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Effects of Potassium Fertilizer on Water-Soluble Carbohydrate Content of Timothy (*Phleum pratense* L.), Silage Fermentation, Nutritive Values, and Nutrient Intake

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Abstract

The objective of this study was to examine the effects of potassium fertilizer (standard (S) and high (H) levels) on water-soluble carbohydrate (WSC) content of timothy (*Phleum pratense* L.), silage fermentation, nutritive values, and nutrient intake. The silage treatments were as follows: S level without inoculant (SC), S level plus inoculant (SI), H level without inoculant (HC), and H level plus inoculant (HI). The K content was increased by 14.5% in timothy grown with the H level compared with the S level. The WSC contents of the S and H treatments were 75.9 and 66.1 g·kg^{-1} dry matter (DM), respectively. The silage fermentation quality was low with both SC and HC treatments. The addition of inoculant significantly improved the fermentation quality in SI and HI treatments. The addition increased the DM and organic matter digestibilities of silage. The total digestible nutrient (TDN) content of silage was highest with the HI treatment. The DM, TDN, and digestible energy intakes with the SI and HI treatments were improved compared with the SC and

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HC treatments, respectively. This study demonstrated negligible effects of K fertilizer levels and significant effects of the lactic acid bacteria inoculant on the fermentation quality, nutritive values, and feed intake of silage.

Keywords

Feed Intake; Fermentation Quality; Nutritive Value; Potassium Fertilizer; Timothy Silage

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1. Introduction

Timothy (*Phleum pratense* L.) is a backbone forage grass in Hokkaido, Japan, and is utilized as silage by most dairy farmers. For the preparation of high-quality grass silage, lactic acid fermentation should be advanced. It is well known that lactic acid fermentation is greatly influenced by the water-soluble carbohydrate (WSC) content [1] [2]. WSC content is affected by several factors, including growth stage, harvest time, wilting treatment, cultivar, and fertilizer management [3]-[6]. Reports have shown that a reduction in WSC can lower the fermentation quality of silage [7]-[12]. Wang *et al*. focused on the effect of fertilizer rate on WSC content via several factors. Research showed a fertilizer rate of nitrogen (N) higher than standard rate reduced the WSC content in timothy, but nutritive value and feed intake were not affected [13].

A potassium (K) fertilizer should be applied at 125 - 183 kg·ha^{-1} to ensure stable yields of timothy in Hokkaido [14]. K is supplied by chemical fertilizer or dairy manure and slurry. K supply reaches the required amount if slurry is applied to pasture grass at 50 ton·ha^{-1}, and K content exceeds 3.0% in dry matter (DM) if slurry is applied to pasture grass at 160 ton·ha^{-1} [15]. K intake, however, should be limited because a dietary K content exceeding 3.0% in DM may cause milk fever, grass tetany, and breast edema in dairy cattle [16]. The WSC content reduces as the K content increases in timothy collected from pasture grass in Hokkaido [17]. Silage prepared using grass with a high K content may influence the fermentation quality of the silage. Thus, the relationship between K fertilizer rate levels and the WSC content should be examined.

Timothy was cultivated by altering the K fertilizer level to examine its effects on the WSC content of timothy, and the fermentation quality, nutritive value, and feed intake of silage. During fermentation, the number of lactic acid bacteria that adhere to the ensiled grass affected the fermentation quality and nutritive value of silage. Thus, silage supplemented with a lactic acid bacteria inoculant was compared with non-inoculated silage.

2. Materials and Methods

2.1. Material Grass and Fertilizer Rate

Hokushu, a late variety of timothy, was used as the study material. Planting was performed in a field of the Tenpoku branch, Kamikawa Agricultural Experiment Station (Hokkaido, Japan). Seeding density was 20 kg·ha^{-1}. K fertilizer rate was 125 and 375 kg·ha^{-1} per year in the standard (S) and high level (H) K fertilizer level treatments, respectively. For the K content of pasture grass to reach 3.0% DM by slurry spraying, three times the standard slurry level is required [15].

Two-thirds of the fertilizer amount was applied on May 7, 2008 and the remaining third was applied on June 20, 2008. The total annual N and phosphate (P) fertilizer rates were 160 and 26 kg·ha^{-1}, respectively. The distribution and periods of treatment were similar to those of K fertilizer. Each fertilizer treatment was applied over a field area of 100 m^2 (10 m long × 10 m wide) and three replicates were randomly set in each of the S and H treatment areas. Potassium sulphate (K_2SO_4), urea, and superphosphate ($Ca(H_2PO_4)_2$) were used as K, N, and P fertilizers, respectively. Pasture grass was harvested on June 18, 2008. The study material was prepared by mixing three replicates to prepare silage.

2.2. Silage Preparation

Silage was prepared using the following four treatments: standard fertilizer level without inoculant (SC), standard fertilizer level plus inoculant (SI), high fertilizer level without inoculant (HC), and high fertilizer level plus inoculant (HI). The grass for each treatment was cut into approximately 1 - 4 cm lengths and ensiled in 220 L plastic silos. Three replicates were prepared. The filling weight (mean ± standard deviation) was 111.7 ± 6.9 kg of fresh matter. An inoculant containing *Lactobacillus plantarum* and *Enterococcus faecium* (11F25, Pioneer Hi-Bred Japan Co. Ltd., Tokyo, Japan) was added to the fresh crop at 1 $mg \cdot kg^{-1}$ (*Lactobacillus* concentration was 1.0×10^5 $cfu \cdot g^{-1}$). The silages were evaluated for fermentation quality and used for feeding and digestion trials in sheep.

2.3. Feeding and Digestion Trials

Four male Corriedale sheep were used in the feeding and digestion trials at the animal shed of Tokyo University of Agriculture (Abashiri, Hokkaido, Japan). The weight (mean ± standard deviation) of the sheep was 83.8 ± 9.3 kg. The trials were conducted between August 3 and September 19, 2008. Whole feces collecting method was carried out in a feces and urine separating metabolic cage. A 4 × 4 Latin square design was used with the four silage treatments and four animals. Feces samples were collected 5 days after a 7 day adaption period. Feeding and digestion trials were conducted with the approval of the Animal Experiment Committee of Tokyo University of Agriculture. Water and mineral blocks were freely accessible. Digestibility and total digestible nutrient (TDN) content and digestible energy (DE) were calculated in accordance with JGAFSA [18].

2.4. Chemical Analysis

The grass material, silage, and feces were dried in an electric drying oven (FS-620; Advantec Co. Ltd., Tokyo, Japan) at 60˚C for 48 h and ground through a 1 mm screen using a cutting mill (1029-13; Yoshida Co. Ltd., Tokyo, Japan). The contents of dry matter (DM), crude protein (CP), ether extract (EE), and gross energy (GE) of the grass material, silage, and feces were analyzed in accordance with the methods of the AOAC [19]. The contents of acid detergent fiber (ADF) and neutral detergent fiber (NDF) were analyzed in accordance with Van Soest *et al*. [20]. The WSC content of grass material was analyzed using the procedures in Deriaz [21]. The contents of K, calcium (Ca), and magnesium (Mg) were measured using an atomic absorption spectrophotometer (Z-8200; Hitachi Co. Ltd., Tokyo, Japan) [22]. The contents of glucose and fructose were measured by high performance liquid chromatography (LC-10AT; Shimadzu Co. Ltd., Kyoto, Japan) [23]. The fermentation quality of silage was determined using water extracts from macerated material of 50 g of silage with 100 mL of water using a blender. The pH value was determined using a pH meter (HM-25G; Toa DKK Co. Ltd., Tokyo, Japan). The content of lactic acid was determined using the colorimetric method of Barker-Summerson [24]. The content of volatile fatty acids was measured using gas chromatography (GC-14A; Shimadzu Co. Ltd., Kyoto, Japan) [25]. The content of ammonium-nitrogen (NH_3-N) was measured using steam distillation [26]. The V-score was calculated to evaluate the fermentation quality based on the values of organic acids and percentage of NH_3-N to total N (NH_3-N ratio) [18].

2.5. Statistical Analysis

All data were subjected to analysis using SAS statistical software (SAS Institute Japan Ltd., Tokyo, Japan). For the chemical composition of material grass, the significance of difference between the S and H treatments was analyzed using a t-test. Two-way ANOVA was used to test the effects of the main factors (K fertilizer rate and addition of inoculant) and their interactions on the fermentation quality, chemical composition, digestibility, nutritive value, and feed intake of silage.

3. Results

3.1. Chemical Composition of Grass

The chemical composition of the grass is shown in **Table 1**. The K content of grass from the H treatment was significantly higher ($P < 0.01$) than that from the S treatment. The level of organic matter (OM) in the H treatment was significantly lower ($P < 0.01$) than that of the S treatment. The WSC content of the S and H treatments were 75.9 and 66.1 $g \cdot kg^{-1}$ DM, respectively; that from the H treatment was significantly lower ($P < 0.01$) than from the S treatment. The glucose content of grass from the H treatment was significantly lower ($P < 0.05$) than that from the S treatment. The sucrose content was substantially lower than the glucose content, while the sucrose content from the S treatment was significantly higher ($P < 0.01$) than that from the H treatment.

Table 1. Chemical composition of grass material.

Item	S	H	SEM	Significance
DM ($g \cdot kg^{-1}$)	201	203	1.7	NS
OM ($g \cdot kg^{-1}$ DM)	933	925	1.8	**
CP ($g \cdot kg^{-1}$ DM)	140	142	0.8	NS
EE ($g \cdot kg^{-1}$ DM)	47	40	2.1	*
ADF ($g \cdot kg^{-1}$ DM)	353	358	2.9	NS
NDF ($g \cdot kg^{-1}$ DM)	683	722	8.9	**
WSC ($g \cdot kg^{-1}$ DM)	75.9	66.1	2.24	**
Glucose ($g \cdot kg^{-1}$ DM)	52.9	44.2	2.11	*
Sucrose ($g \cdot kg^{-1}$ DM)	10.1	2.2	1.83	**
K ($g \cdot kg^{-1}$ DM)	25.5	29.2	0.84	**
Ca ($g \cdot kg^{-1}$ DM)	1.6	2.1	0.18	*
Mg ($g \cdot kg^{-1}$ DM)	0.8	0.8	0.02	NS
GE ($MJ \cdot kg^{-1}$ DM)	19.2	19.2	0.03	NS

S: standard level potassium fertilizer rate (150 $kg \cdot ha^{-1}$); H: high level potassium fertilizer rate (450 $kg \cdot ha^{-1}$); DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; ADF: acid detergent fiber; NDF: neutral detergent fiber; WSC: water-soluble carbohydrate; GE: gross energy; SEM: standard error of the mean; NS: not significant; *: $P < 0.05$; **: $P < 0.01$.

3.2. Fermentation Quality and Chemical Composition of Silage

The fermentation quality and chemical composition of the silages are shown in **Table 2**. Regarding the fermentation quality of silage, significant differences in pH and NH_3-N ratio were observed in relation to K fertilizer level (fertilizer) and addition of a lactic acid bacteria inoculant (addition) ($P < 0.01$ and $P < 0.01$). The fertilizer × addition of inoculant interaction was significant for the pH and NH_3-N ratio ($P < 0.01$).

Differences in the pH and NH_3-N ratio were observed in relation to fertilizer treatment. However, with the S and H fertilizer levels treatment without addition of inoculant (SC and HC treatments, respectively), the pH (5.56 and 5.13), NH_3-N ratio (174.6 and 209.4 $g \cdot kg^{-1}$ TN), and butyric acid contents (23.0 and 29.3 $g \cdot kg^{-1}$ DM) were high, which resulted in low V-scores (51.4 and 46.9). All the items showed small differences between the SC and HC treatments. Fermentation quality was low with both treatments.

The lactic and butyric acid contents showed significant differences owing to the addition of inoculant ($P < 0.01$). In the S and H fertilizer level treatments with addition of inoculant (SI and HI treatments, respectively), the pH (4.33 and 4.24), butyric acid contents (0.8 and 1.1 $g \cdot kg^{-1}$ DM), and NH_3-N ratios (57.8 and 48.5 $g \cdot kg^{-1}$ TN) were low, whereas the lactic acid content (52.9 and 55.5 $g \cdot kg^{-1}$ DM) and V-scores (97.0 and 96.9) were high. V-scores were high with the SI and HI treatments compared with the SC and HC treatments, respectively. The addition of inoculant significantly improved the fermentation quality in the SI and HI treatments.

Regarding the chemical composition of the silages, significant differences in the CP and K contents were observed in relation to fertilizer and the addition of inoculant ($P < 0.05$ and $P < 0.05$). The fertilizer × addition of inoculants interaction was significant for the CP contents ($P < 0.01$). The K content was higher with the HC treatment than with the SC treatment ($P < 0.05$), and higher with the HI treatment than with the SI treatment ($P < 0.05$). The K content with the SI treatment was lower than that with the SC treatment; the K content with the HI treatment was lower than with the HC treatment ($P < 0.05$).

3.3. Digestibility, Nutritive Value, and Feed Intake of Silage

The digestibility, nutritive value, and feed intake of the silages are shown in **Table 3**. Regarding the digestibility of the silages, significant differences were observed in the digestibility of DM, OM, and CP in relation to fertilizer and the addition of inoculant ($P < 0.05$, $P < 0.05$ and $P < 0.05$). The fertilizer × addition of inoculant interaction was significant for the CP digestibility ($P < 0.01$). The DM and OM digestibility were higher with the HI treatment than with the HC treatment ($P < 0.05$), and higher with the HI treatment than with the SI treatment ($P < 0.05$). A significant difference in the GE digestibility was observed in relation to the addition of inoculant ($P < 0.01$). The GE digestibility was higher with the HI treatment than with the HC treatment.

For the nutritive value of the silages, significant differences in relation to the addition of inoculant were

Table 2. Fermentation quality and chemical composition of silages.

Item	S		H		SEM	Significance		
	C	I	C	I		F	I	F × I
Fermentation quality								
pH	5.56	4.33	5.13	4.24	0.17	**	**	NS
Lactic acid (g·kg^{-1} DM)	2.0	52.9	1.9	55.5	7.24	NS	**	NS
Acetic acid (g·kg^{-1} DM)	21.7	7.1	19.7	7.4	2.24	NS	**	NS
Propionic acid (g·kg^{-1} DM)	14.2	0.2	15.4	0.3	2.24	NS	**	NS
Butyric acid (g·kg^{-1} DM)	23.0	0.8	29.3	1.1	4.25	NS	**	NS
NH_3-N (g·kg^{-1} TN)	174.6	57.8	209.4	48.5	2.14	**	**	**
V-score	51.4	97.0	46.9	96.9	7.43	NS	**	NS
Chemical composition								
DM (g·kg^{-1})	188	222	181	218	5.7	NS	**	NS
OM (g·kg^{-1} DM)	924	934	913	922	2.4	**	**	NS
CP (g·kg^{-1} DM)	132	127	113	142	3.3	*	*	**
EE (g·kg^{-1} DM)	49	41	45	44	1.0	NS	*	*
ADF (g·kg^{-1} DM)	423	394	430	390	5.5	NS	**	NS
NDF (g·kg^{-1} DM)	666	683	680	665	4.0	NS	NS	NS
K (g·kg^{-1} DM)	31.4	26.2	34.6	29.8	0.98	*	*	NS
Ca(g·kg^{-1} DM)	1.7	1.0	1.4	1.5	0.08	*	*	**
Mg (g·kg^{-1} DM)	0.9	0.7	0.8	0.7	0.02	**	**	**
GE (MJ·kg^{-1} DM)	19.8	19.2	19.3	19.1	0.09	NS	NS	NS

S: standard level potassium fertilizer rate; H: high level potassium fertilizer rate; C: non-inoculant; I: inoculant; SEM: standard error of the mean; F: fertilizer rate; NS: not significant; *: $P < 0.05$; **: $P < 0.01$; DM: dry matter; NH_3-N: ammonium-nitrogen; TN: total nitrogen; V-score (X): $X > 80$, good; $60 < X \leq 80$, middle; $X \leq 60$, poor; OM: organic matter; CP: crude protein; EE: ether extract; ADF: acid detergent fiber; NDF: neutral detergent fiber; GE: gross energy.

Table 3. Digestibility, nutritive value and feed intake of silages.

Item	S		H		SEM	Significance		
	C	I	C	I		F	I	F × I
Digestibility								
DM	0.661	0.682	0.673	0.719	0.007	*	*	NS
OM	0.665	0.688	0.674	0.722	0.007	*	*	NS
CP	0.674	0.699	0.654	0.769	0.013	*	*	**
EE	0.715	0.693	0.730	0.755	0.010	NS	NS	NS
ADF	0.694	0.687	0.711	0.720	0.006	NS	NS	NS
NDF	0.702	0.711	0.707	0.738	0.007	NS	NS	NS
GE	0.647	0.664	0.656	0.704	0.007	NS	**	NS
Nutritive value								
TDN (g·kg^{-1} DM)	658	678	656	707	7.0	NS	**	NS
DE (MJ·kg^{-1} DM)	12.8	12.8	12.7	13.4	0.12	NS	NS	NS
Feed intake								
DM (g·kg$^{-0.75}$·day^{-1})	23.4	29.8	22.6	29.8	1.31	NS	*	NS
TDN (g·kg$^{-0.75}$·day^{-1})	15.9	20.8	15.2	21.8	0.99	NS	*	NS
DE (MJ·kg$^{-0.75}$·day^{-1})	0.31	0.39	0.29	0.41	0.02	*	*	NS

S: standard level potassium fertilizer rate; H: high level potassium fertilizer rate; C: non-inoculant; I: inoculant; SEM: standard error of the mean; F: fertilizer; NS: not significant; *: $P < 0.05$; **: $P < 0.01$; DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; ADF: acid detergent fiber; NDF: neutral detergent fiber; GE: gross energy; TDN: total digestible nutrients; DE: digestible energy.

observed in the TDN content ($P < 0.01$). The TDN content with the HI treatment was higher than that with the HC treatment; the difference between the HI and HC treatments was 51 g·kg^{-1} DM. For the feed intake of the silages, significant differences were shown in the DE intake in relation to fertilizer and the addition of inoculant ($P < 0.05$), and shown in DM and TDN intakes in relation to the addition of inoculant. The DM, TDN, and DE intakes were higher with the SI treatment than with the SC treatment, and higher with the HI treatment than with the HC treatment.

4. Discussion

The K content of grass in the H treatment was increased by 14.5% compared with that with the S treatment. Previous studies on the effects of K fertilizer levels on the K content in alfalfa demonstrated that, as the amount of K fertilizer increased, the K content also increased [27], and the K absorption and content of a Sudan and Rye grass mixture also increased [28]. The WSC content of grass in the H treatment was reduced by 13.2% compared with the S treatment. A large amount of glucose and a low amount of sucrose were contained in the WSC of grass. A lower amount of WSC is contained in timothy with a high K content cultivated in Hokkaido pastures, suggesting that the K content influences the accumulation of WSC [17].

Usually, silage is prepared by using forage crop and grass and these WSC play a major role during silage fermentation [1]. Therefore, it is important to study the relationship between amount of applied K fertilizer in pasture grass and WSC content of grass material. K influences photosynthesis, carbohydrate metabolism, and protein synthesis [29]-[31]. K deficiency increases the WSC content and decreases polymer material synthesis from low molecular weight compounds [31]. Few reports have been published on the effects of excessive K supply on the WSC content of pasture grass, however, their mechanism is unclear. In the present study, the relationship between the WSC of grass material and K contents in pasture grass or K supply was phenomenologically examined. The WSC content of timothy was reduced in conditions of excessive K supply. In contrast with this result, the WSC content of perennial ryegrass was increased by K supply in pasture grass. The most plausible explanation lies in the grasses with different sugar compositions. Timothy mainly contains long-chain fructosan, while perennial ryegrass mainly contains short-chain fructosan [32] [33]. Excessive K supply inhibits long-chain carbohydrate synthesis, increasing low-molecular-weight carbohydrates. Therefore, excessive K supply in pasture grass may increase the WSC content of perennial ryegrass, but decreased the WSC content of timothy. However, the difference between the S and H treatments was as small as 9.8 $g \cdot kg^{-1}$ DM. Oven dried (60˚C) forage crops had 19% lower water-soluble sugar content than freeze-dried forage crops [34]. Freeze drying will be required for measuring WSC contents in grass material.

To achieve a high fermentation quality for silage prepared from temperate grass, a WSC content of more than 65 $g \cdot kg^{-1}$ DM is needed [35]. The WSC contents of the timothy grass in the present study were 75.9 and 66.1 $g \cdot kg^{-1}$ DM with the S and H treatments, respectively. Specifically, the WSC content with the H treatment was lower than that with the S treatment, but it exceeded the required amount of 65 $g \cdot kg^{-1}$ DM. The WSC contents in the grass in the S and H treatments exceeded the value required to achieve a high fermentation quality. However, the fermentation quality in the SC and HC treatments was low, with a small difference between the two treatments. This is probably because the number and species of lactic acid bacteria were insufficient for lactic acid fermentation [1]. A high fermentation quality cannot be achieve with a small number of lactic acid bacteria, even if the WSC content in the grass material is sufficient [6].

In the SI and HI treatments with the addition of inoculant, the pH, butyric acid content, and NH_3-N ratio were low, and the lactic acid content and V-score were high, and fermentation quality was significantly improved compared with the SC and HC treatments, respectively. This was consistent with the result that the fermentation quality of timothy silage with additional 11F25, as in the present study, was better than that without inoculant [36]. In addition, in the present study, the DM contents in the SI and HI treatments were higher than those in the SC and HC treatments, because of later packaging in the SI and HI treatment areas. This may have influenced the fermentation quality.

The K content in the HC treatment was increased by only 10.2% compared with the SC treatment, which was a smaller difference than that in grass material. The effects of K fertilizer levels on the K content of silage may be attributable to the K content in the source grass. However, the K contents of the silage were reduced by 16.6% with the SI treatment compared with the SC treatment and by 13.9% with the HI treatment compared with the HC treatment, suggesting that the fermentation quality influences the amount of residual K. Only a few studies have been conducted on this relationship.

The addition increased the DM and OM digestibilities of silage. This is explained by the high fermentation quality in the SI and HI treatments and low loss of readily available carbohydrates, such as nitrogen-free extracts, in silage. The DM digestibility of grass silage is significantly improved by the addition of inoculant [37]-[39].

The TDN content of silage was significantly increased in the HI treatment. The digestibility in the HI treatment was high, ranging from 0.704 to 0.769 (**Table 3**), and the high digestibility may have increased the TDN content. The DM, TDN, and DE intakes in silage were high with the SI and HI treatments. The DM, TDN, and DE intakes with the HI treatment were significantly increased by 31.9%, 43.4%, and 41.4%, respectively, compared with the HC treatment. In studies in sheep fed on grass silage, the DM intake was positively correlated with the DM content and percentage of total organic acids to lactic acid, and was negatively correlated with the acetic acid content and NH_3-N ratio [40]-[42]. Similar results were obtained in a study of cattle fed on grass silage [43]. In the present study, the DM, TDN, and DE intakes in the SI and HI treatments were improved. This is explained by the significantly improved fermentation quality, high lactic acid content, low butyric acid content, and low NH_3-N ratio after the addition of inoculant. The TDN intake is improved by the synergistic effects of the increased TDN content and DM intake. The DE intake is improved by the increased DM intake.

5. Conclusion

The high K fertilizer rate (H treatment) increased the K content and decreased the WSC content in the grass. The decrease in the WSC content in the H treatment was as small as 9.8 $g \cdot kg^{-1}$ DM. The fermentation quality of silage in the SC and HC treatments was low, with a small difference between the treatments. The addition of inoculant (SI and HI treatments) significantly improved the fermentation quality and decreased the K content compared without the addition of inoculant (SC and HC treatments). The TDN contents and DM, TDN, and DE intakes with the SI and HI treatments were improved compared with the SC and HC treatments, respectively. A comparison of the effects of the fertilizer and the inoculant demonstrated negligible effects of the K fertilizer level and significant effects of the lactic acid bacteria inoculant on the fermentation quality, nutritive value, and feed intake of silage.

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