

Article

Not peer-reviewed version

Sustainable Feeding Strategies: The Role of Corn and Barley in Alfalfa-Based Sheep Ration

[Krešimir Bošnjak](#) and [Marina Vranić](#) *

Posted Date: 2 July 2025

doi: 10.20944/preprints202507.0096.v1

Keywords: alfalfa haylage; corn; barley; intake; digestibility; N retention; sustainable



Preprints.org is a free multidisciplinary platform providing preprint service that is dedicated to making early versions of research outputs permanently available and citable. Preprints posted at Preprints.org appear in Web of Science, Crossref, Google Scholar, Scilit, Europe PMC.

Copyright: This open access article is published under a Creative Commons CC BY 4.0 license, which permit the free download, distribution, and reuse, provided that the author and preprint are cited in any reuse.

Disclaimer/Publisher's Note: The statements, opinions, and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions, or products referred to in the content.

Article

Sustainable Feeding Strategies: The Role of Corn and Barley in Alfalfa-Based Sheep Ration

Krešimir Bošnjak and Marina Vranić *

University of Zagreb Faculty of Agriculture, Department of Field Crops, Forage and Grassland, Svetošimunska cesta 25, 10000 Zagreb, Croatia

* Correspondence: mvranic@agr.hr; Tel.: 00385 1 2393 999

Abstract

Forages like alfalfa haylage (AH), which are high in crude protein (CP), need energy-rich supplements to optimize nutrient intake, improve digestibility, and enhance nitrogen use efficiency for better sustainability in livestock production. The objective of the study was to find whether corn or barley is better supplement to AH to achieve these goals. A feeding trial was conducted as an change over design with four treatments, four periods and four animals in each period. The concentrate was fed at a rate of 30 g kg⁻¹ M^{0.75} wether sheep d⁻¹ and consisted of: (i) 100% corn (CG) (ii) 67% corn and 33% barley (CG67), (iii) 33% corn and 67% barley (BG67), (iv) 100% barley (BG). CG67 had lower dry matter intake (DMI) (P<0.001) but higher dry matter (DM) digestibility (P<0.01). BG had the highest intake of neutral detergent fibre (NDF) (P<0.01), crude protein (CP) (P<0.001) and CP digestibility (P<0.001). BG and BG67 had a higher N balance than CG and CG67 (P<0.001). The results suggest that the quality of the AH and the moderate amount of concentrate used in this study make the addition of barley to AH-based diet more beneficial in enhancing sustainability in sheep nutrition than the addition of corn.

Keywords: alfalfa haylage; corn; barley; intake; digestibility; N retention; sustainable

1. Introduction

Sustainable feeding systems aim to enhance nutrient intake, improve nitrogen balance, and reduce overall emissions. Forages high in crude protein (CP), such as alfalfa haylage (AH), require energy-rich supplement feedstuffs to optimize nutrient intake, improve ration digestibility, and maximize nitrogen use efficiency as key components of sustainable livestock production [1]. A more synchronised release of energy and nitrogen promotes the synthesis of microbial proteins and fatty acids in the rumen [2,3]. This, in turn, enhances microbial nutrient assimilation [4], which subsequently increases ration intake, digestibility and N utilisation [5–8].

Both, dried corn grains (corn) and barley grains (barley) serve as effective energy supplements for ruminants. Compared to barley, corn contains at least 15% more starch (5–10 % more energy), but has about 3% less CP. When ground or dry-rolled, corn can be a better source of energy than barley in forage-based diets [9–11]. In contrast, the feeding value of barley remains constant regardless of processing [12]. The reason for this is that the starch in the vitreous endosperm region of corn is surrounded by zein, which is resistant to microbial invasion and is only 55 to 70% digested in the rumen [13]. Barley, however, has highly digestible protein and easily accessible starch, resulting in a faster fermentation rate, with starch digestibility in the rumen often exceeding 90% [14] and can even reach 98% [15]. As a result, the overall digestibility of corn is lower than that of barley, potentially reducing microbial protein synthesis when corn is added to protein-rich forages. Conversely, completely replacing corn with barley can negatively affect the growth performance of lambs [16,17] while no significant differences in dry matter intake (DMI) have been noted in beef cattle fed high-concentrate corn or barley diets [18].

Some research suggests that partially replacing corn with barley in cereal mixtures with different fermentation rates [4,19] can improve growth performance in lambs [17], heifers [20] and milk production in dairy cows [21,22].

However, intake and digestibility results vary due to differences in the supplementation rate, animal type, and forage quality [11,23]. Given that AH proteins are highly soluble in the rumen, readily available energy sources like barley may enhance microbial protein synthesis and nitrogen (N) utilisation as key factors in reducing nutrient losses and promoting nutritional sustainability.

This study hypothesised that barley, compared to corn, is a more effective supplement to AH in improving feed intake, digestibility, and N retention in wether sheep. The objective was to evaluate how partial or complete replacement of corn with barley in an AH-based diet affects intake, *in vivo* digestibility, N retention, and water intake, contributing to strategies for improved animal performance and sustainable feed use.

2. Materials and Methods

2.1. Alfalfa Haylage

The first cut of alfalfa was harvested at the beginning of flowering (30-40% of the crop in full bloom). The crop was allowed to wilt for at least 24 hours to a dry matter (DM) content of about 500-600 g kg⁻¹ fresh weight. The wilted crop was baled without additives using a John Deere round baler (bale diameter 1.2 m). The bales were wrapped with 4-6 layers of white plastic film using a Pöttinger bale wrapping machine. The plastic-wrapped bales were transported to the Grassland Research Centre of the University of Zagreb, where they were stored for at least 60 days before being used. A total of 6 bales of alfalfa haylage (AH) were selected for this study. Before the start of each trial period, the AH was chopped to a chop length of 3-5 cm using a commercial chopper and compressed by hand into plastic bags (each with a capacity of 20 litres). The bags were sealed with plastic film and stored in a cold chamber at a temperature of 4 °C and weighed in a plastic bag on the day of feeding.

2.2. Concentrate

Corn grains (corn) and barley grains (barley) were produced using a standard technology, dried at approximately 40 °C to 87-87% DM and stored separately in two 50-litre plastic containers at room temperature until needed for the experimental purposes. The concentrates (corn, barley and their mixtures) for daily feeding were weighed separately in plastic bags and fed separately from AH to ensure that no refusals were left behind.

The animals were fed twice daily, approximately half of the feed at 9 am and the other half at 4 pm. Apart from the AH and the concentrate, no other feed was given. The animals were given fresh and clean water *ad libitum* throughout the experiment.

2.3. Dietary Treatments

The concentrate was fed to the animals at a rate of 30 g kg⁻¹ M^{0.75} wether sheep d⁻¹, ranging from approximately 555.6 g to 635.10 g day⁻¹. The trial consisted of four feeding treatments in which AH was supplemented with: (i) 100% corn (treatment CG), (ii) 67% corn and 33% barley (treatment CG67), (iii) 33% corn and 67% barley (treatment BG67), (iv) 100% barley (treatment BG). The four treatments were randomly assigned to one of the four sheep in each period.

2.4. Animals

Suffolk wether sheep were used for this study. They were about 2 years old and had a body weight (BW) of 49-56 kg (mean 54 kg, s.d. 5.0 kg). Before the experiment, they were treated against parasites, examined for health and the fleece around the tail was removed. They received daily light from 8 am to 8 pm.

The BW of the animals was determined twice during an experimental period using a Trutest balance. This was done before the period of adaptation to the feeding treatment and before the period

of determination of *ad libitum* intake, in vivo digestibility and nitrogen balance. The amount of concentrate was adjusted individually for each animal taking BW into account.

2.5. Adaptation to the Feeding Treatment

The animals were kept in individual pens (1.5 × 2.2 m) during the 12-day adaptation to the feeding treatment. They were only allowed to move to a limited extent and lie down. Sawdust was used as bedding. The animals had individual feeders and drinkers, which were cleaned daily. The feeders were designed to ensure separation between the voluminous part and the concentrated feed.

2.6. Determination of Ad Libitum Intake, In Vivo Digestibility and Nitrogen Balance

After the animals were adapted to the feeding treatments, they were given faecal bags and housed in individual cages (1.36 m × 1.53 m × 1.49 m). The cages are equipped with individual feeders and drinkers, which were cleaned daily. The floor of the cage is made of perforated hard plastic with openings to prevent the animals from injuring their paws. Under the floor is an inclined metal surface through which the excreted urine flows into a plastic container for collection (40 cm × 20 cm × 30 cm).

During the measurement period, the animals were given AH *ad libitum* for the first 4 days. Thereafter, for the next 7 days, they were offered AH amounting to 10-15% of the AH remaining on the previous day. The exact amount of water intake, AH residues, excreted faeces and urine were determined every morning before the animals were fed.

2.7. Samples for Chemical Analysis

The researchers collected corn and barley samples before each study period (approximately 1.5 kg each) and stored at room temperature until chemical analysis. We collected AH samples daily, approximately 100 g per day, while weighing the relative daily feed amount, and stored them in plastic bags.

We also took samples of AH residues daily before the morning feeding. Additionally, we collected fecal and urine samples daily, gathering 10% of the excreted feces and urine. To preserve the daily excreted urine, we added 100 ml of 2 mol/l sulfuric acid to the urine collection container to achieve a pH of 2-3.

We stored all samples of fed AH, AH residues, feces, and urine at 4 °C until the end of each experimental period. Afterward, we transferred them to -20 °C storage before conducting chemical analysis.

2.8. Determination of In Vivo Digestibility and N-Balance

The digestibility of DM, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and starch was calculated by subtracting the amount of fecal excretion from the amount of feed ingested and dividing by the amount ingested [24].

Nitrogen balance was calculated by subtracting the amount of nitrogen excreted in feces and urine from the amount of nitrogen ingested. The water balance was calculated by subtracting the amount of water excreted in urine and feces from the total water intake (AH, concentrate and water).

The experiment was conducted in accordance with the Ethical Guidelines for the Use of Animals in Research [25] and the Council Directive of the European Economic Community (EEC) (1986). The study was approved by the Ethics Committee for Scientific Research of the University of Zagreb Faculty of Agriculture, Croatia (Ethical reference number: 380-71-01-06-1).

2.9. Chemical Analyzes

The DM content was determined by drying the samples for 48 hours in a fan dryer (ELE International) at a temperature of 60 °C to a constant sample weight. The organic matter (OM) content was determined by burning the samples in a microwave oven (Milestone PIYRO, Italy) at a temperature of 550 °C for 3 hours.

Nitrogen content was determined using the Kjeldahl method [26] and CP content was determined by multiplying the N content by a factor of 6.25. The NDF and ADF content was determined using the Ankom filter bag technique [27]. The pH value was determined using the pH meter 315i (WTW). The content of volatile fatty acids was determined using a gas chromatograph and a liquid-liquid extraction with two different solvents (dichloromethane and methyl tert-butyl ether) [28]. The lactic acid content was determined enzymatically using the Express Auto biochemical analyzer. The starch content in the samples was determined using the enzymatic method [29].

2.10. Statistical Analyses

Descriptive statistics for means of four measurements and standard deviation were calculated for the parameters investigated. The experiment was statistically analysed as an change over design with 4 treatments, 4 animals and 4 time periods, using models in SAS software [30]. Model applied: $Y_i = \mu + T_i + e_i$ where Y is the overall model, μ = grand mean, T = treatment, P = time period, e = experimental error and i = number of treatments. The experimental data are presented as the mean across the different treatments together with the standard error of the mean (SEM). Effects were determined to be significant at $P < 0.05$.

3. Results

The chemical composition of the alfalfa haylage (AH), corn grain (corn) and barley grain (barley) used in the study is presented in Table 1.

Table 1. Chemical composition of alfalfa haylage, corn grains and barley grains (g kg⁻¹ DM, unless otherwise stated).

Chemical parameter	AH		Corn		Barley	
	Mean value	SD	Mean value	SD	Mean value	SD
DM	624.6	21.1	916.8	7.8	908.1	6.6
OM	923.2	9.1	946.8	20.1	952.6	22.4
CP	166.2	3.0	88.7	0.7	100.5	6.0
starch	23.2	5.2	688.3	7.64	536.5	14.7
NDF	496.0	22.4	215.8	46.0	205.7	39.4
ADF	370.2	32.7	28.6	1.1	90.3	8.6
Butyric acid	3.35	0.72	ND		ND	
Lactic acid	23.4	3.72	ND		ND	
Acetic acid	3.7	1.58	ND		ND	
NH ₃ -N (g kg ⁻¹ total N)	20.37	4.0	ND		ND	

pH	5.49	0.12	ND	ND
----	------	------	----	----

AH, alfalfa haylage; Corn, corn grain; Barley, barley grain; SD, standard deviation; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; ND, not determined.

The AH (Table 1) had an acceptable crude protein (CP) content for high protein forage and a high neutral detergent fibre (NDF) and acid detergent fibre (ADF) content. The parameters of AH fermentation quality in the silo were consistent with the high DM content. Lactic acid was the main organic acid in the AH, while the ammonia content was low.

The high-energy feeds used in the study, corn and barley, had a high DM content and a high starch content. The starch content was higher, while the CP content was lower in corn than in barley grain (Table 1).

Table 2 presents the effect of partial or complete replacement of corn by barley on the daily voluntary feed intake and digestibility of the ration based on AH fed to wether sheep.

Table 2. The effect of partial or complete replacement of corn by barley on the daily voluntary feed intake and digestibility of the ration based on alfalfa haylage fed to wether sheep.

Item	CG	CG67	BG67	BG	SEM	p-value
Intake of total g d ⁻¹						
Full diet DM	1438 ^b	1270 ^c	1453 ^{ab}	1583 ^a	51.06	<.0001
DM haylage	913 ^b	755 ^c	926 ^b	1045 ^a	50.52	<.0001
CP	181.7 ^b	164.3 ^c	193.5 ^b	222.7 ^a	8.29	<.0001
NDF	341 ^{ab}	294 ^b	385 ^{ab}	417 ^a	26.81	0.0001
ADF	146.3 ^c	134.9 ^c	210.8 ^b	276.1 ^a	22.04	<.0001
Starch	383.2 ^a	338.1 ^b	340.8 ^b	312.5 ^c	5.52	<.0001
Intake of g kg ⁻¹ M ^{0.75}						
Full diet DM	74.4 ^a	66.9 ^b	74.1 ^a	79.4 ^a	2.82	<.0001
DM haylage	47.2 ^a	39.9 ^b	47.2 ^a	52.5 ^a	2.79	0.0004
CP	9.4 ^{bc}	8.7 ^c	9.9 ^b	11.2 ^a	0.48	<.0001
NDF	17.6 ^{bc}	15.5 ^c	19.6 ^{ab}	20.9 ^a	1.36	0.0010
ADF	7.5 ^c	7.1 ^c	10.6 ^b	13.7 ^a	1.05	<.0001
Starch	19.8 ^a	17.8 ^b	17.4 ^b	15.6 ^c	0.23	<.0001
Digestibility (g kg ⁻¹ DM)						
DM	643 ^b	730 ^a	684 ^{ab}	670 ^b	24.36	0.0058
CP	750 ^b	696 ^b	763 ^b	828 ^a	20.96	<.0001
NDF	532 ^{ab}	459 ^b	522 ^b	615 ^a	41.94	<.0001
ADF	457 ^{ab}	417 ^b	425 ^b	549 ^a	49.16	<.0001
Starch	926 ^a	943 ^a	845 ^b	923 ^a	15.24	<.0001

CG, corn only; CG67, corn grain 67%, barley grain 33%; BG67, barley grain 67%, corn grain 33%; BG, barley supplement; DM, dry matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; SEM, standard error of the mean; numbers in the same row marked with the same letters does not differ significantly ($P>0.05$).

DM intake (DMI) (g d⁻¹ and g kg⁻¹ M^{0.75}) of CG67 was lower ($P<0.001$) than other feeding treatments that achieved the same level of DMI. However, DM digestibility was higher ($P<0.001$) in CG67 than in the CG and BG treatments, while it remained the same in the BG67 treatment. Voluntary intake and digestibility of CP was higher ($P<0.001$) in BG than in the other feed treatments. Intake of dietary fibre (NDF and ADF) was higher in BG and BG67 than in CG and CG67. Digestibility of dietary fibre was higher in BG than in CG67 and BG67.

Starch intake decreased as the proportion of CG in the concentrate decreased and was lowest in BG ($P<0.001$). Treatment BG67 had the lowest starch digestibility ($P<0.001$) compared with the other

feeding treatments, between which there were no statistically significant differences in starch digestibility ($P>0.05$).

Table 3 shows the effect of partial or complete replacement of corn by barley on the nitrogen and water balance of the ration based on AH fed to wether sheep.

Table 3. The effect of partial or complete replacement of corn grains by barley grains on the nitrogen balance (g d^{-1}) and water balance (ml d^{-1}) of the ration based on alfalfa haylage fed to wether sheep.

Item	CG	CG67	BG67	BG	SEM	p-value
N intake haylage	29.07 ^b	26.29 ^c	30.95 ^b	35.63 ^a	1.32	<.0001
N intake concentrate	13.01 ^c	14.69 ^a	14.12 ^b	14.73 ^a	0.18	<.0001
Total N intake	42.07 ^c	40.97 ^c	45.07 ^b	50.36 ^a	1.41	<.0001
Faecal N	7.16 ^a	7.75 ^a	7.37 ^a	6.14 ^b	0.48	0.0097
Urinary N	12.27 ^a	8.79 ^b	9.04 ^{ab}	12.44 ^a	1.66	0.0425
N balance	22.64 ^c	24.43 ^{bc}	28.66 ^{ab}	31.79 ^a	2.23	0.0004
Water intake by water	2748	2702	2883	3029	187.63	0.3051
Water intake by the ration	591.5 ^c	504.3 ^a	604.8 ^{bc}	666.6 ^b	32.98	<.0001
Total water intake	3339 ^{ab}	3206 ^b	3487 ^{ab}	3695 ^a	199.61	0.0942
Total water intake $\text{g kg}^{-1} \text{M}^{0.75}$	142.5	142.1	147.6	151.6	10.02	0.7470
Urinary water excreted	1017	1215	1297	1102	163.98	0.0606
Faecal water excreted	1151 ^a	765 ^b	798 ^b	1299 ^a	84.05	<.0001
Total water excreted	2169 ^a	1980 ^b	2095 ^{ab}	2402 ^{ab}	157.0	0.0406
Water balance	1170	1226	1393	1294	179.07	0.6368

CG, corn only; CG67, corn grain 67%, barley grain 33%; BG67, barley grain 67%, corn grain 33%; BG, barley supplement; N, nitrogen; SEM, standard error of the mean; numbers in the same row marked with the same letters does not differ significantly ($P>0.05$).

The BG treatment had higher N intake from AH ($P<0.001$), higher total N intake ($P<0.001$) and lower faecal N excretion ($P<0.01$) compared to the other treatments where there were no differences in the amount of N excreted via faeces. BG67 had a higher total N intake ($P<0.001$) compared to CG and CG67. This also resulted in a higher N balance in treatment BG, which was comparable to BG67. Treatments CG and CG67 treatments had the same N balance, while BG67 had a higher N balance compared to treatment CG ($P<0.001$).

Treatments BG and BG67 had a higher water intake via feed compared to CG67 ($P<0.001$). Total water intake (feed + water) was lower in CG67 ($P<0.05$) compared to other treatments, between which there were no statistically significant differences in total water intake ($P>0.05$). More water was excreted via faeces in BG and CG than in CG67 and BG67. No significant differences in water balance were observed between the feeding treatments.

4. Discussion

Feed Chemical Composition

The chemical composition of alfalfa haylage (AH) corresponds to a haylage with a high dry matter (DM) content and limited fermentation. The applied technology of ensiling in bales wrapped in plastic film requires a higher forage DM content (more than 40%) and longer chopping. These are prerequisites for the bales to retain their shape and be stored on top of each other without damaging the plastic film [31]. As a result, fewer acids are formed during fermentation and the final pH value of the ensiled forage is higher. Lactic acid was the main organic acid in the AH, while the ammonia content was below 50 g ammonia N kg^{-1} total N, indicating a good quality of the AH. The average crude protein (CP) content in the AH was lower than the value of 204.4 g kg^{-1} DM determined for high quality forage [32]. It was also lower than the value of 183 g kg^{-1} DM determined for alfalfa harvested at the fool bloom stage or the value of 252 g kg^{-1} DM at the early bloom maturity stage [33]. This indicates that AH was of medium quality, although the neutral detergent fibre (NDF) and acid

detergent fibre (ADF) values were consistent for alfalfa harvested at the early or late bloom maturity stage [33].

DM content of corn grain (corn) and barley grain (barley) is similar to previous reports, while starch content is slightly lower and CP is higher [11,34]. Corn generally contain more starch but lower CP concentrations compared to barley [9], which is confirmed by the present results (Table 1).

Feed Intake and Digestibility

Voluntary dry matter intake (DMI) in this study ranged between 50–80 g kg⁻¹ M^{0.75} d⁻¹, consistent with earlier studies [15], and exceeded values reported for 50 kg male lambs (800–1100 g d⁻¹; AFRC, 1993), likely due to the higher average body weight (54 ± 5.0 kg) of the animals in this experiment. DMI is directly influenced by body weight, production level, and diet quality in terms of palatability and digestibility. Adult sheep typically consume around 1.5% of body weight for maintenance, and up to 2–3% when fed ad libitum [35], which corresponds to the current results.

At high neutral detergent fibre (NDF) levels, rumen fill can limit intake, whereas at low NDF levels, energy intake becomes the limiting factor [36]. In this study, barley had lower NDF and higher ADF compared to corn. Neither partial (67%) nor complete replacement of corn with barley reduced DMI, which can otherwise occur when dietary NDF drops significantly [37]. Animals fed the CG, BG67 and BG diets had similar DMI and dry matter digestibility, supporting previous evidence that similar intake generally results in similar digestibility [38]. This is relevant in promoting nutritional efficiency and sustainability in feeding strategies.

While some researchers reported lower DMI in cows fed barley versus corn due to faster rumen fermentation and pH reduction [39], such effects were not observed in this study. Corn's slower fermentation supports a higher rumen pH and propionate production, potentially enhancing carcass quality [40].

However, DMI can also be affected by the level, not just type, of concentrate used. Previous studies used higher supplementation (1100 g d⁻¹ from 25 to 47 kg BW) than the moderate levels used here (500–700 g d⁻¹ for 54 kg BW) [41], which aligns with more sustainable and cost-effective feeding practices.

Treatment CG67 showed the lowest DMI but highest digestibility, indicating that nutrient absorption can improve with reduced intake. Similar findings in goats and cattle suggest improved fibre digestibility with smaller feed amounts or lower-quality forage, which slows passage through the gut [42,43]. However, higher intake often boosts total digestible intake even if nutrient digestibility drops slightly [44].

Barley contained less starch compared to corn, resulting in the lowest starch intake in the BG. While whole-tract starch digestibility remained unchanged with 67% corn replacement, full replacement caused a slight decline (92.28% vs. 90.50%) [16], likely due to rumen pH reduction and limited starch degradation in the gut [16,45]. Other studies confirm improved total tract starch digestibility with higher barley levels due to its rapid rumen degradation [46].

All diets provided sufficient crude protein (around 160 g CP kg⁻¹ DM) to support DMI and efficiency [47]. Nitrogen intake was influenced more by energy source than CP concentration alone. Sheep fed the barley-only supplement consumed more nitrogen and showed higher CP digestibility. Similar trends were observed in cattle, where barley-fed animals exhibited greater CP digestibility [48].

Although rapid barley fermentation can reduce fibre digestibility when pH drops [49] this study did not observe such effects—possibly due to moderate grain intake and the lower digestibility of corn components [20,50]. Barley's higher rumen degradability, better nitrogen utilisation, and potential to reduce health issues point to its role in supporting both animal productivity and sustainability. Lower veterinary costs and reduced reliance on high-input supplements also enhance farm profitability over time.

Nitrogen –Balance

Nitrogen intake was primarily driven by alfalfa hay content, though barley's higher CP contributed to greater overall nitrogen intake in BG. Despite differences in intake, nitrogen excretion was not significantly affected by treatment. BG showed lower faecal N excretion, and CG67 had lower urinary N excretion likely due to better energy-protein balance.

Corn-based treatments (CG, CG67, BG67) increased faecal N loss, consistent with studies showing enhanced intestinal fermentation from fermentable substrates in corn diets [20]. Postruminal starch fermentation can elevate hindgut microbial activity and nitrogen loss [51,52]. These findings support the view that improved nitrogen efficiency with barley can help reduce environmental nitrogen waste, contributing to agricultural sustainability.

Water- Balance

Ad libitum water intake showed that 15–18% of total water came from the feed, aligning with previous findings [53], which is consistent with this study where 15-18% of water was consumed through the diet. The DMI and diet composition, especially energy content, influence *ad libitum* water intake [54]. Starch-rich diets like corn may require more water for metabolism [55], while barley's fibre content enhances rumen fermentation and indirectly affects water intake [56]. Although BG had slightly higher water intake due to greater DMI, no significant differences between treatments were found. Water intake also depends on animal behaviour and physiological needs, making it relatively stable [56].

5. Conclusions

Barley supplementation in alfalfa haylage-based diets improved intake and digestibility of dry matter, crude protein, and fibre, as well as nitrogen balance, compared to corn. While corn was superior in starch content and digestibility, the overall benefits of barley suggest it as a cost-effective and sustainable alternative. Barley supports better nutrient utilisation and nitrogen efficiency which are key components of sustainable livestock production.

These results are particularly relevant in regions where protein-rich forages are common and need carbohydrate-rich supplementation. However, grain choice should consider local availability, cost, and livestock needs. Future studies should explore long-term impacts of barley and corn feeding on sheep health and meat quality, while expanding to different breeds, climates, and feeding systems. This would improve understanding of how cereal choice influences farm sustainability and productivity.

Author Contributions: M.V.: project administration, methodology, investigation and writing original draft. K.B.: methodology, resources, software, formal analysis and investigation, review and editing. The authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Scientific Ethic Committee of the University of Zagreb Faculty of Agriculture (Ethical Reference No: 380-71-01-06-1 from 2nd of March 2006.)

Informed Consent Statement: Not applicable.

Data Availability Statement: All data sets collected and analysed during the current study are available from the corresponding author on fair request.

Acknowledgments: The publication was supported by the Open Access Publication Fund of the University of Zagreb Faculty of Agriculture.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Zhang, J.; Zheng, N.; Shen, W.; Zhao, S.; Wang, J. Synchrony Degree of Dietary Energy and Nitrogen Release Influences Microbial Community, Fermentation, and Protein Synthesis in a Rumen Simulation System. *Microorganisms* **2020**, *8*, doi:10.3390/microorganisms8020231.
2. Mohammadabadi, T.; Jolazadeh, A. Replacement of alfalfa hay (*Medicago sativa* L.) with subabul (*Leucaena leucocephala*) leaf meal in diets of Najdi goats: effect on digestion activity of rumen microorganisms. *Tropical animal health and production* **2017**, *49*, 1309-1316, doi:10.1007/s11250-017-1330-8.
3. Lengowski, M.B.; Witzig, M.; Mohring, J.; Seyfang, G.M.; Rodehutschord, M. Effects of corn silage and grass silage in ruminant rations on diurnal changes of microbial populations in the rumen of dairy cows. *Anaerobe* **2016**, *42*, 6-16, doi:10.1016/j.anaerobe.2016.07.004.
4. Kazemi-Bonchenari, M.; Mirzaei, M.; HosseinYazdi, M.; Moradi, M.H.; Khodaei-Motlagh, M.; Pezeshki, A. Effects of a Grain Source (Corn Versus Barley) and Starter Protein Content on Performance, Ruminal Fermentation, and Blood Metabolites in Holstein Dairy Calves. *Animals-Basel* **2020**, *10*, doi:Artn 172210.3390/Ani10101722.
5. Vranic, M.; Knezevic, M.; Bosnjak, K.; Leto, J.; Perculija, G.; Matic, I. Effects of replacing grass silage harvested at two maturity stages with maize silage in the ration upon the intake, digestibility and N retention in wether sheep. *Livest Sci* **2008**, *114*, 84-92, doi:10.1016/j.livsci.2007.04.011.
6. Vranic, M.; Knezevic, M.; Bosnjak, K.; Leto, J.; Perculija, G. Feeding value of low quality grass silage supplemented with maize silage for sheep. *Agr Food Sci* **2007**, *16*, 17-24, doi:Doi 10.2137/145960607781635831.
7. Lu, Z.; Xu, Z.; Shen, Z.; Tian, Y.; Shen, H. Dietary Energy Level Promotes Rumen Microbial Protein Synthesis by Improving the Energy Productivity of the Ruminal Microbiome. *Frontiers in microbiology* **2019**, *10*, 847, doi:10.3389/fmicb.2019.00847.
8. Vranic, M.; Bosnjak, K.; Luksic, B.; Cacic, I.; Pukec, N.P.; Vranic, I.; Krapinec, K.; Gantner, R.; Starcevic, K.; Masek, T.; et al. Effect of a partial replacement of ground corn grain with dried whey on the quality of the ration based on different forage species in wether sheep. *J Cent Eur Agric* **2024**, *25*, 22-34, doi:10.5513/Jcea01/25.1.4086.
9. Johnson, J.A.; Sutherland, B.D.; Mckinnon, J.J.; McAllister, T.A.; Penner, G.B. Use of barley or corn silage when fed with barley, corn, or a blend of barley and corn on growth performance, nutrient utilization, and carcass characteristics of finishing beef cattle. *Transl Anim Sci* **2020**, *4*, 129-140, doi:10.1093/tas/txz168.
10. Casper, D.P.; Schingoethe, D.J. Lactational Response of Dairy-Cows to Diets Varying in Ruminal Solubilities of Carbohydrate and Crude Protein. *J Dairy Sci* **1989**, *72*, 928-941, doi:DOI 10.3168/jds.S0022-0302(89)79186-4.
11. Arbaoui, A.; de Vega, A. Does Replacing Maize with Barley Affect the Animal Performance and Rumen Fermentation, including Methane Production, of Beef Cattle Fed High-Concentrate Diets On-Farm? *Animals : an open access journal from MDPI* **2023**, *13*, doi:10.3390/ani13193016.
12. Economides, S.; Koumas, A.; Georghiadis, E.; Hadjipanayiotou, M. The Effect of Barley Sorghum Grain Processing and Form of Concentrate Mixture on the Performance of Lambs, Kids and Calves. *Anim Feed Sci Tech* **1990**, *31*, 105-116, doi:Doi 10.1016/0377-8401(90)90117-Q.
13. Xia, Y.; Kong, Y.H.; Seviour, R.; Yang, H.E.; Forster, R.; Vasanthan, T.; McAllister, T. identification and quantification of starch-hydrolyzing bacteria attached to barley and corn grain in the rumen of cows fed barley-based diets. *Fems Microbiol Ecol* **2015**, *91*, doi:10.1093/femsec/fiv077.
14. Amanzougarene, Z.; Yuste, S.; Fondevila, M. Effect of pre-activated or malate salts on fermentation of ground barley grain under in vitro conditions simulating intensive ruminant feeding. *Span J Agric Res* **2021**, *19*, doi:ARTN e06SC0210.5424/sjar/2021192-17728.
15. Morgan, E.K.; Gibson, M.L.; Nelson, M.L.; Males, J.R. Utilization of Whole or Steamrolled Barley Fed with Forages to Wethers and Cattle. *Anim Feed Sci Tech* **1991**, *33*, 59-78, doi:Doi 10.1016/0377-8401(91)90046-U.
16. Ma, X.; Zhou, W.; Guo, T.; Li, F.; Li, F.; Ran, T.; Zhang, Z.; Guo, L. Effects of Dietary Barley Starch Contents on the Performance, Nutrient Digestion, Rumen Fermentation, and Bacterial Community of Fattening Hu Sheep. *Frontiers in nutrition* **2021**, *8*, 797801, doi:10.3389/fnut.2021.797801.

17. Haddad, S.G.; Nasr, R.E. Partial replacement of barley grain for corn grain: Associative effects on lambs' growth performance. *Small Ruminant Res* **2007**, *72*, 92-95, doi:10.1016/j.smallrumres.2006.08.005.
18. Martín-Orue, S.M.; Balcells, J.; Vicente, F.; Castrillo, C. Influence of dietary rumen-degradable protein supply on rumen characteristics and carbohydrate fermentation in beef cattle offered high-grain diets. *Anim Feed Sci Tech* **2000**, *88*, 59-77, doi:10.1016/S0377-8401(00)00191-7.
19. Ghoorchi, T.; Lund, P.; Larsen, M.; Hvelplund, T.; Hansen-Moller, J.; Weisbjerg, M.R. Assessment of the mobile bag method for estimation of starch digestibility. *Animal* **2013**, *7*, 265-271, doi:10.1017/S1751731112001504.
20. Sutherland, B.D.; Johnson, J.A.; McKinnon, J.J.; McAllister, T.A.; Penner, G.B. Effects of barley and corn as sources of silage and grain on dry matter intake, ruminal fermentation, and total-tract digestibility in growing beef heifers. *Can J Anim Sci* **2021**, *101*, 447-458, doi:10.1139/cjas-2020-0025.
21. Chibisa, G.E.; Gorka, P.; Penner, G.B.; Berthiaume, R.; Mutsvangwa, T. Effects of partial replacement of dietary starch from barley or corn with lactose on ruminal function, short-chain fatty acid absorption, nitrogen utilization, and production performance of dairy cows. *Journal of dairy science* **2015**, *98*, 2627-2640, doi:10.3168/jds.2014-8827.
22. Lehmann, M.; Meeske, R. Substituting maize grain with barley grain in concentrates fed to Jersey cows grazing kikuyu-ryegrass pasture. *S Afr J Anim Sci* **2006**, *36*, 175-180.
23. Capstaff, N.M.; Miller, A.J. Improving the Yield and Nutritional Quality of Forage Crops. *Front Plant Sci* **2018**, *9*, doi:10.3389/fpls.2018.00535.
24. Rymer, C. The Measurement of Forage Digestibility In Vivo. In: D. I. Givens, E. Owen, R. F. E. Axford, & H. M. Omed (Eds.), *Forage Evaluation in Ruminant Nutrition*. . CABI publishing. pp. 113-135. **2000**.
25. NENT, T.N.N.C.f.R.E.i.S.a.T. Ethical Guidelines for the Use of Animals in Research. **2018**, ISBN: 978-82-7682-085-0 (printed edition) 978-82-7682-086-7 (digital edition, pdf).
26. Freitas, D.; Capuchinho, R.S.R.C.; Santos, I.R.; Silva, A.M.; Marin, M.L.M. Determination of Total Nitrogen in Parenteral Nutrition Solutions for Micro-Kjeldahl Method. *Ann Nutr Metab* **2013**, *63*, 1820-1820.
27. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science* **1991**, *74*, 3583-3597, doi:10.3168/jds.S0022-0302(91)78551-2.
28. Fussell, R.J.; Mccalley, D.V. Determination of Volatile Fatty-Acids (C2-C5) and Lactic-Acid in Silage by Gas-Chromatography. *Analyst* **1987**, *112*, 1213-1216, doi:10.1039/An9871201213.
29. Norris, J.R.; Rooney, L.W. Enzymatic Determination of Starch in Sorghum Grain. *Cereal Science Today* **1970**, *15*, 304-&.
30. SAS. SAS Software. *SAS Institute Inc., Cary North Carolina, USA* **1999**.
31. Coblenz, W.K.; Akins, M.S.; Kieke, B.A. Storage characteristics of baled alfalfa-grass forages treated with a propionic-acid-based preservative or wrapped in stretch plastic film. *Appl Anim Sci* **2021**, *37*, 505-518, doi:10.15232/aas.2021-02193.
32. Liebhardt, P.; Maxa, J.; Bernhardt, H.; Aulrich, K.; Thurner, S. Comparison of a Conventional Harvesting Technique in Alfalfa and Red Clover with a Leaf Stripping Technique Regarding Dry Matter Yield, Total Leaf Mass, Leaf Portion, Crude Protein and Amino Acid Contents. *Agronomy-Basel* **2022**, *12*, doi:10.3390/agr12061408.
33. Balde, A.T.; Vandersall, J.H.; Erdman, R.A.; Reeves, J.B.; Glenn, B.P. Effect of Stage of Maturity of Alfalfa and Orchardgrass on in-Situ Dry-Matter and Crude Protein Degradability and Amino-Acid-Composition. *Anim Feed Sci Tech* **1993**, *44*, 29-43, doi:10.1016/0377-8401(93)90035-I.
34. Yahaghi, M.; Liang, J.B.; Balcells, J.; Valizadeh, R.; Alimon, A.R.; Ho, Y.W. Effect of replacing barley with corn or sorghum grain on rumen fermentation characteristics and performance of Iranian Baluchi lamb fed high concentrate rations. *Anim Prod Sci* **2012**, *52*, 263-268, doi:10.1071/AN11181.
35. Khattab, I.M.; Anele, U.Y. Dry matter intake, digestibility, nitrogen utilization and fermentation characteristics of sheep fed Atriplex hay-based diet supplemented with discarded dates as a replacement for barley grain. *J Anim Physiol Anim Nutr (Berl)* **2022**, *106*, 229-238, doi:10.1111/jpn.13577.

36. Allen, M.S.; Sousa, D.O.; VandeHaar, M.J. Equation to predict feed intake response by lactating cows to factors related to the filling effect of rations. *Journal of dairy science* **2019**, *102*, 7961-7969, doi:10.3168/jds.2018-16166.
37. Mertens, D.R. Predicting Intake and Digestibility Using Mathematical-Models of Ruminant Function. *J Anim Sci* **1987**, *64*, 1548-1558, doi:DOI 10.2527/jas1987.6451548x.
38. Ramezani-Afarani, O.; Zali, A.; Ganjkanlou, M.; Nasrollahi, S.M.; Moslehifar, P.; Ahmadi, F. Effects of varying proportions of corn grain to barley grain in corn silage-based diet on feed sorting behaviour and productivity of dairy cows. *Anim Prod Sci* **2021**, *61*, 1575-1583, doi:10.1071/AN21048.
39. Mccarthy, R.D.; Klusmeyer, T.H.; Vicini, J.L.; Clark, J.H.; Nelson, D.R. Effects of Source of Protein and Carbohydrate on Ruminant Fermentation and Passage of Nutrients to the Small-Intestine of Lactating Cows. *Journal of dairy science* **1989**, *72*, 2002-2016, doi:DOI 10.3168/jds.S0022-0302(89)79324-3.
40. Orskov, E.R.; Fraser, C.; Gordon, J.G. Effect of Processing of Cereals on Rumen Fermentation, Digestibility, Rumination Time, and Firmness of Subcutaneous Fat in Lambs. *Brit J Nutr* **1974**, *32*, 59-69, doi:Doi 10.1079/Bjn19740058.
41. Petit, H.V. Effect of whole and rolled corn or barley on growth and carcass quality of lambs. *Small ruminant research : the journal of the International Goat Association* **2000**, *37*, 293-297, doi:10.1016/s0921-4488(99)00118-2.
42. Helal, A.; Puchala, R.; Detweiler, G.D.; Gipson, T.A.; Sahlu, T.; Goetsch, A.L. Effects of restricted feed intake on heat energy by different goat breeds. *J Anim Sci* **2011**, *89*, 4175-4187, doi:10.2527/jas.2011-3907.
43. Albornoz, R.I.; Aschenbach, J.R.; Barreda, D.R.; Penner, G.B. Feed restriction reduces short-chain fatty acid absorption across the reticulorumen of beef cattle independent of diet. *J Anim Sci* **2013**, *91*, 4730-4738, doi:10.2527/jas.2012-6223.
44. Colucci, P.E.; MacLeod, G.K.; Grovum, W.L.; McMillan, I.; Barney, D.J. Digesta kinetics in sheep and cattle fed diets with different forage to concentrate ratios at high and low intakes. *Journal of dairy science* **1990**, *73*, 2143-2156, doi:10.3168/jds.S0022-0302(90)78895-9.
45. Owens, F.N.; Zinn, R.A.; Kim, Y.K. Limits to starch digestion in the ruminant small intestine. *J Anim Sci* **1986**, *63*, 1634-1648, doi:10.2527/jas1986.6351634x.
46. Johnson, J.A.; Sutherland, B.D.; McKinnon, J.J.; McAllister, T.A.; Penner, G.B. Effect of feeding barley or corn silage with dry-rolled barley, corn, or a blend of barley and corn grain on rumen fermentation, total tract digestibility, and nitrogen balance for finishing beef heifers. *J Anim Sci* **2020**, *98*, doi:10.1093/jas/skaa002.
47. Sileshi, G.; Mitiku, E.; Mengistu, U.; Adugna, T.; Fekede, F. Effects of dietary energy and protein levels on nutrient intake, digestibility, and body weight change in Hararghe highland and Afar sheep breeds of Ethiopia. *Journal of advanced veterinary and animal research* **2021**, *8*, 185-194, doi:10.5455/javar.2021.h501.
48. Beauchemin, K.A.; McGinn, S.M. Methane emissions from feedlot cattle fed barley or corn diets. *J Anim Sci* **2005**, *83*, 653-661.
49. Johnson, J.A.; Sutherland, B.D.; McKinnon, J.J.; McAllister, T.A.; Penner, G.B. Effect of feeding barley or corn silage with dry-rolled barley, corn, or a blend of barley and corn grain on rumen fermentation, total tract digestibility, and nitrogen balance for finishing beef heifers. *J Anim Sci* **2020**, *98*, doi:ARTN skaa00210.1093/jas/skaa002.
50. Khorasani, G.R.; Okine, E.K.; Kennelly, J.J. Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield, and milk composition of Holstein cows. *Journal of dairy science* **2001**, *84*, 2760-2769, doi:10.3168/jds.S0022-0302(01)74730-3.
51. Kung, L.; Tung, R.S.; Carmean, B.R. Rumen Fermentation and Nutrient Digestion in Cattle Fed Diets Varying in Forage and Energy-Source. *Anim Feed Sci Tech* **1992**, *39*, 1-12, doi:Doi 10.1016/0377-8401(92)90027-4.
52. Benchaar, C.; Hassanat, F.; Gervais, R.; Chouinard, R.Y.; Petit, H.V.; Massé, D.I. Methane production, digestion, ruminal fermentation, nitrogen balance, and milk production of cows fed corn silage- or barley silage-based diets. *Journal of dairy science* **2014**, *97*, 961-974, doi:10.3168/jds.2013-7122.
53. NRC. Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. In *Nutrient Requirements of Dairy Cattle: Eighth Revised Edition*, National Academy of Sciences, W.D.C., USA., Ed.; Washington (DC), 2021.

54. Alqaisi, O.; Al-Jazmi, F.; Al-Abri, M.; Al Kalaldehy, M.; Al-Sabahi, J.; Al-Marzooqi, W. Effect of diet quality and shearing on feed and water intake, in vitro ruminal methane production, and blood parameters of Omani sheep. *Tropical animal health and production* **2020**, *52*, 1115-1124, doi:10.1007/s11250-019-02108-5.
55. NRC, N.R.C. Nutrient Requirements of Small Ruminants: sheep, goats, cervids, and New World camelids. *1st rev. ed. National Academy Press, Washington, DC.* **2007**.
56. Patra, A.K.; Dos Santos Ribeiro, L.P.; Yirga, H.; Puchala, R.; Goetsch, A.L. Influence of the concentration and nature of total dissolved solids in brackish groundwater on water intake, nutrient utilization, energy metabolism, ruminal fermentation, and blood constituents in different breeds of mature goats and sheep. *The Science of the total environment* **2024**, *907*, 167949, doi:10.1016/j.scitotenv.2023.167949.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.