^{b}	3.69^{b}	3.50^{b}	4.39^{a}	4.49^{a}	0.099	< 0.001	< 0.001	0.224
CK U/mL	0.18	0.14	0.16	0.13	0.11	0.14	0.006	0.029	0.008	0.333
LDH U/L	3034.04^{abc}	2802.83^{bc}	3193.64^{ab}	2578.03^{c}	2893.06^{bc}	3384.39^{a}	69.849	0.011	0.275	0.023
AST U/L	11.18	11.36	13.04	10.80	12.75	11.92	0.295	0.181	0.339	0.547
ALT U/L	2.97^{ab}	2.76^{ab}	3.40^{a}	2.27^{b}	3.32^{a}	2.57^{ab}	0.118	0.044	0.524	0.669

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
Glu: glucose; CK: creatine kinase; LDH: lactate dehydrogenase; AST: azelaic transaminase and ALT: alanine aminotransferase.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The number of OTUs specific to groups A, B, C, and D was higher than that of group CK1, and the number of OTUs specific to groups C and D was higher than that of group CK2, indicating that more bacterial species were detected in groups A, B, C, and D. The number of OTUs specific to groups A, B, C, and D was higher than that of group CK2. According to Table 9, the relative abundance of the top 10 phyla in the rumen fluid of ewes was higher in group D than that in groups CK1 and CK2 (P<0.05), and in group C the abundance of the phylum Actinomycetes was significantly higher than that in groups CK1 and CK2 (P<0.05). According to Table 10, the top 10 species in terms of relative abundance of rumen fluid genus levels in ewes were *Prevotella*, Succiniclasticum, *Rikenellaceae*_RC9_gut_group, uncultured_rumen_bacterium, unclassified_ *Selenomonadaceae*, NK4A214_group *Selenomonas*, *Desulfovibrio*, Butyrivibrio, Veillonellaceae_UCG_001, others the relative abundance of gram *Prevotella* in group A was significantly higher than that in groups CK1 and CK2 (P<0.05); the relative abundance of Selenomonas in groups A, B, and D was significantly higher than that in groups CK1 and CK2 (P<0.05).

Table 8 Effects of different proportions of barley seedlings on alpha diversity (%) in rumen fluid of ewes (N=8)

Items	Groups						SEM	P-value
	CK1	CK2	A	B	C	D		
Feature	1356.33	1278.25	1177.75	1128.00	1155.80	1117.75	38.903	0.545
ACE	1371.74	1295.04	1190.37	1137.83	1164.35	1126.81	39.588	0.524
Chao1	1362.54	1286.15	1182.77	1130.75	1157.94	1121.14	39.235	0.530
Simpson	0.99	0.99	0.99	0.99	0.98	0.99	0.002	0.296
Shannon	8.81	8.74	8.37	8.46	7.98	8.16	0.118	0.282
PD whole tree	91.95	89.95	88.08	90.73	98.06	86.20	2.097	0.648
Goods coverage	1.00^{b}	1.00^{b}	1.00^{ab}	1.00^{ab}	1.00^{a}	1.00^{a}	0.000	0.089

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

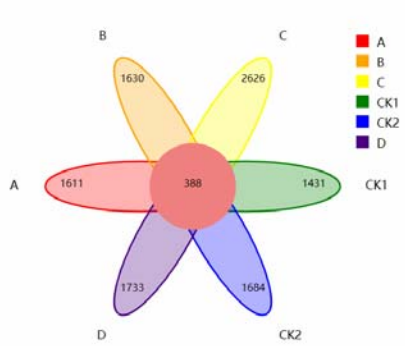

Figure 1 OUT-based Venn diagram analysis

According to Table 11, the top 10 families of rumen fluid bacteriophage levels in ewes were *Prevotellaceae*, *Lachnospiraceae*, *Selenomonadaceae*, *Acidaminococcaceae*, *Rikenellaceae*, *Oscillospiracea*, F082, *Succinivibrionaceae*, *Desulfovibrionaceae*, *Ruminococcaceae*, *Ruminovibrionaceae*, *Ruminococcaceae*, There was no significant effect of different barley seedlings ratios on the relative abundance of species at the level of ewe rumen fluid family (P>0.05).

According to Table 12, the top 10 species in the relative abundance of ewe rumen fluid population species level were uncultured rumenbacterium, unclassified *Prevotella*, unclassified *Selenomonadaceae*, unclassified Selenomonas, unclassified F082, unclassified NK4A214_group, unclassified Butyrivibrio, unclassified *Rikenellaceae* RC9 gut group, unclassified Lachnospiraceae, unclassified *Prevotellaceae*, others unclassified *Prevotella* species level relative abundance was significantly higher in group A than in groups CK1 and CK2 (P<0.05); group B the relative abundance of unclassified *Selenomonas* species level was significantly higher than that of groups CK1 and CK2 (P<0.05).

LEfSe analysis was able to find statistically different species between groups, as shown in Figures 1, there were seven species with significant differences between groups, two species with significant differences in group CK1 were uncultured rumen bacterium, *Succinivibrionaceae*_UCG_002; Group A had significant species were *Prevotella*; two species with significance in group B were unclassified *Selenomonas*, *Selenomonas*; species with significance in group C were *Acidaminococcaceae*; And species with significance in group D were Firmicutes.

Hydroponically grown barley seedlings are a very palatable and easily digestible source of high-quality green forage that can improve diet palatability and ruminant growth performance (Saidi and Omar, 2015; Majid *et al*. 2016). In lambs diets, replacing 50% of the mixed concentrate with hydroponically grown barley seedlings can improve the growth performance of lambs, and replacing 30% of barley seeds can well improve certain rumen traits as well as the digestibility and feed conversion of most nutrients in lambs (Devendar *et al*. 2020).

In calf diets, the inclusion of 21.08% hydroponic barley seedlings (fresh weight) increased the average daily weight gain (Bari *et al*. 2021). Gebrenedhin (2015) reported that feeding 60% cereal grass + 20% hydroponically grown maize + 20% hydroponically grown barley seedlings diet to Konkan Kanyar goats during the fattening period increased their dry matter intake, feed conversion ratio, and total weight gain.

In this experiment, the average daily weight gain of ewes in the alternative group reached to highest in group C as the substitutive percentage of hydroponic barley seedlings reached to highest during the formal experiment. the lowest average daily weight gain was observed in group B, it may be due to the lower dry matter intake of ewes in this group, the nutritional level of the diet did not meet their needs resulting in a significantly lower daily weight gain in this group than in the other groups.

Table 9 Effects of different proportions of barley seedlings on the relative abundance of species at phylum level (%) in rumen fluid of ewes (n=8)

Items	Groups						SEM	P-value
	CK1	CK2	A	B	C	D		
Firmicutes	52.48^{bc}	58.81^{ab}	48.92^{bc}	55.34^{bc}	55.51^{bc}	64.56^{a}	1.420	0.017
Bacteroidota	32.92	28.32	36.81	31.46	29.37	25.40	1.387	0.244
Proteobacteria	7.12	4.42	7.05	3.23	5.44	2.56	0.688	0.312
Desulfobacterota	2.88	3.46	2.58	5.82	3.53	3.07	0.362	0.114
Patescibacteria	2.68	2.32	1.37	1.25	0.93	1.62	0.190	0.054
Actinobacteriota	0.19^{b}	0.37^{b}	0.84^{ab}	0.57^{b}	2.41^{a}	1.26^{ab}	0.246	0.049
Cyanobacteria	0.67	0.96	0.94	0.95	0.85	0.58	0.071	0.568
Verrucomicrobia	0.42	0.59	0.52	0.48	0.48	0.44	0.047	0.949
unidentified_*Bacteria*	0.16	0.33	0.31	0.31	0.33	0.05	0.039	0.182
Fibrobacterota	0.26	0.08	0.25	0.37	0.27	0.20	0.038	0.429
Others	0.22	0.33	0.40	0.23	0.89	0.26	0.094	0.213

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 10 Effects of different proportions of barley seedlings on the relative abundance of genus-level species (%) in rumen fluid of ewes (n=8)

Items	Groups						SEM	P-value
	CK1	CK2	A	B	C	D		
Prevotella	11.88^{b}	10.09^{b}	17.94^{a}	11.89^{b}	7.80^{b}	10.09^{b}	0.950	0.018
Succiniclasticum	9.72	9.43	8.84	11.14	8.38	12.15	0.761	0.724
Rikenellaceae_RC9_gut_group	7.78	6.40	6.88	5.99	6.02	4.40	0.484	0.580
Uncultured rumen bacterium	7.14	5.10	5.41	6.63	3.10	5.46	0.459	0.125
Unclassified *Selenomonadaceae*	4.22	3.91	3.89	1.10	8.61	7.41	1.721	0.848
NK4A214_group	3.37	4.90	3.80	5.50	3.00	4.64	0.326	0.166
Selenomonas	1.04^{b}	1.42^{b}	3.96^{ab}	6.30^{a}	3.58^{ab}	4.88^{a}	0.527	0.023
Desulfovibrio	2.39	3.06	2.23	5.46	3.27	2.48	0.353	0.067
Butyrivibrio	2.69	4.19	2.90	2.85	1.97	2.61	0.285	0.340
Veillonellaceae_UCG_001	4.64	4.04	2.34	1.80	2.29	2.46	0.406	0.344
Others	45.12	47.47	41.82	41.34	51.98	43.44	1.304	0.087

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 11 Effects of different proportions of barley seedlings on the relative abundance of species (%) at the family level in the rumen fluid microbiota of ewes (n=8)

Items	Groups						SEM	P-value
	CK1	CK2	A	B	C	D		
Prevotellaceae	16.50	14.04	22.47	16.36	16.18	14.42	1.117	0.301
Lachnospiraceae	13.26	15.87	11.97	13.51	13.74	14.66	0.782	0.835
Selenomonadaceae	11.76	10.46	12.20	11.94	15.89	16.64	1.810	0.919
Acidaminococcaceae	9.73	9.43	8.84	11.14	8.39	12.15	0.761	0.727
Rikenellaceae	7.93	6.57	7.06	6.10	6.12	4.50	0.491	0.573
Oscillospiraceae	4.54	5.96	5.01	6.53	3.53	5.57	0.375	0.177
F082	4.79	4.37	3.38	2.89	4.26	2.45	0.455	0.365
Succinivibrionaceae	5.50	3.80	5.68	2.10	2.98	1.85	0.618	0.070
Desulfovibrionaceae	2.45	3.15	2.26	5.49	3.30	2.50	0.353	0.070
Ruminococcaceae	3.53	3.53	2.61	2.65	2.47	3.75	0.199	0.212
Others	20.02	22.82	18.49	21.29	23.12	21.51	0.827	0.622

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 12 Effects of different proportions of barley seedlings on the relative abundance of species (%) at the family level in the rumen fluid microbiota of ewes (n=8)

Items	Groups						SEM	P-value
	CK1	CK2	A	B	C	D		
Uncultured rumen bacterium	39.64	36.18	33.34	37.15	29.64	34.53	1.151	0.168
Unclassified *Prevotella*	6.10^{b}	5.34^{b}	11.24^{a}	6.79^{ab}	4.19^{b}	5.25^{b}	7.259	0.045
Unclassified *Selenomonadaceae*	3.51	3.00	3.64	0.94	8.55	7.19	1.710	0.814
Unclassified *Selenomonas*	0.97^{b}	1.32^{b}	3.77^{ab}	5.88^{a}	3.41^{ab}	4.84^{a}	0.506	0.026
Unclassified F082	2.86	3.05	2.43	2.56	3.60	2.21	0.421	0.949
Unclassified NK4A214 group	2.11	2.79	2.53	4.07	1.92	3.36	0.255	0.107
Unclassified *Butyrivibrio*	2.63	4.08	2.83	2.83	1.90	2.51	0.281	0.339
unclassified_*Rikenellaceae*_RC9_gut_group	3.34	2.93	2.72	2.45	1.86	1.52	0.254	0.354
Unclassified *Lachnospiraceae*	2.28	2.00	2.37	2.70	1.45	2.30	0.219	0.665
Unclassified *Prevotellaceae*	1.71	1.40	2.00	1.51	3.63	1.46	0.518	0.794
Others	34.84	37.90	33.14	33.11	39.84	34.81	1.268	0.557

CK1: control +; CK2: control -; A: hydroponic barley seedlings 5%; B: hydroponic barley seedlings 10%; C: hydroponic barley seedlings 15% and D: hydroponic barley seedlings 20%.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

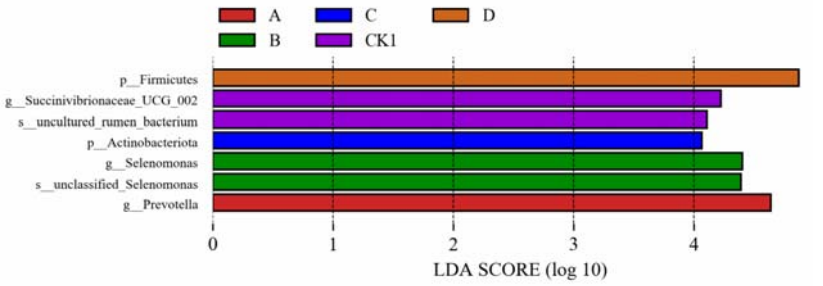

Figure 2 The Venn diagram for the number of OTUs shared among the six groups and the number of OTUs unique to each group

There is a complex network of antioxidant enzymes in mammalian cells, as well as non-enzymatic antioxidants that effectively scavenge reactive oxygen species. The degree of antioxidants in a cell determines its sensitivity to oxidative damage and varies with stress (Bhor *et al*. 2004). The phenolic content of hydroponically grown barley seedlings, a green fodder, varies considerably at different growth periods, and their polyphenol content and antioxidant enzyme activities are significantly higher than those of ungerminated barley seeds, which are closely related to antioxidant capacity (Peer and Leeson, 1985). In this experiment, hydroponically grown barley seedlings substituting for different ratios of basal diets increased the serum SOD and GSH-px enzyme contents and decreased the serum MDA content in lactating ewes. At the same time, IL-1β, IL-2β, IFN-γ, and CD4 levels increased between groups as the amount of substitutive hydroponic barley seedlings increased. The experiment showed that the polyphenol content of hydroponic barley seedlings indirectly increased the content of related antioxidant enzymes in the ewes' organism, thus improving the antioxidant and immune capacity of the organism.

The blood biochemical level reflects protein, amino acid, sugar, and lipid nutrition and organ metabolism of the organism of the animal, and blood biochemical indexes are affected by the healthy condition of the organism firstly, and the nutrition status of the organism and the nutrition level of the diet secondly. Total plasma protein (TP) and urea nitrogen (BUN) levels reflect the body's ability to digest and absorb protein in diet and nitrogen deposition, as well as the body's protein metabolism and the balance between amino acids (Zhu *et al*. 2013; Kisadere *et al*. 2019). The TP and BUN contents did not differ significantly between the groups as the amount of substitutive hydroponic barley seedlings increased (Stanley *et al*. 2002). In this experiment, using hydroponically grown barley seedlings to replace proportionally the basal diet increased the serum TP content of lactating ewes, but had no significant effect on

the serum BUN content. This indicates that the hydroponic barley seedlings designed in this experiment can meet the basic protein and amino acid demand of lactating ewes by substituting different ratios of basal diets. Plasma glucose is an important indicator of glucose absorption, transport, and basal metabolic rate of the body. Serum cholesterol level reflects the lipid metabolism of the body, which can be divided into LDL cholesterol and HDL cholesterol. High levels of LDL cholesterol can cause narrowing or blockage of blood vessels, leading to atherosclerosis. High-density lipoprotein cholesterol, in contrast to low-density lipoprotein cholesterol, resists atherosclerosis and protects the blood vessels (Palinski and Napoli, 2002). In this experiment, with an increased amount of hydroponic barley seedling substitution, the differences in TC, HDL-c, and LDL-c contents between the groups were not significant, but the TG content decreased, which may be caused by the important regulatory role of folic acid protein and lipid and other metabolism. The serum levels of CK, LDH, AST, and ALT in the lactating ewes were not significantly different and were within the normal range, indicating that the practice was meant to substitute different proportions of basal diets with hydroponic barley seedlings without any effect on their liver metabolism,specific mechanisms require further in-depth study.

Alpha diversity analysis can mainly determine the abundance of sample microorganisms and microbial diversity, as well as information on the coverage of sample microorganisms under this sequencing condition. In this study, the OTUs coverage of samples in all groups was above 99%, indicating that the number of sequenced rumen fluid samples in each group was reasonably selected and the data acted accurately upon the composition of rumen microflora. In this study, the number of OTUs specific to group C was found to be higher than the number of OUTs specific to other groups in the Venn diagram analysis based on OTU levels, indicating that the diversity level of rumen microorganisms in group C was higher than that in other groups, indicating that the addition of different levels of hydroponic barley seedlings to the diet could increase the diversity of rumen microorganisms.

Gut microbes are an important component of the digestive system in the gut, and the structure and type of ration are one of the key factors affecting the intestinal microecological balance (Respondek *et al.* 2018). The thick-walled phylum mainly plays a role in the decomposition of proteins and other substances, and the relative abundance of the thick-walled phylum was significantly higher in group D especially in this study than in other groups, and Huang *et al.* (2019) found that hydroponically grown barley grass contains proteins that are very easily utilized by the rumen fermentation of ruminants because of its low lignin content, which was reflected in the results of total protein content in the blood of groups C and D in this study, thus leading to the increase in the relative abundance of the thick-walled phylum. In the present study, the relative abundance of Actinomycetes in group C was significantly higher than that in the control group, and Peng *et al.* (2021) found that different substitution ratios of hydroponically grown barley grass significantly increased the total protein content in rumen fluid. In current study the significantly increased total volatile fatty acid (VFA), acetic acid and propionic acid in rumen fluid, indicated that the addition of hydroponically grown barley seedlings could improve the fermentation efficiency of carbohydrates by rumen microorganisms.

CONCLUSION

Hydroponic barley seedlings can be used as supplementary green feed, which can not only replace part of the basic diet but also reduce the breeding cost. Most time it can also improve the immune performance of female animals and alleviate oxidative stress. It can be concluded that in the ewes' diet 10%-15% inclusion of hydroponic barley seedlings is helpful to improve the overall performances of the animal.

ACKNOWLEDGEMENT

The authors thank all the teams who worked on the experiments and provided results during this study.

REFERENCES

Badran E., Qaisy J.L., Amsha R.A., Qaisy R., Amsha M., Aub R., Qadri M. and Jammal A.L.A. (2017). Milk yield and quality and performance of Awassi ewes fed two levels of hydroponic barley. *J. New Sci. Agric. Biotechnol*. **39(6),** 2136-2143.

Bari S., Abbassi M., Abloudlou L., Nadir M., El M., Mannas H., Assbbane A., Ait I.I. and Kaaya A. (2021). Study of the hydroponic barley effect on the performance of feedlot of calves in the region Souss Massa (Southern Morocco). *J. Biol. Agric. Healthcare*. **11(14),** 30-37.

Bhor V.M., Raghuram N. and Sivakami S. (2004). Oxidative damage and altered antioxidant enzyme activities in the small intestine of streptozotocin-induced diabetic rats. *Int. J. Biochem. Cell. Biol*. **36(1),** 89-97.

Devendar R., Kumari N.N., Reddy Y.R., Rao K.S., Reddy K.K., Raju J. and Sridhar K. (2020). Growth performance, nutrient utilization and carcass characteristics of sheep fed hydroponic barley fodder. *Anim. Nutr. Feed Technol*. **(2),** 20-28.

Gebrenedhin W.K. (2015). Nutritional benefit and economic value of feeding hydroponically grown maize and barley fodder for Konkan Kanyal goats. *J. Agric. Vet. Sci*. **8(7),** 24-30.

Huang W.L.,Wang J.P. and Liu N. (2019). Nutritional value and CNCPS components of barley seedlings under different hydroponics. *China Pra Sci.* **36(7),** 1811-1818.

Kisadere I., Bayraktar M. and Salykov R. (2019). Some hematological and biochemical reference values of the thoroughbred Appaloosa horse breeds reared in Kyrgyzstan. *Comp. Clin. Pathol*. **28(6),** 1651-1660.

Majid A., Saadi I., Ibrahim A. and Al-Zubiadi H. (2016). Effects of substitution barley by 10%, 30% of sprouted barley on rumen characters, digestibility and feed efficiency in diet of Awassi male lambs. *Int. J. Sci. Res*. **5,** 2228-2233.

Morales M.A., Fuente B., Juárez M. and Avila E. (2009). Short communication: Effect of substituting hydroponic green barley forage for a commercial feed on performance of growing rabbits. *World Rabbit Sci*. **17(1),** 35-38.

NRC. (2007). Nutrient Requirements of Small Ruminants, Sheep, Goats, Cervids, and New World Camelids. National Academy Press, Washington, D.C., USA.

Palinski W. and Napoli C. (2002). The fetal origins of atherosclerosis: Maternal hypercholesterolemia, and cholesterol-lowering or antioxidant treatment during pregnancy influence in utero programming and postnatal susceptibility to atherogenesis. *FASEB J.* **16(11),** 1348-1360.

Peng R., Juan F. and Ruocheng L. (2021). Effects of replacing alfalfa and oat with hydroponic barley seedlings on rumen fermentation characteristics of dairy cows by *in vitro* gas production method. *Chinese J. Anim. Sci.* **58(9),** 233-237.

Raeisi Z., Tahmasbi R., Dayani O. and Mehrgardi A.A. (2018). Digestibility, microbial protein synthesis, rumen and blood parameters in sheep fed diets containing hydroponic barley fodder. *J. Livest. Sci. Technol*. **6(1),** 9-17.

Respondek F., Goachet A.G. and Julliand V. (2018). Effects of dietary short-chain fructooligosaccharides on the intestinal microflora of horses subjected to a sudden change in diet. *J. Anim. Sci.* **86(2),** 316-323.

Saidi A. and Omar J.A. (2015). The biological and economical feasibility of feeding barley green fodder to lactating Awassi ewes. *J. Anim. Sci*. **5(2),** 99-105.

SPSS Inc. (2011). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc., USA.

Stanley C.C., Williams C.C., Jenny B.F., Fernandez J.M., Nipper W.A., Lovejoy J.C., Gantt DT. and Goodier G.E. (2002). Effects of feeding milk replacer once versus twice daily on glucose metabolism in Holstein and Jersey calves. *J. Dairy Sci*. **85(9),** 2335-2343.

Zhu L.L., Zhao C.X., Su H.X., Xu K., Shi D.Y. and Jiang G.J. (2013). Study on the relationship between phenols and antioxidant capacity in barley and malt. *Chinese Food Sci. Technol.* **38(8),** 5-17.