



Title	Effects of grazing adaptation on intake, ruminal fermentation, blood metabolites, and body weight change in dairy cows after turning out to pasture in early spring
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1 **Title:**

2 Effects of grazing adaptation on intake, ruminal fermentation, blood metabolites, and
3 body weight change in dairy cows after turning out to pasture in early spring

4

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1 ABSTRACT

2 This study investigated the effect of adaptation to grazing in early spring on herbage
3 intake, ruminal fermentation parameters, blood metabolite concentrations, and body
4 weight change in dairy cows. The experiment was conducted on eight rumen-cannulated
5 non-lactating cows in the early spring period. Four cows were adapted to grazing by
6 stocking for four hours for one week (ADP group). The other cows were kept in a barn
7 during the period (CON group). Then, both groups of cows were stocked together
8 throughout a day on a 1 ha pasture for three weeks (experimental period). In the first week
9 of the experimental period, compared to the CON group, the ADP group had a higher
10 herbage intake, ruminal NH₃-N and total VFA concentration, and blood urea
11 concentration, but the NEFA concentration was lower in the ADP group ($P < 0.01$).
12 During the subsequent weeks, there were little differences in ruminal fermentation
13 parameters and blood metabolites. Cows in the ADP group maintained their body weight,
14 but cows in the CON group lost 60 kg of body weight in the first week of the experimental
15 period.

16

17 Key words:

18 blood metabolites, body weight change, grazing adaptation, herbage intake, ruminal
19 fermentation

20

1 INTRODUCTION

2 Feeding management incorporating stocking for dairy cows has been widely used in
3 temperate regions all over the world. In recent years, grazing management for dairy cows
4 has been reevaluated due to many aspects such as animal welfare, low costs of feed, labor,
5 manure handling, and low environmental loads. However, in temperate regions, sufficient
6 stocking throughout the year is impossible because of the low growth of temperate grass
7 in the winter season. Moreover, in snowy areas, cows cannot be stocked during winter
8 and are necessarily in a barn and fed conserved feeds. In using a grazing system in such
9 situations, grazing cows are exposed to dramatic changes in feeding management between
10 indoor feeding and stocking in early spring and in autumn.

11 When cows suddenly changed to stocking from indoor feeding, a temporary decrease
12 in dry matter (DM) intake, body weight (BW), and milk production were observed
13 (Charmley, Jannasch & Boyd, 2003; Khanal, Dhiman & Boman, 2008). These decreases
14 were more remarkable and it took more time for the recovery of milk compositions
15 compared to the feeding change in autumn (Hartwiger et al., 2018a; Schären et al., 2016a).
16 When cows turned out to stocking without adaptation, excessively decreased milk yields
17 (Schären et al., 2016a), drastically changed milk compositions (Mitani et al., 2011;
18 Khanal et al., 2008), and temporary decreased body weight for steers (Charmley et al.,
19 2003) were reported in spring. These results indicated that a temporary energy deficit
20 could occur in the early period after starting stocking.

21 To reduce production depression, adaptation to grazing in early spring has been
22 empirically recommended for cows to transfer smoothly from indoor feeding to stocking
23 rather than not adapting to grazing. However, there is no unified view for the effectiveness
24 of adaptation to grazing in the early spring period for stocking cows. When cows were
25 gradually adapted to grazing, milk yields immediately increased after a day of stocking;

1 however, when cows suddenly turned out to one-day grazing, it took one week to increase
2 milk yield (Sato et al., 1981). When the cows were fed with poor quality feeds, such as
3 low-quality hay before starting one-day grazing, milk yields suddenly increased after
4 starting one-day grazing (Coulon, D'Hour & Petit, 1988). In a series of grazing studies
5 conducted in Germany, milk production changes were compared between cows gradually
6 adapted to grazing and cows fed a total mixed ration (TMR) continuously (Hartwiger et
7 al., 2018a; Schären et al., 2016a). In these studies, even though cows were adapted
8 gradually to grazing before starting one-day grazing, these cows either recovered after
9 three weeks (Schären et al., 2016a) or did not (Hartwiger et al., 2018a), to similar milk
10 yield, as cows fed TMR.

11 To construct an efficient grazing adaptation practice, we should clarify the
12 mechanisms by which cows adapt to grazing circumstances. Therefore, investigating
13 changes in ruminal fermentation and blood metabolites in cows during the transition
14 period from indoor to stocking is necessary. Investigating the change in herbage DM
15 intake is also necessary because herbage DM intake is a primary factor in the change of
16 ruminal fermentation and blood metabolites. It is known that herbage DM intake
17 temporarily decreases during the transition period from indoors to stocking. In this study,
18 by comparing cows that have adapted to grazing and those that have not adapted, we
19 investigated the effect of short time-grazing prior to turning out from indoor feeding to
20 stocking on the changes in intake, body weight, ruminal fermentation and blood
21 metabolites in the early spring period, using cannulated non-lactating cows.

22

23 **2 MATERIALS AND METHODS**

24 **2.1 Animals and experimental design**

25 The methods for feeding management and ruminal cannulation surgery for the cows used

1 in this study were approved by the Animal Care and Welfare Committee of Hokkaido
2 University (No. 15-0123).

3 This study was conducted between May 1 and May 29, 2017 at the Experiment Farm
4 of the Field Science Center for Northern Biosphere, Hokkaido University (Sapporo,
5 Japan). Eight ruminal cannulated, non-lactating cows that were adapted to grazing for
6 several years (mean BW \pm standard deviation [SD] at the start of experiment: 741 ± 56
7 kg) were considered for the experiment. The cows were kept in a barn prior to the
8 experiment and were supplied with 35 kg of fresh matter (FM)/day consisting of a mixed
9 silage in which corn silage and hay were available at a ratio of 93:7 on an FM basis. Eight
10 cows were divided into two groups based on their initial BW. Cows in one group were
11 subjected to a 1-week adaptation to grazing (ADP group), stocked for 4 h (15:00 to 19:00)
12 and supplied with 15 kg FM/day of mixed silage from May 1 to May 7 (adaptation period).
13 During this period, the other cows were kept in the barn and supplied with 35 kg FM/day
14 of mixed silage (CON group). The cows were stocked on a 1.0 ha pasture that consisted
15 primarily of kentucky blue grass (*Poa pratensis* L.) and white clover (*Trifolium repens*
16 L.). Both the cow groups were turned out to one-day grazing together on the pasture from
17 May 8 to May 29 (experimental period). All cows could freely access water and mineral
18 blocks on the pasture.

19

20 **2.2 Vegetation survey**

21 Stocking on the pasture was based on a continuous stocking method. Herbage mass
22 and sward surface height of the pasture were measured once a week using a 0.5×0.5 m
23 quadrat. Herbage mass and grass sward height did not drastically change and were
24 sufficient for stocking for eight cows (mean \pm SD: 1.7 ± 0.2 t of DM/ha and 7.2 ± 1.8 cm,
25 respectively). Herbage samples were hand-plucked, mimicking grazing behavior at 16:00

1 hours in the adaptation period and at 08:00 and 19:00 hours in the experimental period.
2 Herbage samples were composited for a week and stored at -20°C . Samples of mixed
3 silage supplied in the adaptation period were also collected every day, and composited for
4 a week and stored at -20°C . They were lyophilized and ground through a 1-mm screen.
5 The feed samples were analyzed for DM, organic matter (OM), and CP using the method
6 of the Association of Official Analytical Chemists (AOAC, 1990). Ash-free neutral
7 detergent fiber (NDFom) was measured according to the method described by Van Soest,
8 Robertson & Lewis (1991). Water-soluble carbohydrates (WSC) were determined using
9 the anthrone method (Yemm & Willis, 1954).

10

11 **2.3 Feeding time and herbage intake**

12 Feeding time of herbage (min/day) was measured using the Kenz Lifecorder EX
13 (Suzuken Co. Ltd., Nagoya, Japan) according to the method described by Ueda et al.
14 (2011). The device was attached under the cow's necktie, which recorded the intensity of
15 the physical activity of the cow's head. The intensities every 4 s were recorded during the
16 4 weeks of the experimental period. The cumulative time of the intensity over 1 of 11
17 scales in 24 h was regarded as the feeding time of herbage.

18 Herbage DM intake during in the adaptation and experimental periods was measured
19 using the double-indicator method, using the lanthanum (La) maker as an external marker
20 and C_{33} alkane as an internal marker (Ueda, Mitani & Kondo, 2016a). The La maker was
21 prepared by soaking the $\text{LaCl}_3 \cdot 7\text{H}_2\text{O}$ solution into sugar beet-pulp. Fifty grams of the La
22 maker was thrown into the rumen via the ruminal cannula at 08:00 and 19:00 hours daily.
23 The feces in the rectum were collected at 08:00 and 19:00 hours, and were composited
24 on days 1-3, days 4-7 in the adaptation period, and days 1-3, days 4-7, days 8-11, days
25 15-17, and days 18-21 in the experimental period. The concentrations of La in the fecal

1 samples and the La marker were determined using an inductively coupled plasma mass
2 spectrometer (ERAN DRACE; Perkin Elmer, Tokyo, Japan) after acid digestion. The
3 concentrations of C₃₃ alkane in the herbage, mixed silage, and fecal samples were
4 determined using gas chromatography (GC-2010; Shimadzu, Kyoto, Japan) after alkaline
5 saponification. Herbage DM intake was estimated using the following equation: herbage
6 intake (kg of DM/day) = [C₃₃ alkane concentration in feces (mg/kg of DM) × La intake
7 from marker (mg/day)]/[C₃₃ alkane concentration in herbage (mg/kg of DM) × Fecal La
8 concentration (mg/kg of DM)]. The herbage DM intake during the adaptation period was
9 calculated after adjusting the C₃₃ alkane intake from mixed silage.

11 **2.4 Body weight, ruminal fermentation and blood metabolites**

12 The body weight of each cow was measured at 19:00 h on the day before the adaptation
13 period started, days 4, and 7 during the adaptation period, and days 1, 2, 3, 4, 5, 6, 7, 10,
14 14, 17, and 21 in the experimental period using an electronic scale (Orionkikai Co. Ltd.,
15 Nagano, Japan). Rumen fluid and blood samples were also collected. Rumen fluid was
16 collected using a 50 mL syringe via the rumen cannula. Immediately after collection, the
17 rumen fluid pH was measured using a portable pH meter (D-51T; Horiba, Kyoto, Japan).
18 The fluid samples were centrifuged (4°C, 3000 rpm, 20 min), and the supernatants were
19 stored at – 80°C until further analysis. NH₃-N concentration was determined using the
20 indophenol method (Wetherburn, 1967), and each volatile fatty acid (VFA) concentration
21 was determined using a gas chromatograph (GC-2010; Shimadzu, Kyoto, Japan)
22 according to the method (Ueda, Mitani & Kondo, 2016b). Blood samples were collected
23 from the jugular vein using vacuum blood tubes containing heparin. The blood samples
24 were centrifuged (4°C, 3000 rpm, 20 min), and the blood plasma was stored at – 80°C
25 until analysis. The urea concentration of the plasma (BUN) was determined using the

1 urease-indophenol method (Wetherburn, 1967). The non-esterified fatty acid (NEFA) and
2 blood glucose concentrations of the plasma were determined using a commercial kit
3 (NEFA C-TEST Wako and Glucose CII-TEST Wako, respectively; Wako Pure Chemicals,
4 Osaka, Japan).

5

6 **2.5 Statistical analysis**

7 Statistical analysis was conducted using JMP Pro 14.3 (SAS Institute Inc., Cary, NC,
8 USA). The data in the experimental period were analyzed with a repeated MIXED model
9 using the Fit Model Platform in the JMP. The model included treatment (ADP or CON),
10 days of sampling, and interactions between those as fixed effects, cows as a random effect,
11 and day of sampling as a repeated effect adjusting a first-order autoregressive structure
12 as a correlation structure. If the probability were less than 0.05 or 0.10, the result was
13 regarded as significant or tendency, respectively. The results are shown as least square
14 means and standard errors of means.

15

16 **3 RESULTS**

17 **3.1 Feed chemical compositions, herbage intake, and body weight change**

18 The chemical compositions of the mixed silage in the adaptation period and herbage are
19 shown in Table 1. The CP content of herbage maintained more than 25% of DM
20 throughout the experiment but decreased from 32.7% of DM in the adaptation period to
21 25.2% of DM at the end of the experiment. The NDF content of herbage increased from
22 29.9% in the adaptation period to 43.2% of DM at the end of the experiment. The WSC
23 content of herbage showed the highest level at 19.6% of DM at the start of experiment
24 but decreased to 10.0% of DM at the end of the experiment. The mixed silage had a lower
25 CP content and higher NDF content compared to herbage.

1 The changes in herbage intake and herbage feeding time are shown in Table 2. During
2 the experimental period, the herbage intake of cows in the ADP group tended to be higher
3 than that of cows in the CON group ($P = 0.06$) but the change in herbage intake between
4 the ADP and CON groups was significantly different ($P < 0.01$). Cows in the ADP group
5 consumed 6 to 7 kg DM/day of herbage and 4.7 kg DM/day of mixed silage (data not
6 shown) during the adaptation period. After the start of the experimental period, cows in
7 the ADP group consumed 9.2 kg DM/day of herbage during the first 3 days and
8 maintained more than 9 kg DM/day until the end of the experiment. In the CON group,
9 the cows consumed 10.6 kg DM/day of mixed silage in the adaptation period (data not
10 shown), and they consumed only 4.0 kg DM/day of herbage at the start of the
11 experimental period. Cows in the CON group required one week to reach the herbage
12 intake as the cows in the ADP group.

13 The change in herbage feeding time was similar to the change in herbage intake. The
14 feeding time of the ADP group was between 116 and 154 min/day in the adaptation period,
15 doubled at the start of the experimental period, and then increased until the end of the
16 experiment. In the CON group, the cows spent half the time of the ADP group at the start
17 of the experimental period and increased until the end of the experimental period. Cows
18 in the CON group took one week to reach the same level of herbage feeding time as those
19 in the ADP group.

20 The change in BW is shown in Figure 1. During the adaptation period (days – 7 to –
21 1 in figure), there was no difference in BW between the ADP and CON groups. In the
22 experimental period, the average BW throughout the experimental period did not differ
23 between the ADP and CON groups (767 and 732 kg, respectively; $P = 0.42$), but the
24 changes were significantly different between the ADP and CON groups ($P < 0.01$). Cows
25 in the ADP group maintained their BW for seven days after the start of the experimental

1 period, and then slightly increased. While cows in the CON group lost 60 kg of BW four
2 days after turning out from indoor to stocking, they gained BW gradually. However, BW
3 of cows in the CON group did not reach a similar level of BW of cows in the ADP group
4 even at the end of the experimental period.

6 **3.2 Ruminal fermentation**

7 The changes in ruminal pH, NH₃-N, and total VFA concentrations are shown in Figure 2.
8 During the adaptation period (days - 3 and - 1 in figure), the ruminal pH in the ADP group
9 was lower than that in the CON group. During the experimental period, the average
10 ruminal pH in the ADP group tended to be lower than that in the CON group (6.0 and 6.2,
11 respectively; $P = 0.09$), but the changes were significantly different between the ADP and
12 CON groups ($P < 0.01$). Ruminal pH in the ADP group remained at a low level one week
13 after starting the experimental period and then gradually increased. While the ruminal pH
14 in the CON group was higher than that of the ADP group during days 2 and 4, it changed
15 similarly to that in the ADP group until the end of the experiment.

16 In the adaptation period, ruminal NH₃-N concentrations in the ADP group were higher
17 than those in the CON group. In the experimental period, the average ruminal NH₃-N
18 concentration in the ADP group (36.0 mg/dL) was higher than that in the CON group
19 (19.9 mg/dL, $P = 0.03$), and the changes in ruminal NH₃-N concentration also differed
20 significantly between the ADP and CON groups ($P < 0.01$). After the start of the
21 experimental period, the ruminal NH₃-N concentration of the ADP group continued to
22 increase and reached 50 mg/dL on day 4 in the period, and then gradually decreased until
23 day 10 and remained constant until the end of the experiment. In the CON group, ruminal
24 NH₃-N concentration drastically increased on day 1 in the experimental period from that
25 in the adaptation period but maintained a low concentration compared with that in the

1 ADP group throughout the experimental period.

2 During the adaptation period, the total VFA concentrations in the rumen were higher
3 in the ADP group than in the CON group. During the experimental period, the average
4 total VFA concentrations in the rumen were also higher in the ADP group (16.8 mmol/dL)
5 compared with that in the CON group (14.8 mmol/dL: $P = 0.05$), and the changes in total
6 VFA concentration in the rumen also differed significantly between the ADP and CON
7 groups ($P < 0.01$). In the ADP group, the total VFA concentration in the rumen remained
8 constant after the start of the experimental period. In the CON group, the total VFA
9 concentration in the rumen temporarily increased to a level similar to that in the ADP
10 group but decreased until day 4 in the experimental period. Total VFA concentrations in
11 the rumen of the CON group were similar to those of the ADP group from day 7 to the
12 end of the experiment.

13 During the adaptation period, the proportions of acetate were lower but those of
14 propionate and butyrate were higher in the ADP group than in the CON group. In the
15 experimental period, compared to the CON groups, the average ruminal VFA proportion
16 for ADP groups tended to be lower in acetate and, higher in butyrate, but did not differ in
17 propionate (acetate: 56.8 and 59.9; $P = 0.06$, propionate: 21.1, and 20.0; $P = 0.36$,
18 butyrate: 16.8 and 14.8, mmol/100 mmol; $P = 0.04$, respectively). The changes in each
19 ruminal VFA proportion significantly differed between the ADP and CON groups ($P <$
20 0.01). The proportions of ruminal acetate in the ADP group were also lower than those in
21 the CON group at the start of the experimental period. The proportions of ruminal acetate
22 in the CON group gradually decreased after turning out from indoor to stocking, and there
23 was no difference in those between the ADP and CON groups after day 6 until the end of
24 the experiment. The changes in ruminal propionate and butyrate proportions in the ADP
25 and CON groups were opposite to the change in the ruminal acetate proportion.

1

2 **3.3 Blood metabolites**

3 The changes in BUN, NEFA, and glucose concentration in the plasma are shown in Figure
4 3. During the adaptation period, BUN concentrations in the ADP group were higher than
5 those in the CON group. During the experimental period, the average BUN concentration
6 in the ADP group (21.8 mg/dL) was higher than that in the CON group (16.8 mg/dL, $P <$
7 0.01), and the changes in BUN concentration also significantly differed between the ADP
8 and CON groups ($P < 0.01$). In the ADP group, the BUN concentration was maintained
9 at a high level throughout the experimental period. While the BUN concentration in the
10 CON group rapidly increased from day 1 to day 2, it was maintained at a lower level than
11 that in the ADP group throughout the experiment.

12 During the adaptation period, NEFA concentrations in the ADP group were higher
13 than those in the CON group. During the experimental period, there was no difference in
14 the average NEFA concentration between the ADP and CON groups (168 and 202 μ Eq/L,
15 respectively: $P = 0.23$), but the changes in NEFA concentration significantly differed
16 between the ADP and CON groups ($P < 0.01$). In the ADP group, the NEFA concentration
17 drastically decreased after turning out from indoor to stocking and was maintained at a
18 constant level until the end of the experiment. In the CON group, the NEFA concentration
19 drastically increased from day 1 to day 2 in the experimental period and maintained a
20 high level for five days and then decreased. Following that, there was no difference in
21 NEFA concentration between the ADP and CON groups until the end of the experiment.

22 During the adaptation period, the blood glucose concentrations in the ADP group were
23 lower than those in the CON group. During the experimental period, there was no
24 difference in the average blood glucose concentration between the ADP and CON groups
25 (66.2 and 65.3 mg/dL, respectively). Although the changes in blood glucose differed

1 statistically between the ADP and CON groups ($P = 0.02$), the blood glucose level, both
2 in the ADP and CON groups, changed similarly throughout the experimental period.

3 4 **4 DISCUSSION**

5 The results from the present study clearly indicate the effectiveness of maintaining
6 herbage intake and BW for the adaptation to grazing before turning out to one-day grazing.

7 The results of changes in ruminal fermentation and blood metabolites supported the
8 usefulness of the adaptation to grazing. The effectiveness and mechanisms can be
9 explained by the change and difference in herbage intake between the ADP and CON
10 groups. The present study first clarified the effectiveness of the adaptation to grazing in
11 terms of maintaining herbage intake by continuously measuring herbage intake, not
12 conducted in previous studies (Charmley et al., 2003; Khanal et al., 2008, Schären et al.,
13 2016a).

14 15 **4.1 Ruminal fermentation and blood metabolites**

16 The effect of grazing adaptation on ruminal fermentation was observed for one week after
17 turning out to one-day grazing. High $\text{NH}_3\text{-N}$ and VFA concentrations in the ADP group
18 resulted from high intake of early spring herbage. In early spring, herbage generally has
19 extremely high CP, and the ruminal degradation rate is also high, although early spring
20 herbage has high WSC, which is easily degradable in the rumen (Wales, Dellow & Doyle,
21 1999). Therefore, when cows consumed much of the early spring herbage such as in the
22 ADP group, ruminal $\text{NH}_3\text{-N}$ and total VFA concentrations elevated.

23 The high proportion of ruminal propionate in the ADP group for one week after
24 turning out to one-day grazing also resulted from high intake of early spring herbage
25 which contained extremely high WSC, especially sugar. On the other hand, the high

1 proportion of ruminal acetate in the CON group during this period could be due to the
2 supply of mixed silage in the adaptation period, which remained in the rumen for some
3 days after turning out to one-day grazing.

4 BUN and NEFA concentrations were also affected mainly by the change in
5 herbage intake between the ADP and CON groups. High BUN concentration in the ADP
6 group after turning out to one-day grazing resulted from a high ruminal $\text{NH}_3\text{-N}$
7 concentration, which was converted into urea in the liver. In the ADP group, the blood
8 NEFA concentration was lower than that in the CON group and maintained at a constant
9 level. This result indicated that cows in the ADP group smoothly adapted to one-day
10 grazing and maintained sufficient energy supply until the end of the experiment. However,
11 elevated blood NEFA concentration in the CON group during the first week of the
12 experiment indicated that cows in the CON group had an extreme negative energy status
13 during this period.

14 More than one week was required to stabilize ruminal fermentation parameters
15 and blood metabolites in both groups. This was because the WSC content in the herbage
16 decreased and was maintained at a constant level, and the herbage intake in both groups
17 was similar after one week in the experimental period. In addition, ruminal microbes
18 might also have adapted to herbage digestion. It has been reported that two to three weeks
19 were needed for the ruminal microbial flora to adapt to herbage degradation during the
20 transition from indoor feeding to stocking (Hartwiger et al., 2018b; Schären et al., 2016b).

21

22 **4.2. Herbage intake**

23 It is difficult to explain why cows in the CON groups could not ingest herbage
24 satisfactorily after turning out to one-day grazing. It is a well-known theory that voluntary
25 intake of ruminants is controlled by the physical signal from ruminal extension and the

1 metabolic signal from fermentation products in the rumen (Forbes, 2007). There was a
2 possibility that the physical fullness of the rumen limited herbage intake in the CON
3 group, although the ruminal degradability of spring herbage was so high and the herbage
4 intake in the CON group was low. This was because the ruminal microbes could not adapt
5 to digest early spring herbage, and some of mixed silage ingested during the adaptation
6 period continued to remain in the rumen. The nutritive status of the CON group was
7 clearly lower than that of the ADP group because, in the CON group, the total VFA
8 concentration in the rumen decreased and the NEFA concentration increased for one week
9 after turning out to one-day grazing. In this situation, it is unlikely that the metabolic
10 feedback limited herbage intake in the CON group.

11 It is empirically known that a temporary dropping intake of cows occurs when
12 the cow's feeds suddenly switch to other feeds, or when the cows are suddenly exposed
13 to another environment. However, only a few studies support this phenomenon (Forbes,
14 2007). Forbes (2007) speculated that this phenomenon occurred because the animals were
15 not familiar with the new food and exhibited neophobia. The present study is the first to
16 reveal a sudden decrease in herbage intake during the transition period from indoor to
17 stocking. Further research is necessary to investigate the change in herbage intake during
18 the transition period in other situations such as in other seasons and other grazing
19 adaptations.

20 The sudden decrease in BW in the CON group was caused by a decrease in the
21 contents of the rumen and gut resulting from the sudden decrease in herbage intake.
22 Similar results were reported in studies that estimated the changes in BW during the
23 transition period from indoor to stocking (Charmley et al., 2003; Hartwiger et al., 2018a;
24 Schären et al., 2016a). Once the production level dropped drastically, a long time was
25 required for recovery (Jørgensen et al., 2016). Therefore, sudden nutrient deficiency, such

1 as in the CON groups, should be avoided even if temporary.

2

3 **5 CONCLUSION**

4 The present study suggested that a short time-grazing adaptation before turning out for
5 one-day grazing was effective for stabilizing herbage intake, ruminal fermentation
6 parameters, and blood metabolites of grazing dairy cows. The grazing adaptation should
7 need at least one week, because herbage intake and BW drastically decreased in the one
8 week following turning out to one-day grazing when cows were not sufficiently adapted
9 to grazing.

10

11 **Conflict of interest**

12 Authors declare no Conflict of Interests for this article.

13

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6

1 **Figure legends**

2 Figure 1. Change in body weight in grazing cows subject to one-week adaptation to
3 grazing (ADP) and non-adaptation (CON) during the trial.

4 Probability of the effects in the experimental period ($P =$): Treatment, 0.42; Day, < 0.01;
5 Interaction, < 0.01.

6 Plots at day – 8: data measured on the day before starting the experiment (basal data).

7 Error bar: standard error of means.

8

9 Figure 2. Changes in the ruminal pH, NH₃-N, and total volatile fatty acid (VFA)
10 concentrations, and proportion of each VFA of grazing cows subject to one-week
11 adaptation to grazing (ADP) and non-adaptation (CON) during the trial.

12 Probability of the effects in the experimental period ($P =$): Treatment, Day, and
13 Interaction; 0.09, < 0.01, and < 0.01 in pH, 0.03, < 0.01, and < 0.01 in NH₃-N, 0.05, <
14 0.01, and < 0.01 in total VFA, 0.06, < 0.01, and < 0.01 in acetate, 0.36, < 0.01, and 0.01
15 in propionate, 0.04, < 0.01, and < 0.01 in butyrate, respectively.

16 Error bar: standard error of means.

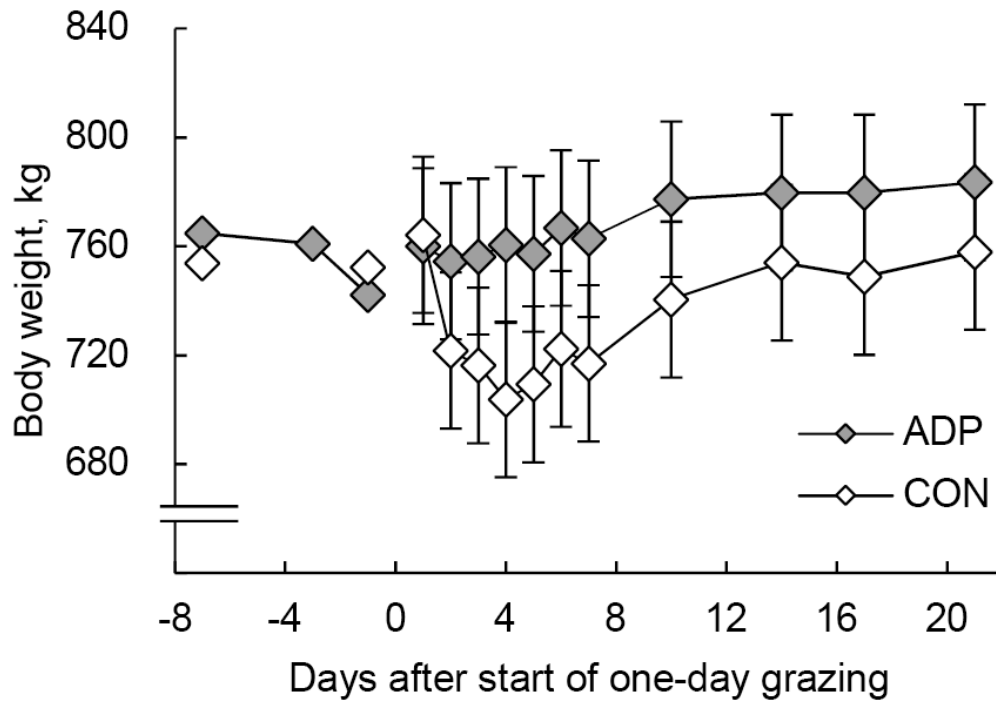
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18 Figure 3. Changes in urea-N (BUN), non-esterified fatty acid (NEFA), and glucose
19 concentrations in the blood of grazing cows subject to one-week adaptation to grazing
20 (ADP) and non-adaptation (CON) during the trial.

21 Probability of the effects in the experimental period ($P =$): Treatment, Day, and
22 Interaction; < 0.01, < 0.01, and < 0.01 in BUN, 0.23, < 0.01, and < 0.01 in NEFA, 0.72,
23 < 0.01, and 0.02 in glucose, respectively.

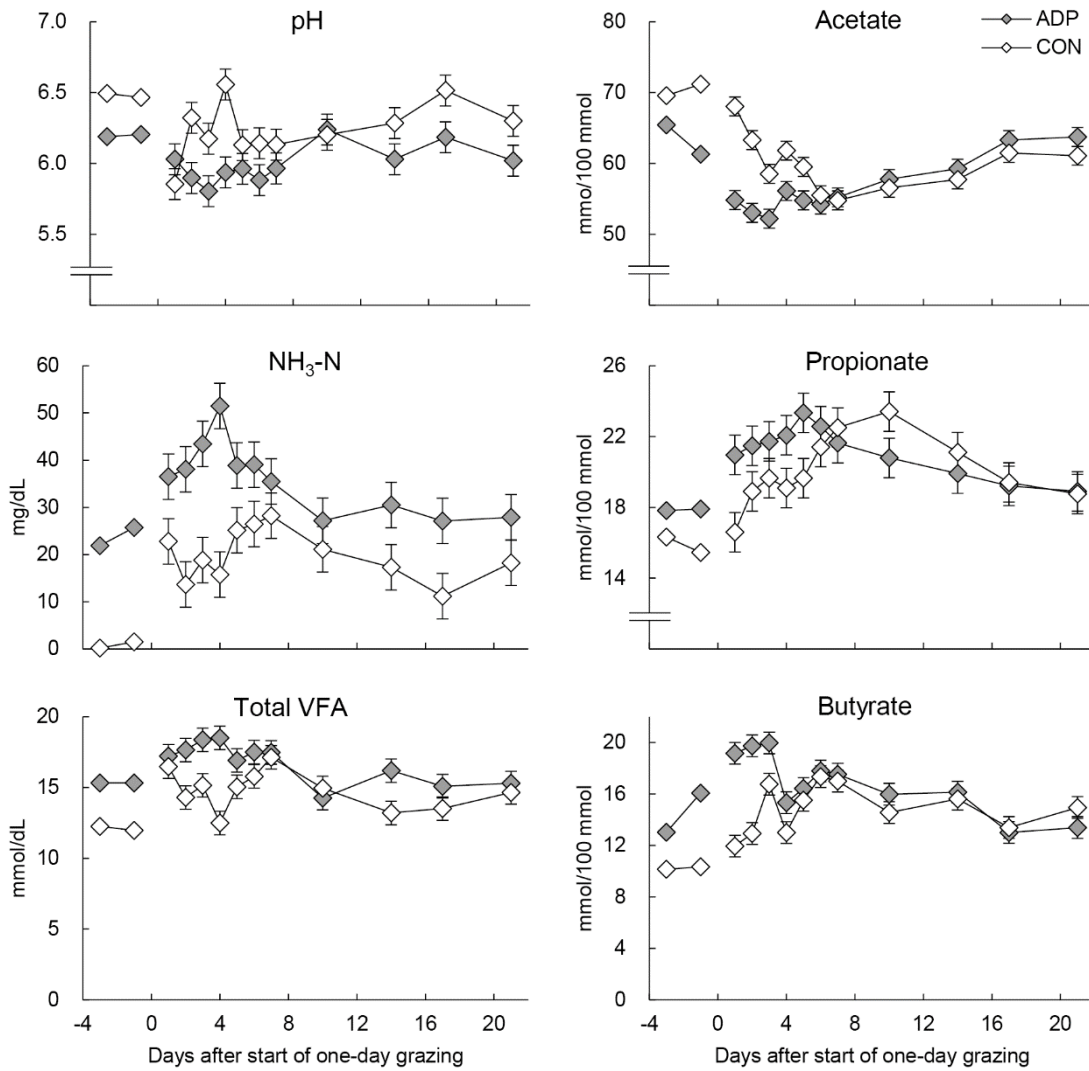
24 Error bar: standard error of means.

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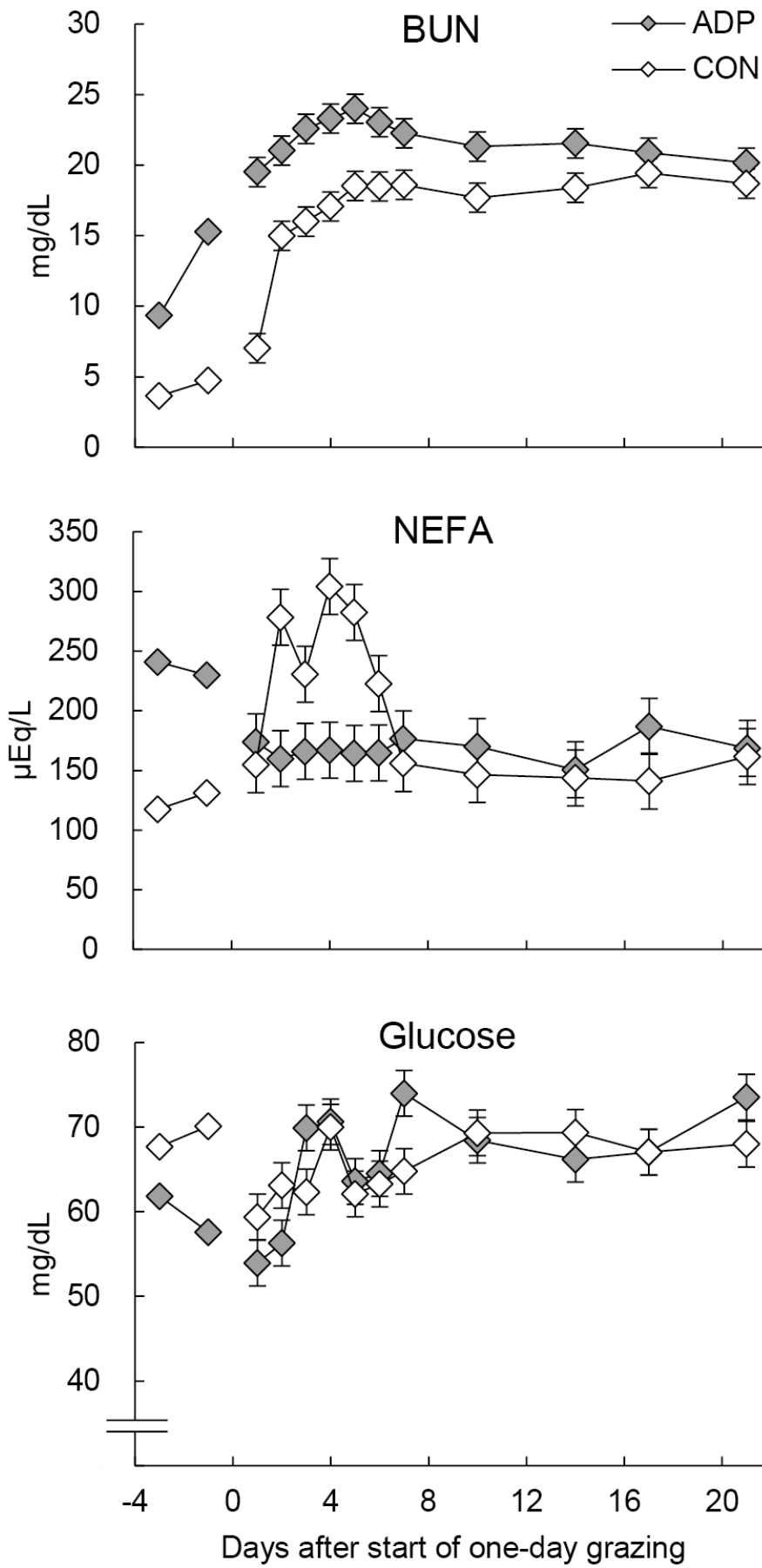
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Table 1. Chemical compositions of mixed silage and herbage during trial

	Mixed silage	Herbage			
		Adaption Period	Experimental period		
			Day 1-7	Day 8-14	Day 15-21
DM, % of FM	30.3	21.4	19.3	20.6	20.3
Chemical compositions, % of DM					
OM	93.0	93.0	90.6	90.2	89.8
CP	7.8	32.7	28.6	26.8	25.2
NDFom	46.3	29.9	32.6	37.4	43.2
WSC	–	19.4	19.6	14.0	10.0

DM: dry matter, FM: fresh matter, OM: organic matter, CP: crude protein, NDFom: neutral detergent fiber, WSC: water soluble carbohydrate

2

3

Table 2. Changes in herbage dry matter intake and herbage feeding time of grazing cows treated by one week adaption to grazing (ADP) and non-adaption (CON) during the trial

	Adaption period		Experimental period						SEM	Significance ($P =$)		
	Day 1-3	Day 4-7	Day 1-3	Day 4-7	Day 8-10	Day 11-14	Day 15-17	Day 18-21		Trt.	Day	Int.
Herbage dry matter intake, kg/day/cow												
ADP	6.2	7.5	9.2	10.8	9.8	9.6	11.5	10.3	0.8	0.06	<.01	<.01
CON	-	-	4.0	7.0	9.3	8.8	10.5	10.5				
Feeding time of herbage, min/day												
ADP	116	154	327	310	350	397	483	550	30	0.11	<.01	<.01
CON	-	-	150	266	330	346	449	528				

¹ Trt.: ADP vs. CON, Day: collection day in the experimental period, Int.: interaction between Trt. and Day.