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Effects of Long-Term Grazing on Feed Intake and Digestibility of Cattle in Meadow Steppe

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Abstract: (1) Estimation of grazing livestock intake is the basis for studying animal–plant relationships and the nutritional status of grazing livestock and has important implications for grassland composition and productivity. (2) We used the saturated alkanes method to determine the feed intake and vegetation nutrient digestibility of livestock at different grazing intensities and in different months. (3) We found that C_{31} had the highest concentration in both pasture and fecal output, and the average recovery of C_{31} was 77.99%. The different grazing intensities significantly affected livestock intake. As the grazing intensity increased, there was a decreasing trend of livestock intake and the highest livestock feed intake was 6.11 kg DM/day in light grazing. With the increase in grazing season months, the highest livestock intake was 6.67 kg DM/day in the cold period in September. The month also had a significant effect on the digestibility of livestock for all nutrient variables when compared to the grazing intensity. Livestock weight and medium palatability species are more important for livestock intake. (4) Our study provides a more accurate measurement of grazing livestock intake, which can be used as a reference for the scientific management of grazing livestock and the rational use of grazing pastures.

Keywords: grassland; grazing intensity; cattle; herbage intake and digestibility

1. Introduction

Grassland above-ground net primary production (ANPP) is considered to be a key aspect of ecosystem functioning due to its decisive influence on ecosystem structure and biodiversity [1]. Most productivity studies have used maximum biomass as a proxy, ignoring livestock foraging in grazing ecosystems [2], but an accurate estimation of livestock foraging is beneficial for the assessment of livestock nutrition and digestibility which offers

potential ecological and economic benefits. However, estimating grazing livestock intake has always been challenging due to the limitations of available measurement methods and environmental conditions in ecology [3,4]. Currently, the estimation methods of livestock feed intake mainly include the herbage disappearance method, animal performance method, and internal or external markers. However, no methods have been approved as the golden standard for the estimation of herbage intake [5,6]. The alkane technique has been developed principally for use in grazing ruminants. Long-chain n-alkanes (C_{21}~C_{36}), which occur naturally in the waxes of the plant cuticle and are relatively indigestible in the gastrointestinal tract and can be recuperated in the feces, are one of the widely and successfully used markers to estimate dry matter intake (DMI), diet composition, and digestibility of animals [7–9]. Long-chain n-alkanes have an obvious trend for recovery to increase with chain length, though it should be noted that even with C_{35} and C_{36} alkanes, observed recoveries are still incomplete, especially in cattle, where alkane recoveries are more variable and more work needs to be done to obtain further estimates of alkane recovery [10]. So, observational studies mostly use plant wax markers to estimate the diet composition and dry matter intake of animals with a correction for fecal marker recovery [4,11–14]. This is a great advantage of this technique to support studies of plant–animal interactions in rangeland environments.

Livestock foraging is a complex, dynamic process in which biotic and abiotic factors interact. In the case of grazing livestock, palatability selection and grassland vegetation community structure are important factors influencing foraging [15]. Grassland aboveground biomass determines the supply of grass, while the grass layer structure (including plant height, carrying capacity, and spatial distribution within the canopy) determines the grazing tolerance of the grass [16]. Long et al. [17] showed that differences in the composition of grassland vegetation and differences in the foraging behavior of livestock resulted in differences in intake. Other factors that influence intake include the physiological characteristics of the livestock (e.g., gender, weight, etc.). The growth of pastures varies between seasons with different ambient temperatures and rainfall. Some studies have shown that suitable temperatures can increase livestock intake, while high temperatures can reduce livestock intake [18]. Grazing intensity is one of the policies of grazing management; accurate estimation of livestock DMI is also a key indicator of judgment whether the stocking rate is reasonable. However, little information exists about cattle intake under different grazing intensities and seasons, especially using the saturated alkanes method to determine cattle intake. We hypothesize that the alkanes would not be fully recovered under grazing cattle and that livestock intake would decrease with increasing grazing intensity. This study will assess the recovery of alkanes in cattle at different grazing intensities, more accurately determine grazing livestock intake and provide a database for the estimation of grassland ANPP on the one hand, and to more rationally manage grazing areas according to livestock forage preferences on the other hand, thus achieving both improvements in livestock production and grassland optimization.

Meadow steppe is the most productive grassland type of Eurasian steppe, with rich plant species and relatively high vegetation productivity, and is the main production base for forage resources in China [19], making it particularly important to accurately estimate livestock intake. The present study was conducted in a meadow steppe with a 10-year grazing history, where community composition has shifted at different grazing intensities, for example, from tall grasses to short forbs at heavy grazing intensities, with the objectives of (1) measuring the alkanes recovery in grazing cattle, (2) quantifying the intake and digestibility of cattle at different grazing intensities, and (3) understanding the cattle foraging palatability, by analyzing the relationship between forage intake and community composition.

2. Materials and Methods

2.1. Location of the Study Site

The study area is located at Hulunbuir Grassland Ecosystem Observation and Research Station (49°32′~49°34′ N, 119°94′~119°96′ E), with an altitude of 670~677 m. It belongs to the temperate semi-arid continental climate, with an average annual temperature of −3~1 °C, a frost-free period of about 110 days, and an average annual precipitation of 350~400 mm. The precipitation is mostly concentrated from July to September. The soil type is chernozem, and the vegetation type is *Leymus chinensis*—weeds meadow grassland. The main species are *Leymus chinensis*, *Stipa baicalensis*, *Carex pediformis*, *Galium verum*, and *Bupleurum scorzonerifolium* [20].

2.2. Experiment Design

2.2.1. Experiment Platform

The grazing experiment was established in 2008 on relatively flat terrain and consistent soil as well as vegetation conditions steppe. Based on the daily feed intake of livestock and the utilization rate of local forage, 1 Au is equal to 500 kg adult cattle, with 0.46 cattle units per hectare (0.46 cow. Au/ha) as the theoretical stocking rate. There were six grazing intensities in total, with stocking rates ranging from 0, 0.23, 0.34, 0.46, 0.69, to 0.92 cow.Au/ha, replicated three times. Each experimental plot was 5 ha. Three grazing intensities were selected as the experiment units, namely light (G0.23: 0.23 cow. Au/ha), moderate (G0.46: 0.46 cow. Au/ha), and heavy (G0.92: 0.92 cow. Au/ha). The plots were simulated with two, four, and eight 250–300 kg adult cattle per plot. Wire fences were established in the experimental plot without herders; there was no supplementary feeding and sufficient water was available throughout the free grazing season (June–September) each year [20].

2.2.2. Animals–Plant Sampling

- Animals

Livestock from each experimental plot was weighed from June to the end of September 2018. From July to September (i.e., days 14–18 of the month), three cattle were selected from each plot to follow during the day, with the exception of light grazing (only two cattle), and as soon as the livestock excreted fecal output, it was shoveled into buckets to collect the volume of excrement. Each plot had a separate enclosure (with an area large enough for one livestock to move around for one night), and each livestock was whisked into the enclosure at night and released early the next morning to continue the tracking and collect the manure from the enclosure.

- Plants

Plant community surveys were based on five randomly selected 1 m × 1 m quadrats per plot at the beginning of each month from July through September 2018. The height and abundance of each plant species were recorded. The aboveground component of each species was cut, collected, and dried to constant weight at 65 °C for 48 h. The sum of the dry weights of individual species in the quadrats was termed aboveground biomass (AGB). Species richness, which in the present study is defined as a total number of species occurring per unit area (e.g., 1-m^2 plot), is a simple and easily interpretable indicator of biological diversity [21]. Species abundance is the study of how common a particular species occurs in a given community. The plants were sampled in the morning, mid-day, and evening simulating the livestock foraging pattern (tongue roll) while tracking the livestock. The samples were mixed, stored at −20 °C, and then lyophilized. Based on the palatability of the vegetation combined with expert experience, the vegetation species were classified as high, medium, low, or poisonous palatable vegetation [22].

2.2.3. Recovery Experiment

In early August, one cattle from each of the light, moderate, and heavy grazing plots was placed in a paddock (the pasture was removed from the paddock) and fed above the

stem to simulate the feeding pattern of the cattle. Three feedings time per day (8:00, 12:00, 18:00) were carried out with a total feeding intake of approximately 1.0 kg DM/100 kg LW [23]. The fresh weight of the forage was weighed before each feeding, as well as the fresh weight of the forage left over from the previous feeding, and a portion was taken to determine the dry matter content of the forage. The amount of manure excreted by the cattle was collected in a bucket at the same time as feeding. The trial period was four days for acclimatization and four days for testing. One part of each forage and manure sample was lyophilized to determine the alkanes content and the other part was dried to determine the dry matter content. The recovery was calculated according to Formula (1):

$$\text{Recovery} = C_n(\text{fecal}) * \text{Total Fecal}/C_n(\text{grass}) * \text{Feeding intake} \quad (1)$$

where C_n is the concentration of alkanes in the grass and fecal samples.

2.3. Alkane and Nutrient Analysis

2.3.1. Alkane Analysis

Alkane was determined according to the method of Mayes et al. [7], Sun et al. [24], and Dove et al. [25], with some modifications. Briefly, 2 g of plant or 1 g of fecal sample was weighed into a pyrex bottle with three replicates; two internal standards (2 mg C_{22} and 2 mg C_{34}) and 15 mL ethanolic KOH (1.5 mol/L) were added to each sample. The tubes were capped tightly and heated for 4.5 h at 90 °C. The extraction of alkanes was performed by adding 7 mL heptane plus 5 mL distilled water, with ultrasonic treatment for 5 min, followed by transferring the heptane layer to an evaporating dish. The extraction was repeated twice with 5 mL heptane. The evaporating dish was heated in a water bath at 60 °C and the heptane solution was evaporated to approximately 1 mL. The solution was then transferred into a silica gel column (70–230 mesh), and the dish was rinsed four times with 2-, 2-, 3-, and 4-mL heptane. The lipid in the sample was absorbed into the gel, and alkanes were eluted. The eluate was collected in a tube, blown dry using nitrogen, and reconstituted in 1 mL heptane for the determination of alkane concentrations (Figure 1).

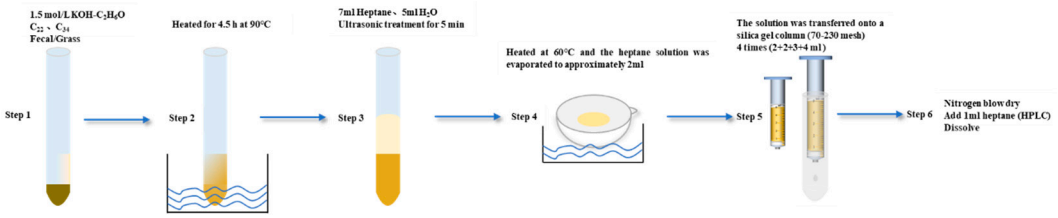

Figure 1. Alkane extraction process.

The identification of alkanes was determined by using a Gas Chromatograph–Mass Spectrometer (GC-MS, Agilent 7000C, Santa Clara, CA, USA), which offers higher sensitivity and more definitive compound identification. The initial column temperature was set at 200 °C, held for 1 min, increased to 250 °C at a rate of 20 °C/min, then ramped to 300 °C at 10 °C/min. Then, 1 µL of the reconstitute was injected at split mode. Individual alkanes were identified from their retention times and quantitated according to their peak areas in reference to the internal standards C_{22} (n-docosane) and C_{34} (tetratriacontane). Figure 2 shows that there were almost no impurities in the extraction process of this method.

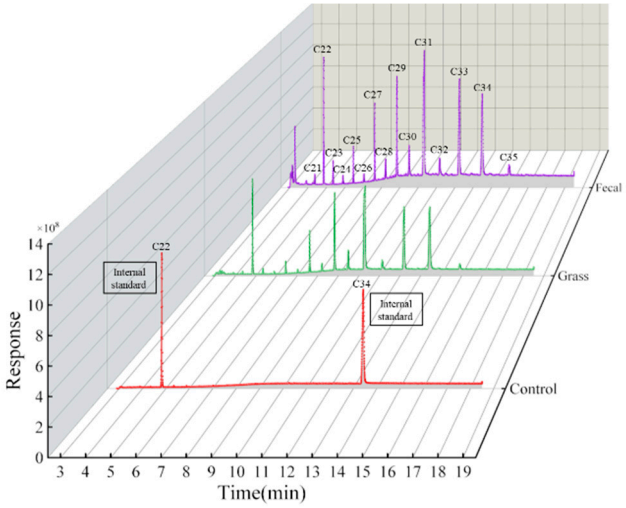

Figure 2. Gas chromatograph spectra of control, grass, and fecal samples.

2.3.2. Nutrient Analysis

Plant and fecal samples were analyzed for C, N, neutral detergent fiber (NDF), acid detergent fiber (ADF), gross energy (GE), dry matter (DM), and organic matter (OM) contents (Table A1). Dry matter was determined by drying the samples to a constant weight at 65 °C for 48 h. The C and N concentrations were determined with an elemental analyzer (Vario EL *III*; Elementar Analysensysteme GmbH, Langenselbold, Germany). Crude protein (CP) was calculated as N × 6.25 [26]. ADF and NDF were determined with an ANKOM 200 automatic fiber analyzer. Ash was determined by muffle furnace combustion at 550 °C for 6 h. GE was determined with the MTZW-A4 high-precision dual-purpose automatic oxygen bomb calorimeter (Shanghai Mitong, Shanghai, China). Metabolizable energy (ME) content was then calculated as 0.82 ∗ Digestible energy content which was the difference between gross energy intake (Dry matter intake × GE) and the fecal energy (fecal output × energy content in feces) [24].

2.4. Estimation of Feed Intake and Digestibility

Livestock feed intake and digestibility was calculated according to Formulas (2) and (3):

$$\text{Dry matter intake (kg/d·cattle)} = C_n(\text{fecal}) * \text{Fecal}/C_n(\text{grass}) * \text{recovery} \quad (2)$$

$$\text{Digestibility (\%)} = 1 - \text{Fecal}/\text{Intake} \quad (3)$$

where Fecal is the total dry fecal output.

2.5. Statistical Analysis

All data were plotted with Origin v. 2021 (OriginLab, Northampton, MA, USA). The data were subjected to two-way ANOVA to analyze the effect of the month and grazing intensity on cattle intake and digestibility using SPSS Statistics v. 23 (IBM Corp., Armonk, NY, USA). Regression analysis was used to examine the plant–livestock factors which influence cattle intake. Duncan's multiple range test was used to determine significance at the 95% confidence interval.

3. Results

3.1. Chemical Composition and n-Alkanes Pattern of Plant and Fecal Matter

The chemical composition of plant and fecal output differed significantly at different grazing intensities and months ($p < 0.05$) (Tables A1 and A2). The plants DM, OM, C, N, CP, and GE were significantly different ($p < 0.05$) across months with the lowest DM content

of 39.36% in August. As grazing intensity increased, plant and fecal nutrients gradually decreased, but the highest values of CP were observed under heavy grazing.

The concentration of alkanes was higher in the fecal output of cattle compared to the grasses at different grazing intensity. The highest concentrations of C_{31} were found in grass and fecal output at different grazing intensities, with mean values of 237.13 mg/kg and 128.37 mg/kg, respectively (Table 1). In addition, odd alkane concentrations were greater than even alkane concentrations, with feces and grass samples accounting for 89.34% and 91.56% of total alkanes, respectively. There was the same trend in different grazing intensities.

Table 1. Alkane concentrations (mg/kg DM) in fecal and grass samples.

	Fecal Alkane Concentrations			**Grass Alkane Concentrations**		
	G0.23	**G0.46**	**G0.92**	**G0.23**	**G0.46**	**G0.92**
C_{21}	7.22	7.91	7.49	1.68	1.85	2.16
C_{22}	200.97	200.92	200.87	100.38	100.43	100.39
C_{23}	15.32	15.08	13.15	4.73	3.93	3.84
C_{24}	6.08	7.40	6.32	1.66	1.80	1.81
C_{25}	30.95	34.56	30.90	9.80	11.24	10.88
C_{26}	8.01	8.82	6.99	3.32	3.59	2.60
C_{27}	75.13	77.54	74.87	30.14	32.07	28.83
C_{28}	15.51	17.35	15.85	5.30	6.58	5.96
C_{29}	131.64	134.55	140.73	62.94	74.85	75.37
C_{30}	26.29	30.55	32.30	8.59	13.40	13.94
C_{31}	224.84	242.02	244.55	118.71	134.68	131.71
C_{32}	18.50	18.53	20.15	4.62	7.16	6.95
C_{33}	160.29	148.72	145.86	65.00	66.90	58.80
C_{34}	200.67	200.70	200.69	100.03	100.08	100.04
C_{35}	12.03	14.59	9.96	5.15	4.24	2.58
Total	731.80	757.62	749.09	321.64	362.28	345.42
Total even chain	657.42	674.97	667.49	298.15	329.75	314.17

3.2. C_{31} Recovery Rate

Based on feeding experiments, it was clear that the amount of fecal output excreted by livestock was directly proportional to the amount of feed. The average digestibility of livestock was 66.90%, with the highest digestibility of 70.33% for heavy grazing. The concentration of alkanes was greater in the fecal output than in the forage, with the highest concentration in C_{31}. The recovery of alkanes increased progressively with increasing alkane length, except for C_{33} and C_{35} where recovery exceeded 100%; we therefore only show the recoveries for C_{31} selected for this study. C_{31} recovery was 77.63%, 75.25%, and 81.09% for light, moderate, and heavy grazing, respectively, but there was no significant difference (Table 2).

Table 2. Daily feed and dry fecal output weight, C_{31} concentration, and C_{31} recovery at different grazing intensities. (n = 3 each grazing intensity).

GI	**Feed Intake (kg/d)**	**Fecal (kg/d)**	**Digestibility (%)**	**C_{31} (Grass) Concentration (mg/kg DM)**	**C_{31} (Fecal) Concentration (mg/kg DM)**	**C_{31} Recovery (%)**
G0.23	4.61 ± 0.15	1.55 ± 0.27	66.15 ± 5.82	106.79 ± 0.72	246.35 ± 1.83	77.63 ± 12.30 [a]
G0.46	4.67 ± 0.24	1.68 ± 0.20	64.23 ± 2.49	145.34 ± 2.89	295.69 ± 3.16	75.25 ± 7.69 [a]
G0.92	3.49 ± 0.29	1.06 ± 0.22	70.33 ± 4.34	120.06 ± 0.78	334.36 ± 2.15	81.09 ± 5.80 [a]

[a] indicates no significant difference between treatments.

3.3. Intake and Digestibility of Cattle under Different Grazing Intensities and Months

The general trend in grazing cattle intake showed the highest intake in September and from light grazing (Figure 3). Significant differences were observed among the grazing intensities and months for all the observed variables except for CP under grazing intensities ($p < 0.001$) (Figure 3). Average values of daily dry matter intake (DMI) were lower under heavy grazing (G0.92) compared to the other treatments, with an average of 4.37 kg DM/ day. In addition, animals in G0.92 showed a relatively low intake of ~1.24% LW, whereas animals in G0.23 showed an intake close to 1.53% LW. Considering the DMI, cattle consumed 27.77% less in G0.92 than in G0.23. Due to the lower DMI at G0.92, the NDF and ADF intake were also lower in this treatment. Grazing intensity and month had a significant effect on the dry matter intake of cattle, as well as on NDF and ADF intake ($p < 0.001$, Figure 3), but there was no significant effect on the interaction of month and grazing intensity.

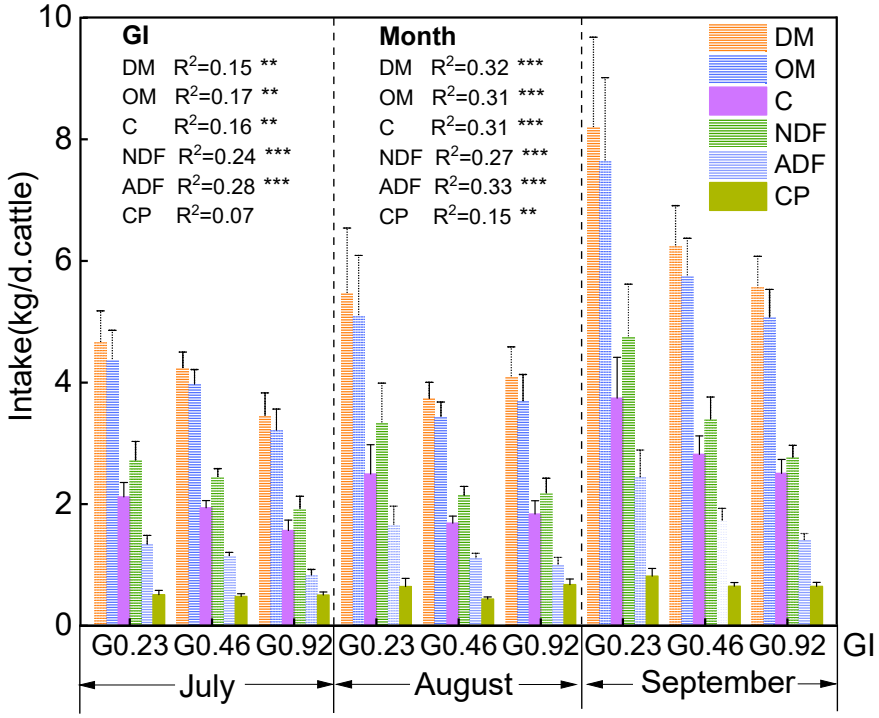

Figure 3. Daily dry matter intake and nutrients intake at different grazing intensities and months. The differences were analyzed using a two-way ANOVA with Duncan post hoc test. **: $p \leq 0.01$, ***: $p \leq 0.001$. (n = 24 each month).

The general trend in grazing cattle digestibility showed that dry matter and nutrient digestibility (except CP) of livestock were lowest during heavy grazing and in September (Table 3). The digestibility of CP increased significantly in August under heavy grazing (64.11%). The month had a significant effect on the digestibility of livestock for all variables compared to the grazing intensity ($p < 0.001$, Table 3). The month explained greater variance in cattle dry matter and nutrient digestibility than grazing intensity. However, the interaction between grazing intensity and month on nutrient digestibility in cattle was not significant.

3.4. Plant Species Composition Influencing Cattle Intake

Livestock intake was not significantly correlated with AGB, while livestock body weight was significantly correlated with livestock intake ($R^2 = 0.46$, $p < 0.01$) (Figure 4a,b). The number of species also showed a significant correlation with livestock intake ($R^2 = 0.34$, $p < 0.01$), with a significant reduction in mainly medium palatable plant species richness ($R^2 = 0.44$, $p < 0.01$), while high and low palatable species richness were not associated with livestock intake (Figure 4c). There was no overall significant trend between livestock

intake and palatable forage abundance, but there was a relationship between palatable species abundance and livestock intake at different grazing intensities. With increasing feed intake of cattle, the high and low palatability species numbers remained constant, but the abundance decreased, e.g., *Leymus chinensis* (high), *Iris tectorum* (low), and the rate of decrease in heavy grazing increased (slope increased); the number of medium palatability species decreased, species abundance decreased in light and medium grazing, and increased in heavy grazing, e.g., *Artemisia frigida* and *Potentilla chinensis* (medium) (Figure 4d).

Table 3. Two-way ANOVA for dry matter (DM) digestibility and nutrient digestibility (%) of cattle at different grazing intensities and months, including NDF, ADF, C, CP, OM, GE, and ME, with ME calculated as ME/DMI. (n = 24 each month).

Month	GI	DM	OM	NDF	ADF	C	CP	GE	ME
7	G0.23	65.3 ± 3.66	68.34 ± 3.35	63.26 ± 3.62	56.18 ± 3.43	66.35 ± 3.67	56.07 ± 7.41	65.54 ± 3.63	9.79 ± 0.55
7	G0.46	65.06 ± 1.3	68.53 ± 1.11	64.11 ± 1.04	53.97 ± 1.05	66.72 ± 1.17	58.79 ± 2.57	65.51 ± 1.23	9.72 ± 0.19
7	G0.92	60.99 ± 1.18	67.23 ± 0.74	60.19 ± 1.23	42.73 ± 2.11	65.39 ± 0.87	62.4 ± 1.44	63.11 ± 1.06	9.32 ± 0.17
8	G0.23	57 ± 4.86	61.84 ± 4.36	57.11 ± 4.93	45.68 ± 5.56	59.89 ± 4.59	54.55 ± 5.76	57.38 ± 4.87	8.48 ± 0.73
8	G0.46	57.5 ± 2.89	62.42 ± 2.56	55.38 ± 2.65	46.31 ± 3.6	60.18 ± 2.71	54.17 ± 3.58	58.56 ± 2.73	8.6 ± 0.39
8	G0.92	56.23 ± 1.4	63.53 ± 1.15	53.07 ± 2.51	33.76 ± 3.82	62.38 ± 1.28	64.11 ± 1.44	59.44 ± 1.35	8.54 ± 0.21
9	G0.23	51.91 ± 6.23	55.97 ± 5.87	50.82 ± 6.53	40.73 ± 7.21	53.69 ± 6.16	48 ± 6.47	52.9 ± 6.13	7.92 ± 0.9
9	G0.46	50.37 ± 2.37	54.87 ± 2.32	46.15 ± 3.28	32.69 ± 4.7	52.05 ± 2.41	46.43 ± 2.22	49.03 ± 2.29	7.05 ± 0.32
9	G0.92	49.68 ± 4.69	57.36 ± 3.78	45.03 ± 3.3	30.31 ± 4.23	55.23 ± 3.98	44.85 ± 5.28	52.35 ± 3.86	7.42 ± 0.53
Main effect									
Month	7	63.60a	68.00a	62.42a	50.31a	66.13a	59.47a	64.62a	9.59a
	8	56.90b	62.69b	54.95b	41.45b	60.93b	58.00a	58.60b	8.55b
	9	50.50c	56.08c	46.90c	33.81c	53.65c	46.23b	51.24c	7.41c
GI	G0.23	58.07A	62.05A	57.06A	47.53A	59.98A	52.88A	58.61A	8.73A
	G0.46	57.65A	61.94A	55.21A	44.32A	59.65A	53.13A	57.70A	8.45A
	G0.92	55.64A	62.71A	52.76A	35.60B	61.00A	57.12A	58.30A	8.43A
p-value	Month	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	GI	0.61	0.93	0.28	<0.001	0.84	0.33	0.93	0.69
	Month * GI	0.97	0.96	0.95	0.67	0.94	0.48	0.82	0.77

Notes: Lower-case letters indicate differences between months and upper-case letters indicate differences between grazing intensities.

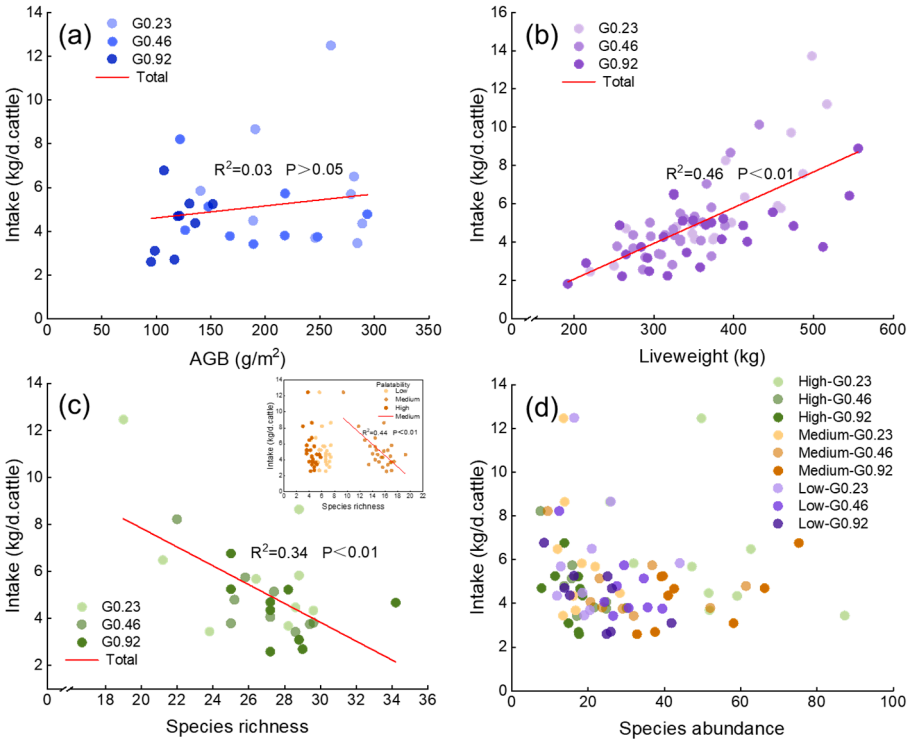

Figure 4. The relationship between livestock intake and vegetation. Cattle intake with AGB (**a**), liveweight (**b**), species richness (**c**), and species abundance (**d**).

4. Discussion

4.1. Chemical Composition and Alkanes Recovery Rate

Grazing intensity is the management tool that truly drives ecosystem functioning. Pasture samples are taken from the remainder of the animal's diet as well as from the new growth under grazing. Plant nutrient composition undergoes dramatic changes across seasons and plant physiological stages. Forage growth and nutrient content were at their peak in August at the Eurasian meadow steppe. In the case of heavy grazing, with few standing dead plants, mainly fresh new-growth grasses, the chemical composition of the fresh grass was characterized by low DM content and high CP (Table A1) [27].

Many studies have shown that the chain alkane patterns of both herbage and fecal output are greater even than odd alkanes. In addition, they show that the concentration of alkanes in fecal output is much greater than in plants, and demonstrate an increase in fecal recovery with increasing carbon chain length [12,23], which was consistent with the results of this study. However, the recoveries increase with the length of the chain and may be greater than 100% due to errors, such as C_{33} and C_{35}. Our study found the highest concentrations of C_{31} and the highest recoveries in grazing cattle. However, different species have different n-alkane patterns—such as *Artemisia frigida* and *Iris ventricose*, which have the highest concentrations of C_{29} (unpublished data)—so the results of this study may only apply to temperate meadow grasslands.

4.2. Animal Intake and Digestibility

Most studies estimate livestock intake based on the analysis of representative samples of daily fecal output because it is laborious and prohibitive for large-scale experiments. Total collection of feces also obviates the need for markers to measure fecal output. Thus, in the current study, we collected total fecal output, fed the livestock without additional markers, and measured the alkanes in the feces to determine the amount of forage taken by the livestock. We found that as grazing intensity increases, the amount of dry matter taken by livestock gradually decreases. This may be explained that as grazing intensity increases, interspecific competition for livestock increases, the grazing resources will decrease, and livestock feed intake will decrease as observed in light grazing. Similarly, as grazing intensity increases, livestock forage time increases, single-mouth intake decreases, and highly palatable forage decrease, so daily intake decreases. Therefore, lower forage intake and animal nutritional status under high stocking rates are explained by lower forage availability [28]. We also found that livestock feed intake was highest in September, probably due to the fact that local temperatures in the study area were already significantly lower in September than in July and August and that livestock need to feed more to meet their energy requirements in cold conditions.

With respect to cattle digestibility, the month had a significant effect on the digestibility of livestock for all variable measuring compared to the grazing intensity. Digestibility reflects the nutritional quality of the forage, and the nutritional quality of forage varies considerably from month to month depending on the stage of growth, resulting in a significant effect of the month on nutrient digestibility for livestock. However, it was shown that grazing management also significantly affects livestock digestibility [29], i.e., livestock dry matter digestibility is significantly lower at low grazing rates than at high grazing rates. Our feeding trials showed that dry matter digestibility was higher in heavily grazed livestock than in light and medium grazing, however, there was no significant difference among them (Table 2). The free grazing trials showed no significant effect of grazing intensity on livestock dry matter digestibility (Table 3), probably because on the one hand, the feeding trials obtained vegetation consisting entirely of fresh grass above plant stems, even in heavy grazing. On the other hand, for livestock under free grazing in heavy intensity, the low digestibility of the forage may be due to the vegetation obtained in the heavy grazing trials being entirely fresh grass. However, the livestock were more likely to forage on ash due to the short vegetation under heavy grazing, resulting in low digestibility. This phenomenon was observed in our experiments from the measurement of acid-insoluble ash in forage and fecal output. In addition, on the

other hand, the apparent nutrient digestibility of animals is, in general, correlated negatively with their dry matter intake. With sufficient precipitation in 2018, the effect of grazing intensity on livestock nutrient digestibility was weaker when herbage was enough available at each grazing gradient.

4.3. Factors Influencing Feed Intake

The most important factors determining intake are the quantity and quality of the forage provided. In pastures, these in turn are influenced by factors such as plant species composition, plant community structure, season (forage maturity), and the grazing history of the site in question. The composition of the plant, the energy level and palatability of the feed, and the physicochemical properties of the feed also affect the animal's intake [30]. The metabolic energy requirements of heavier livestock are also higher, so their feed intake is also relatively high, which is consistent with the results of this study. We usually assume that livestock intake is closely related to grassland AGB, but this study found that livestock intake was poorly correlated with AGB. In our previous study, we found a negative correlation between livestock foraging time and AGB, and foraging intake [31], and therefore suggest that foraging intake may be indirectly related to AGB through livestock behavior (foraging time). Animal feed intake is also influenced by several factors, including age, experience, stress, disease, and external conditions for the same animal. Livestock weight is linearly correlated with livestock feed intake ($R^2 = 0.46$, $p < 0.01$), which is due to the fact that heavier livestock also requires higher metabolic energy and so feed intake is relatively high. Due to the selective foraging of livestock, the palatability of different plants affects livestock foraging [32]. Consequently, we suggest that medium palatability plants were significantly associated with livestock intake, and heavy grazing increased the abundance of medium palatability plants (Figure 4c,d). Related studies have also shown that *Artemisia frigida* and *Potentilla chinensis*, as degradation indicator species, increased significantly under heavy grazing [33]. The abundance of highly palatable species such as *Leymus chinensis* decreased at an accelerated rate with increasing grazing intensity, and we have also found that the importance value of *Leymus chinensis* decreased significantly with increasing grazing intensity [34]. The dominant plant species are usually palatable, and grazing reduces their dominance in meadow steppe, which is consistent with our study [35].

Understanding the impacts of grazing on livestock intake may help improve our prediction for future livestock production and grassland dynamics. However, our study has some limitations. First and foremost, the studies included in our dataset were distributed in temperate regions. Our findings have, therefore, little capacity to predict livestock intake in typical steppe or alpine grasslands. Meanwhile, we measure individual livestock feed intake in the first half of each month, and it is undeniable that feed intake gradually increases as livestock gain weight (Figure 4b), so an underestimation of livestock feed intake throughout the month will occur. The second noteworthy point is that our selected studies were largely shorter than 3 years. This short duration may also influence our findings [32]. The lack of large and complete datasets from long-term studies likely limits our ability to better understand the long-term effects of grazing and seasons on livestock intake. Third, grazing intensity significantly affects livestock intake, but the effect of livestock grazing on ecosystem functioning (e.g., ANPP) was also regulated by environmental fluctuations, such as precipitation and nutrient availability [36]. Furthermore, we found that species richness of different palatability affects livestock intake, that intake is a major component of ANPP under grazing, and that the relationship between ANPP and species richness remains unclear [37].

5. Conclusions

Different grazing intensities and seasons can significantly alter livestock feed intake in temperate meadow grasslands. The livestock intake from light grazing was higher than from heavy grazing, and the daily intake of livestock was significantly higher in September

than in July and August. The months had a greater effect on the dry matter and nutrient digestibility of livestock than grazing intensity. In addition, seasons had a significant effect on the digestibility of livestock for all nutrient variables compared to the grazing intensity. Livestock weight and medium-palatability species are more important for livestock intake. Our study provides a reference basis for the scientific management of grazing livestock and the rational use of grazing pastures. Given the difficulty of collecting fecal output from livestock, it is not always possible to collect fecal output to estimate livestock intake in the future and a model can be introduced to estimate this. This experiment also provides a database of future models of predicted intake.

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Appendix A

Table A1. Chemical composition (mean ± SD, g/kg DM) and Gross energy (MJ/kg DM) of plant under different months and grazing intensities.

Month	GI	DM	NDF	ADF	AIA	OM	C	CP	Gross Energy
7	G0.23	429.5 ± 20.83	592.05 ± 31.61	287.38 ± 26.98	18.22 ± 1.22	938.36 ± 2.73	454.57 ± 2.47	109.14 ± 20.26	18.21 ± 0.11
7	G0.46	429.94 ± 35.01	575.77 ± 21.58	273.5 ± 22.12	20.77 ± 4.97	936.7 ± 3.13	457.43 ± 0.71	112.11 ± 13.09	18.09 ± 0.06
7	G0.92	388.92 ± 28.64	571.93 ± 10.24	238.85 ± 5.86	22.65 ± 1.59	930.53 ± 0.39	453.77 ± 0.89	149.03 ± 14.41	18.01 ± 0.1
8	G0.23	430.36 ± 12.23	607.53 ± 5.22	306.42 ± 11.22	24.92 ± 7.17	932.11 ± 1.98	456.42 ± 2.15	117.53 ± 11.03	18 ± 0.08
8	G0.46	411.56 ± 49.47	565.76 ± 41.03	296.95 ± 20.15	26.75 ± 2.92	919.54 ± 3.89	451.1 ± 2.28	116.62 ± 9.81	17.92 ± 0.13
8	G0.92	338.82 ± 31.18	540.17 ± 33.39	247.84 ± 18.21	29.45 ± 8.08	905.7 ± 13.96	450.2 ± 4.32	162.71 ± 14.08	17.52 ± 0.17
9	G0.23	494.93 ± 15.48	581 ± 12.49	296.85 ± 7.76	27.19 ± 3.85	932.89 ± 3.34	456.45 ± 1.62	101.05 ± 10.74	18.29 ± 0.18
9	G0.46	481.61 ± 49.01	550.17 ± 57.07	277.21 ± 36.13	39.69 ± 3.94	921.8 ± 1.79	451.43 ± 1.73	105.18 ± 9.14	17.53 ± 0.07
9	G0.92	424.52 ± 55.78	507.83 ± 68.8	256.23 ± 33.26	31.83 ± 10.02	911.68 ± 5.05	449.7 ± 1.4	115.5 ± 5.16	17.33 ± 0.25
Main effect									
Month	7	416.122b	579.915a	266.577a	20.544c	935.197a	455.258a	123.425b	18.099a
	8	393.582b	571.152a	283.736a	27.042b	919.114b	452.572b	132.287a	17.817b
	9	467.016a	546.331a	276.763a	32.901a	922.122b	452.526b	107.242a	17.72b
GI	G0.23	451.596A	593.527A	296.883A	23.443A	934.453A	455.811A	109.24B	18.168A
	G0.46	441.037A	563.897AB	282.55A	29.07A	926.013B	453.317B	111.302B	17.848B
	G0.92	384.087B	539.975B	247.643B	27.975A	915.967C	451.227B	142.412A	17.621C
p-value	Month	0.001	0.17	0.293	0.001	<0.001	0.025	0.002	<0.001
	GI	0.002	0.024	0.001	0.107	<0.001	0.001	<0.001	<0.001
	Month ∗ GI	0.818	0.739	0.875	0.382	0.093	0.026	0.145	0.001

Notes: Lower-case letters indicate differences between months and upper-case letters indicate differences between grazing intensities. The same as below.

Appendix B

Table A2. Chemical composition (mean ± SD, g/kg DM) and Gross energy (MJ/kg DM) of fecal under different months and grazing intensities.

Month	GI	DM	NDF	ADF	AIA	OM	C	CP	Gross Energy
7	G0.23	163.91 ± 17.5	615.78 ± 10.87	366.56 ± 8.11	82.55 ± 8.44	856.23 ± 10.35	439.9 ± 7.1	131.19 ± 5.5	18.09 ± 0.18
7	G0.46	177.73 ± 8.87	608.92 ± 26.16	361.05 ± 11.64	94.78 ± 6.75	844.25 ± 8.12	436.05 ± 6.03	130.45 ± 8.94	17.87 ± 0.37
7	G0.92	168.95 ± 12.89	572.88 ± 19.15	350.37 ± 16.5	137.39 ± 17.85	783.33 ± 24.66	403.39 ± 14.27	143.05 ± 6.72	17.04 ± 0.33
8	G0.23	165.11 ± 16.62	616.15 ± 14.06	389.84 ± 13.99	107.74 ± 10.28	827.17 ± 11.63	424.76 ± 19.57	123.05 ± 5.79	17.84 ± 0.58
8	G0.46	179.94 ± 10.41	606.76 ± 32.26	374.72 ± 20.55	118.77 ± 17.26	813.48 ± 18.16	425.56 ± 5.07	124.97 ± 9.17	17.51 ± 0.37
8	G0.92	169.3 ± 10.51	571.93 ± 41.66	372.13 ± 28.56	167.86 ± 20.71	755.25 ± 25.98	422.85 ± 7.35	133.05 ± 8.01	16.24 ± 0.57
9	G0.23	182.14 ± 14.74	587.13 ± 36.56	367.15 ± 14.06	83.17 ± 5.69	852.46 ± 13.9	438.6 ± 5.83	109.55 ± 8.95	17.91 ± 0.2
9	G0.46	195.02 ± 7.54	578.12 ± 38.36	370.12 ± 16.93	90.6 ± 7.33	837.1 ± 10.97	435.68 ± 3.9	113.66 ± 4.93	18.03 ± 0.23
9	G0.92	191.27 ± 10.27	560.02 ± 31.38	360.06 ± 21.5	144.42 ± 15.31	775.4 ± 18.87	401.43 ± 10.25	126.3 ± 4.39	16.55 ± 0.65
Main effect									
Month	7	170.198b	599.192a	359.328b	104.906b	827.937a	426.444a	134.897a	17.665a
	8	171.45b	598.277a	378.893a	131.458a	798.635b	411.84b	127.022b	17.197b
	9	189.476a	575.09b	365.777b	106.063b	821.654a	425.234a	116.503c	17.497a
GI	G0.23	170.387B	606.351A	374.517A	91.152C	845.287A	434.686A	121.262B	17.946A
	G0.46	184.232A	597.932A	368.63AB	101.382B	831.612B	431.525A	123.025B	17.802A
	G0.92	176.505B	568.275B	360.852B	149.893A	771.327C	397.307B	134.134A	16.611B
p-value	Month	<0.001	0.013	0.002	<0.001	<0.001	<0.001	<0.001	0.002
	GI	0.001	<0.001	0.052	<0.001	<0.001	<0.001	<0.001	<0.001
	Month ∗ GI	0.931	0.88	0.75	0.819	0.994	0.993	0.651	0.138

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