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Effect of grain vinegar feeding on milk production and fatty acid profile of Holstein cows

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Abstract

Incorporating organic acids into cattle feed should be carefully considered because dietary organic acids may affect voluntary feed intake and rumen fermentation. We conducted a feeding trial for the practical evaluation of grain vinegar. Lactating Holstein cows (n = 19) were divided into two groups, then were subjected to each of two treatments in a crossover design. The rumen fermentation parameters, blood urea nitrogen and non-esterified fatty acids (NEFA), milk composition, and milk fatty acid content were analyzed. No notable changes were observed in rumen fermentation parameters or blood metabolites. Corn silage intake, milk production, and 4% fat-corrected milk (FCM) were not affected by vinegar supplementation. The proportions of fatty acids in milk originating from de novo synthesis in the mammary gland were 25.2% and 25.4% in control and vinegar-fed groups, respectively. The levels of branched-chain fatty acids iso-C14:0, iso-C15:0, and iso-C16:0 were substantially decreased by vinegar supplementation, are known to be related to rumen environmental stress. This study showed that feeding grain vinegar to lactating dairy cows had no effect on feed intake, rumen fermentation, or milk production, although the proportion of some branched-chain fatty acids in the milk decreased.

Keywords: Dairy cow, Milk fatty acids, Vinegar

INTRODUCTION

Incorporating organic acids into feed has been considered to increase cow performance in terms of both feed quality and additional energy sources. During aerobic exposure to feed, chemical and organoleptic characteristics can change, resulting in a decrease in nutritional value and feed intake [1]. Organic acid supplementation reduces the aerobic deterioration of feed by depressing undesirable microorganisms, consequently stabilizing feed quality and intake [2].

Organic acids sprayed on feed or produced during silage fermentation are consumed together with the feed and are utilized by host animals. However, the feeding level of organic acids should be carefully considered because the response of animals to organic acid feeding is inconsistent and dietary organic acids have been shown to affect voluntary feed intake. Sheperd and Combs [3] reported that additional acetate and propionate were administered via intra-ruminal infusion to increase the body weight

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Authors' contributions
Conceptualization: Oh S, Kawai M, Ueda K.
Data curation: Oh S, Mitani T .
Formal analysis: Oh S, Mitani T.
Methodology: Oh S, Mitani T.
Software: Oh S.
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Ethics approval and consent to participate
The animal protocol was approved by the Institutional Animal Care and Use Committee of Hokkaido University (approval no. 20-0127).

of lactating cows. Intra-ruminal infusion of propionate into lactating cows causes hypophagia by increasing the oxidation of acetyl-CoA in the liver [4]. An increase in butyric acid in silage negatively affects silage intake in cows [5]. Previous studies have suggested that acetic acid in feed is associated with feed intake; however, the reason for this remains unclear [5,6].

Nevertheless, intra-ruminal infusion of acetic acid dose-dependently increases milk fat [3,6] because acetic acid is a lipogenic source for utilization in the mammary glands. Thus, we hypothesized that acetic acid supplementation might also change the fatty acid profile of milk. Therefore, we conducted a feeding trial using lactating Holstein cows to confirm the effects of commercially available vinegar on feed intake, milk composition, and milk fatty acids.

MATERIALS AND METHODS

Ethical approval

All animal experiments were performed in accordance with the Japanese Act on Welfare and Management of Animals. The animal protocol was approved by the Institutional Animal Care and Use Committee of Hokkaido University (approval no. 20-0127).

Animals, experimental design, and sampling

Lactating Holstein cows (n = 19) were divided into two groups (9 vs. 10 heads; 616 ± 42 vs. 642 ± 40 kg; mean ± standard deviation), considering parity numbers (2.6 ± 1.5 vs. 2.4 ± 1.6), milk yield (30.6 ± 6.8 vs. 29.0 ± 6.9 kg), and days in milk (DIM; 99 ± 68 vs. 130 ± 91 days), then were subjected to each of two treatments in a crossover design. Feeding was performed five times a day using an automatic feeder at 08:00, 12:00, 16:15, 20:00, and 23:00. The basal diet consisted of a mixture of corn silage, alfalfa hay, grass hay, and commercial concentrate, and the concentrate was top-dressed onto the forage mixture. Cows in vinegar feeding group were supplemented with 1 L vinegar (4.5% acetic acid, w/w) at 08:00 and 16:15 each (1.5 mol acetic acid/day). It was commercially produced via the fermentation of alcohol (grain-originated) to acetic acid; thus, no other organic acids were incorporated. Each period lasted 3 weeks, consisting of 17 days of adaptation and 4 days of sampling. Milk yield was monitored, and milk samples were collected at 08:30 and 15:30 during the first 3 days of sampling period. Ruminal fluid was collected at 14:00 on the last day of each sampling period. Feed residue was collected daily to calculate feed intake and drinking water intake was monitored using an individually attached water meter.

Rumen fermentation parameters

Volatile fatty acids (VFA) in rumen fluid were analyzed using a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) equipped with a capillary column (ULBON HR-20R, Shinwa Chemical Industries, Kyoto, Japan) and flame ionization detector. The rumen fluid samples were centrifuged at 10,000 rpm for 10 min, and the supernatant was mixed with 25% metaphosphoric acid dissolved in 5 N sulfuric acid at a ratio of 5:1. After 30 min, the samples were centrifuged at 9,000×g for 10 min, and the supernatant was mixed with crotonic acid (3 mmol/dL) as an internal standard in a 1:1 ratio. Ammonia nitrogen was colorimetrically analyzed using the indophenol reaction [7].

Blood urea nitrogen and non-esterified fatty acids

For plasma urea nitrogen analysis, the plasma was treated with urease and then analyzed using an indophenol reaction, similar to the ammonia analysis. Non-esterified fatty acids (NEFA) were analyzed using a commercial kit (NEFA C test Wako, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) according to the manufacturer's guidelines.

Milk composition and fatty acids

Milk composition was analyzed using a Lactoscope (PerkinElmer, Waltham, MA, USA). Milk fatty acids were analyzed as described previously [8]. Briefly, total milk fatty was extracted using Gottlieb method [9], milk fatty acids were then methylated according to International Organization for Standardization and International Dairy Federation [10], then the fatty acid methyl esters were analyzed using gas chromatography equipped with flame ionization detector and fused silica capillary column (SP-2560, 100 m length × 0.25 mm internal diameter, Sigma-Aldrich Japan, Tokyo, Japan). Each fatty acid methyl ester was identified using a standard mix (Supelco 37-Component FAME Mix, Sigma-Aldrich, Tokyo, Japan and GLC-603 FAME mix, Nu-chek-Prep, Elysian, MN, USA).

Statistical analysis

Data obtained from the feeding trial using lactating cows, including feed intake, water intake, rumen fermentation parameters, milk yield, milk composition, and milk fatty acids, were subjected to analysis of variance (ANOVA) using the linear mixed model procedure in SPSS (IBM SPSS Statistics, version 26, Amonk, NY, USA). The model includes the effects of treatment, sequences of different treatments, periods, random effects of animals within sequences, and residual errors. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Feed intake and vinegar supplementation

The chemical composition and intake of the basal diet are shown in Table 1. The intake of corn silage and concentrate by the cows in both groups did not differ. It is likely that the smell of vinegar (4.5% acetic acid) does not affect feed intake, as observed in studies on oral administration and intra-ruminal infusion. Daniel et al. [11] reported temporal feed intake depression when diluted 33% acetic acid (1.5 mol/day) was fed to mid-lactation cows, in which feed intake notably decreased until the initial 3 weeks but for that of entire period did not differ.

Other studies have also reported a decrease in feed intake during intra-ruminal infusion of acetic

Table 1. Chemical composition of basal diet and intake of feed, drinking water, and vinegar

	Control	Vinegar
Ingredients (% as fed basis)		
Corn silage	51.3	
Alfalfa hay	2.85	
Grass hay	2.85	
Commercial concentrate	43.0	
Chemical composition		
Dry matter (%)	53.4	
Organic matter (% of DM)	94.1	
Crude protein (% of DM)	12.1	
Neutral detergent fiber (% of DM)	41.8	
Intake		
Feed (kg DM/d)	18.9	18.8
Drinking water (L/d)	65.6	67.1
Grain vinegar (L/d)	0	2

DM, dry matter.

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acid at a dose of 6 mol/8 h/day [12]. A recent meta-analysis suggested that acetic acid in silage should be less than 17 g/kg dry matter (DM) in dairy cattle to avoid a decrease in feed intake [13]. Buchanan–Smith [14] noted that acetic acid in silage linearly decreases silage intake in sheep, and this phenomenon could be attributed to postprandial effects, including rumen motility and removal of digesta from the rumen. Therefore, excessive amounts of acetic acid supplementation without pH adjustment may change the rumen environment, including the pH, although the maximum allowance is not clear because each study used different basal diets and individuals.

Rumen fermentation parameters

The concentration and molar proportion of VFA were not affected by vinegar supplementation (Table 2). Thus, vinegar was fed twice a day (1.5 mol/day) at 0:800 and 16:00, indicating that 0.75 M of additional acetic acid was diluted or removed from the rumen within 5 h.

A recent study showed that the intraruminal infusion of acetic acid (15 mol/day) decreased VFA concentration and increased ruminal pH in lactating Holstein cows [6]. Gheller et al. [1] fed organic acid-based additives to dairy cows and observed an increase in pH and a decrease in VFA concentration. Sheperd and Combs [3] also reported that intra-ruminal infusion of acetate (36 mol/day) or propionate (20.5 mol/day) increased ruminal pH in lactating cows and that rumen liquid volume was greater with acetate infusion than with propionate infusion. Therefore, it is likely that ruminal fluid is diluted owing to the increase in osmolarity caused by organic acid supplementation, although it is not supported by evidence [15]. However, our results did not support rumen liquid dilution by organic acid feeding, as observed by the results of VFA and drinking water intake. Therefore, vinegar supplementation at a practical level had no effect on rumen dilution.

Milk production, composition, and fatty acids

Milk production and composition are shown in Table 3. The milk production and 4% fat corrected milk were not affected by vinegar intake. The proportions of fat, protein, and solids, but not fat, were also not affected by vinegar feeding. Lactose and milk urea nitrogen were lower in vinegar feeding

Table 2. Effect of grain vinegar feeding on rumen fermentation parameters

	Control[1)]	Vinegar	SEM	*p*-value
Total VFA (mmol/dL)	12.22	11.29	0.38	0.349
Acetate	8.12	7.46	0.26	0.176
Propionate	2.21	2.07	0.06	0.440
iso-Butyrate	0.08	0.09	0.00	0.308
n-Butyrate	1.40	1.30	0.05	0.398
iso-Valerate	0.22	0.20	0.01	0.357
n-Valerate	0.18	0.16	0.01	0.217
Molar ratio (mmol/100 mmo/l)				
Acetate	66.51	66.00	0.30	0.067
Propionate	18.17	18.46	0.25	0.883
iso-Butyrate	0.69	0.79	0.02	0.595
n-Butyrate	11.36	11.48	0.18	0.801
iso-Valerate	1.78	1.80	0.04	0.714
n-Valerate	1.49	1.46	0.02	0.355
A/P ratio	3.68	3.63	0.07	0.351
Ammonia nitrogen (mgN/dL)	7.45	7.64	0.45	0.659

[1)]Control, no treatment; Vinegar; 2 L of 4.5% acetic acid equivalent per day.VFA, volatile fatty acids.

Table 3. Effect of grain vinegar feeding on milk composition, and blood metabolites

	Control[1)]	Vinegar	SEM	*p*-value
Milk				
Production (kg/day)	27.3	26.7	0.300	0.057
4% fat corrected milk (kg/day)	28.7	28.3	0.270	0.130
Fat (%)	4.48	4.50	0.041	0.636
Protein (%)	3.49	3.50	0.017	0.678
Lactose (%)	4.49	4.47	0.009	0.008
Solid not fat (%)	8.98	8.96	0.018	0.301
Urea nitrogen (mgN/dL)	10.6	10.0	0.229	0.012
NE_L (Mcal/kg milk)	1.06	1.07	0.004	0.637
Blood metabolites				
Non-esterified fatty acid (μEq/L)	51.04	47.75	4.26	0.412
Blood urea nitrogen (mgN/dL)	11.44	11.21	0.48	0.693

[1)]Control, no treatment; Vinegar; 2 L of 4.5% acetic acid equivalent per day.

group ($p < 0.05$). Cows in both treatments consumed the same amount of feed, and it appears that vinegar did not act as an additional energy source for fatty acid synthesis in the mammary glands.

NEFA concentrations did not show any apparent differences between the treatments (Table 3). This result is consistent with those of other studies on intra-ruminal infusion of acetate [3,6]. The uptake of NEFA in the mammary gland depends on circulation and is utilized as milk fat [16]. Therefore, in this study, the difference in body fat mobilization was negligible for milk fat.

The milk fatty acid profile is presented as a percentage of total fatty acids (Table 4). Only a few specific fatty acids differed between the groups. The proportion of de novo fatty acids was not affected by grain vinegar feeding (1.5 mol/day). Acetic acid is a lipogenic source used for *de novo* synthesis in the mammary gland [17]. Another trial with cows fed acetic acid (1.5 mol/day) reported no change in milk fat among the treatments [11], although other studies have reported that intra-ruminal injection of acetic acid dose-dependently increased milk fat (36 mol/day [3]; 0–15 mol/day [6]). Therefore, oral administration of acetic acid at 1.5 mol/day does not seem to be effective in improving milk fat. Vinegar supplementation did not affect fatty acids of carbon length 16 (some portion) or longer, which are assumed to be derived from the fatty acids in the feed. Some species of branched-chain fatty acids were substantially decreased in milk from the vinegar-fed group, namely, iso-C14:0, iso-C15:0, and iso-C16:0. Milk odd-and branched-chain fatty acids reflect rumen fermentation and microbial synthesis [18,19]. These fatty acids are derived from the membrane of rumen bacteria and thus show a positive correlation with dietary forage [20] and rumen environmental stresses, such as minimum pH [21]. As corn silage intake was not decreased by vinegar feeding, local environmental stress to specific groups of bacteria may have been caused by vinegar, which has a low pKa value.

For lactating cows, 1.5 mol/day grain vinegar feeding did not improve animal performance. Feed intake, rumen fermentation, and milk production remained unaffected. However, the levels of some branched-chain fatty acids decreased in the vinegar-fed group, implying that some rumen bacteria were affected by the acetic acid of vinegar. More research is needed as a biomarker that reflects the rumen environment. Although smell of vinegar seems not to affect palatability, direct feeding of vinegar at a practical level cannot be expected to improve milk production. Further studies should be conducted to investigate the possibility of improvement by using vinegar to prevent aerobic spoilage of silage.

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Table 4. Effect of grain vinegar feeding on milk fatty acid profile of Holstein cows

% of total FA[1)]	Control[2)]	Vinegar	SEM	*p*-value
C4:0	2.106	2.066	0.04	0.151
C5:0	0.015	0.015	0.00	0.797
C6:0	1.752	1.744	0.02	0.668
C7:0	0.024	0.024	0.00	0.787
C8:0	1.194	1.205	0.01	0.499
C9:0	0.032	0.032	0.00	0.828
C10:0	3.101	3.156	0.06	0.456
C11:0	0.333	0.343	0.01	0.446
C12:0	3.778	3.856	0.01	0.494
iso-C13:0	0.026	0.025	0.00	0.737
iso-C14:0	0.166	0.146	0.01	0.018
C14:0	12.305	12.383	0.17	0.495
iso-C15:0	0.192	0.181	0.00	0.006
t-C14:1	0.009	0.011	0.00	0.018
anteiso-C15:0	0.495	0.484	0.01	0.278
C14:1	0.898	0.945	0.05	0.291
C15:0	1.084	1.073	0.03	0.688
iso-C16:0	0.034	0.032	0.01	0.034
C16:0	32.917	32.996	0.04	0.898
iso-C17:0	0.286	0.278	0.01	0.161
C16:1	1.664	1.689	0.05	0.705
C17:0	0.519	0.508	0.01	0.566
C18:0	10.519	10.287	0.41	0.605
t6-C18:1	0.278	0.270	0.00	0.085
t9-C18:1	0.203	0.200	0.00	0.478
t10-C18:1	0.294	0.290	0.00	0.545
t11-C18:1	1.184	1.128	0.04	0.273
c6-C18:1	0.393	0.382	0.01	0.140
c9-C18:1	17.800	17.911	0.35	0.670
c11-C18:1	0.427	0.432	0.02	0.649
c13-C18:1	0.232	0.226	0.00	0.299
c15-C18:1	0.269	0.264	0.00	0.370
C19:0	0.105	0.102	0.00	0.396
c17-C18:1	0.224	0.222	0.00	0.772
c12,15-C18:2	0.041	0.037	0.00	0.057
n6-C18:2	1.869	1.885	0.03	0.673
C20:0	0.127	0.125	0.00	0.718
n6-C18:3	0.025	0.024	0.00	0.530
t11-C20:1	0.008	0.007	0.00	0.384
c11-C20:1	0.081	0.082	0.00	0.819
n3-C18:3	0.261	0.257	0.01	0.675
c9t11-C18:2	0.576	0.566	0.02	0.774
t10c12-C18:2	0.007	0.006	0.00	0.700
C21:0	0.015	0.015	0.00	0.861
c9c11-C18:2	0.007	0.007	0.00	0.745
C20:2	0.031	0.032	0.00	0.727

Table 4. Continued

% of total FA[1)]	Control[2)]	Vinegar	SEM	*p*-value
C22:0	0.036	0.034	0.00	0.462
n6-C20:3	0.083	0.082	0.00	0.781
n6-C20:4	0.106	0.108	0.00	0.399
C23:0	0.016	0.016	0.00	0.958
n3-20:5	0.022	0.024	0.00	0.034
C24:0	0.024	0.023	0.00	0.557
C22:4	0.018	0.017	0.00	0.526
n3-C22:5	0.047	0.046	0.00	0.742
De novo	25.17	25.38	0.31	0.473

[1)]Proportion of fatty acids from de novo synthesis was calculated as the sum of 4- to 16-carbon fatty acids, odd and branched chain fatty acids were excluded.

[2)]Control, no treatment; Vinegar; 2 L of 4.5% acetic acid equivalent per day.

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