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

Authors:

Tomohiro MITANI [1], Tomoyo KUBOTA [2], Hitoshi Mizuguchi [3, 4], Shiro Kushibiki [5], Koichiro UEDA [6]

Institutions:

[1] Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0811, Japan

[2] Graduate School of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan

[3] DKK-Toa Yamagata Co., Shinjo 996-0053, Japan

[4] Organization for Research Promotion, Iwate University, Morioka 020-8551, Japan

[5] Institute of Livestock and Grassland Science, NARO, Tsukuba 305-0901, Japan

[6] Research Faculty of Agriculture, Hokkaido University, Sapporo, 060-8589, Japan

Corresponding Author:

Tomohiro MITANI

Postal address: Field Science Center for Northern Biosphere, Hokkaido University, Kita 11, Nishi 10, Kita-ku, Sapporo 060-0811, Japan

E-mail: tmitani@fsc.hokudai.ac.jp

ABSTRACT

This study investigated the effect of adaptation to grazing in early spring on herbage intake, ruminal fermentation parameters, blood metabolite concentrations, and body weight change in dairy cows. The experiment was conducted on eight rumen-cannulated non-lactating cows in the early spring period. Four cows were adapted to grazing by stocking for four hours for one week (ADP group). The other cows were kept in a barn during the period (CON group). Then, both groups of cows were stocked together throughout a day on a 1 ha pasture for three weeks (experimental period). In the first week of the experimental period, compared to the CON group, the ADP group had a higher herbage intake, ruminal NH_3-N and total VFA concentration, and blood urea concentration, but the NEFA concentration was lower in the ADP group ($P < 0.01$). During the subsequent weeks, there were little differences in ruminal fermentation parameters and blood metabolites. Cows in the ADP group maintained their body weight, but cows in the CON group lost 60 kg of body weight in the first week of the experimental period.

Key words:

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

1 INTRODUCTION

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

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

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

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

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

2 MATERIALS AND METHODS

2.1 Animals and experimental design

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

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

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

2.2 Vegetation survey

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

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

2.3 Feeding time and herbage intake

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

Herbage DM intake during in the adaptation and experimental periods was measured using the double-indicator method, using the lanthanum (La) maker as an external marker and C_{33} alkane as an internal marker (Ueda, Mitani & Kondo, 2016a). The La maker was prepared by soaking the $LaCl_3$-$7H_2O$ solution into sugar beet-pulp. Fifty grams of the La maker was thrown into the rumen via the ruminal cannula at 08:00 and 19:00 hours daily. The feces in the rectum were collected at 08:00 and 19:00 hours, and were composited on days 1-3, days 4-7 in the adaptation period, and days 1-3, days 4-7, days 8-11, days 15-17, and days 18-21 in the experimental period. The concentrations of La in the fecal

samples and the La marker were determined using an inductively coupled plasma mass spectrometer (ERAN DRACE; Perkin Elmer, Tokyo, Japan) after acid digestion. The concentrations of C_{33} alkane in the herbage, mixed silage, and fecal samples were determined using gas chromatography (GC-2010; Shimadzu, Kyoto, Japan) after alkaline saponification. Herbage DM intake was estimated using the following equation: herbage intake (kg of DM/day) = [C_{33} alkane concentration in feces (mg/kg of DM) × La intake from marker (mg/day)]/[C_{33} alkane concentration in herbage (mg/kg of DM) × Fecal La concentration (mg/kg of DM)]. The herbage DM intake during the adaptation period was calculated after adjusting the C_{33} alkane intake from mixed silage.

2.4 Body weight, ruminal fermentation and blood metabolites

The body weight of each cow was measured at 19:00 h on the day before the adaptation period started, days 4, and 7 during the adaptation period, and days 1, 2, 3, 4, 5, 6, 7, 10, 14, 17, and 21 in the experimental period using an electronic scale (Orionkikai Co. Ltd., Nagano, Japan). Rumen fluid and blood samples were also collected. Rumen fluid was collected using a 50 mL syringe via the rumen cannula. Immediately after collection, the rumen fluid pH was measured using a portable pH meter (D-51T; Horiba, Kyoto, Japan). The fluid samples were centrifuged (4°C, 3000 rpm, 20 min), and the supernatants were stored at – 80°C until further analysis. NH_3-N concentration was determined using the indophenol method (Wetherburn, 1967), and each volatile fatty acid (VFA) concentration was determined using a gas chromatograph (GC-2010; Shimadzu, Kyoto, Japan) according to the method (Ueda, Mitani & Kondo, 2016b). Blood samples were collected from the jugular vein using vacuum blood tubes containing heparin. The blood samples were centrifuged (4°C, 3000 rpm, 20 min), and the blood plasma was stored at – 80°C until analysis. The urea concentration of the plasma (BUN) was determined using the

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

2.5 Statistical analysis

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

3 RESULTS

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

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

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

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

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

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

3.2 Ruminal fermentation

The changes in ruminal pH, NH_3-N, and total VFA concentrations are shown in Figure 2. During the adaptation period (days - 3 and - 1 in figure), the ruminal pH in the ADP group was lower than that in the CON group. During the experimental period, the average ruminal pH in the ADP group tended to be lower than that in the CON group (6.0 and 6.2, respectively; $P = 0.09$), but the changes were significantly different between the ADP and CON groups ($P < 0.01$). Ruminal pH in the ADP group remained at a low level one week after starting the experimental period and then gradually increased. While the ruminal pH in the CON group was higher than that of the ADP group during days 2 and 4, it changed similarly to that in the ADP group until the end of the experiment.

In the adaptation period, ruminal NH_3-N concentrations in the ADP group were higher than those in the CON group. In the experimental period, the average ruminal NH_3-N concentration in the ADP group (36.0 mg/dL) was higher than that in the CON group (19.9 mg/dL, $P = 0.03$), and the changes in ruminal NH_3-N concentration also differed significantly between the ADP and CON groups ($P < 0.01$). After the start of the experimental period, the ruminal NH_3-N concentration of the ADP group continued to increase and reached 50 mg/dL on day 4 in the period, and then gradually decreased until day 10 and remained constant until the end of the experiment. In the CON group, ruminal NH_3-N concentration drastically increased on day 1 in the experimental period from that in the adaptation period but maintained a low concentration compared with that in the

ADP group throughout the experimental period.

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

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

3.3 Blood metabolites

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

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

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

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

4 DISCUSSION

The results from the present study clearly indicate the effectiveness of maintaining herbage intake and BW for the adaptation to grazing before turning out to one-day grazing. The results of changes in ruminal fermentation and blood metabolites supported the usefulness of the adaptation to grazing. The effectiveness and mechanisms can be explained by the change and difference in herbage intake between the ADP and CON groups. The present study first clarified the effectiveness of the adaptation to grazing in terms of maintaining herbage intake by continuously measuring herbage intake, not conducted in previous studies (Charmley et al., 2003; Khanal et al., 2008, Schären et al., 2016a).

4.1 Ruminal fermentation and blood metabolites

The effect of grazing adaptation on ruminal fermentation was observed for one week after turning out to one-day grazing. High NH_3-N and VFA concentrations in the ADP group resulted from high intake of early spring herbage. In early spring, herbage generally has extremely high CP, and the ruminal degradation rate is also high, although early spring herbage has high WSC, which is easily degradable in the rumen (Wales, Dellow & Doyle, 1999). Therefore, when cows consumed much of the early spring herbage such as in the ADP group, ruminal NH_3-N and total VFA concentrations elevated.

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

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

BUN and NEFA concentrations were also affected mainly by the change in herbage intake between the ADP and CON groups. High BUN concentration in the ADP group after turning out to one-day grazing resulted from a high ruminal NH_3-N concentration, which was converted into urea in the liver. In the ADP group, the blood NEFA concentration was lower than that in the CON group and maintained at a constant level. This result indicated that cows in the ADP group smoothly adapted to one-day grazing and maintained sufficient energy supply until the end of the experiment. However, elevated blood NEFA concentration in the CON group during the first week of the experiment indicated that cows in the CON group had an extreme negative energy status duirng this period.

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

4.2. Herbage intake

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

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

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

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

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

5 CONCLUSION

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

Conflict of interest

Authors declare no Conflict of Interests for this article.

References

AOAC. 1990. *Official Methods of Analysis*, 15th edn. Association of Official Analytical Chemists, Arlington, VA.

Charmley, E., Jannasch, R. W. & Boyd, J. (2003). Grazing behaviour and weight change of cattle turned out to pasture in spring. *Canadian Journal of Animal Science*, 83(4), 801–808. https://doi.org/Doi 10.4141/A03-015

Coulon, J. B., D'Hour, P. & Petit, M. (1988). Influence of Transition Feeding Pattern on Milk-Production at the Turnout of Cows to Pasture. *Livestock Production Science*, 20(2), 119–134. https://doi.org/Doi 10.1016/0301-6226(88)90057-7

Forbes, J. M. (2007). Voluntary food intake and diet selection in farm animals (2nd ed.). Oxfordshire, United Kingdam: CABI.

Hartwiger, J., Schären, M., Gerhards, U., Hüther, L., Frahm, J., von Soosten, D., ...

Dänicke, S. (2018a). Effects of a Change from an Indoor-Based Total Mixed Ration to a Rotational Pasture System Combined with a Moderate Concentrate Feed Supply on the Health and Performance of Dairy Cows. *Animals (Basel)*, 8(10), 169. https://doi.org/10.3390/ani8100169

Hartwiger, J., Schären, M., Potthoff, S., Hüther, L., Kersten, S., von Soosten, D., ... Dänicke, S. (2018b). Effects of a Change from an Indoor-Based Total Mixed Ration to a Rotational Pasture System Combined With a Moderate Concentrate Feed Supply on Rumen Fermentation of Dairy Cows. *Animals (Basel)*, 8(11), 205. https://doi.org/10.3390/ani8110205

Jørgensen, C. H., Spörndly, R., Bertilsson, J. & Østergaard, S. (2016). Invited review: Carryover effects of early lactation feeding on total lactation performance in dairy cows. *Journal of Dairy Science*, 99(5), 3241–3249. https://doi.org/10.3168/jds.2014-9043

Khanal, R. C., Dhiman, T. R. & Boman, R. L. (2008). Changes in fatty acid composition of milk from lactating dairy cows during transition to and from pasture. *Livestock Science*, 114(2–3), 164–175. https://doi.org/10.1016/j.livsci.2007.04.020

Mitani, T., Sato, Y., Ueda, K., Takahashi, M., Nakatsuji, H. & Kondo, S. (2011). Change in milk compositions from cows during transition period from barn feeding to grazing. *Animal Science and Agriculture Hokkaido (in japansese)*, 53: 29–34.

Sato, H., Sunagoda, S., Sugiwaka, T., Sato, A. & Miura, Y. (1981). Changes in plasma constituent levels during early period of grazing in lactating dairy cows. *Japanese Journal of Zootechnical Science (in japanese)*, 52(12), 874–877. https://doi.org/10.2508/chikusan.52.874

Schären, M., Jostmeier, S., Ruesink, S., Hüther, L., Frahm, J., Bulang, M., ... Dänicke, S. (2016a). The effects of a ration change from a total mixed ration to pasture on

health and production of dairy cows. *Journal of Dairy Science*, 99(2), 1183–1200. https://doi.org/10.3168/jds.2015-9873

Schären, M., Seyfang, G. M., Steingass, H., Dieho, K., Dijkstra, J., Hüther, L., ... Dänicke, S. (2016b). The effects of a ration change from a total mixed ration to pasture on rumen fermentation, volatile fatty acid absorption characteristics, and morphology of dairy cows. *Journal of Dairy Science*, 99(5), 3549–3565. https://doi.org/10.3168/jds.2015-10450

Ueda, K., Mitani, T. & Kondo, S. (2016a). Effect of increased concentrate allotment before evening grazing on herbage intake, nitrogen utilization and rumen fermentation in dairy cows grazed on perennial ryegrass pasture. *Animal Science Journal*, *87* (10), 1233-1243. https://doi.org/10.1111/asj.12576

Ueda, K., Mitani, T. & Kondo, S. (2016b). Effect of water-soluble carbohydrate content in orchardgrass pasture on grazing time and rumen fermentation in dairy cows. *Animal Science Journal*, *87* (9), 1122-1129. https://doi.org/10.1111/asj.12533

Ueda, Y., Akiyama, F., Asakuma, S. & Watanabe, N. (2011). Technical note: the use of a physical activity monitor to estimate the eating time of cows in pasture. *Journal of Dairy Science*, 94(7), 3498–3503. https://doi.org/10.3168/jds.2010-4033

Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Symposium: Carbohydrate methodology, metabolism, and nutritional implications in dairy cattle Methods for dietary fiber, neutral detargent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74, 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Yemm, E. W. & Willis, A. J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, 57, 508-514. https://doi.org/10.1042/bj0570508

Wales, W. J., Dellow, D. W. & Doyle, P. T. (1999). Degradabilities of dry matter and crude

protein from perennial herbage and supplements used in dairy production systems in Victoria. *Australian Journal of Experimental Agriculture*, 39 (6), 645-656. https://doi.org/10.1071/EA98156

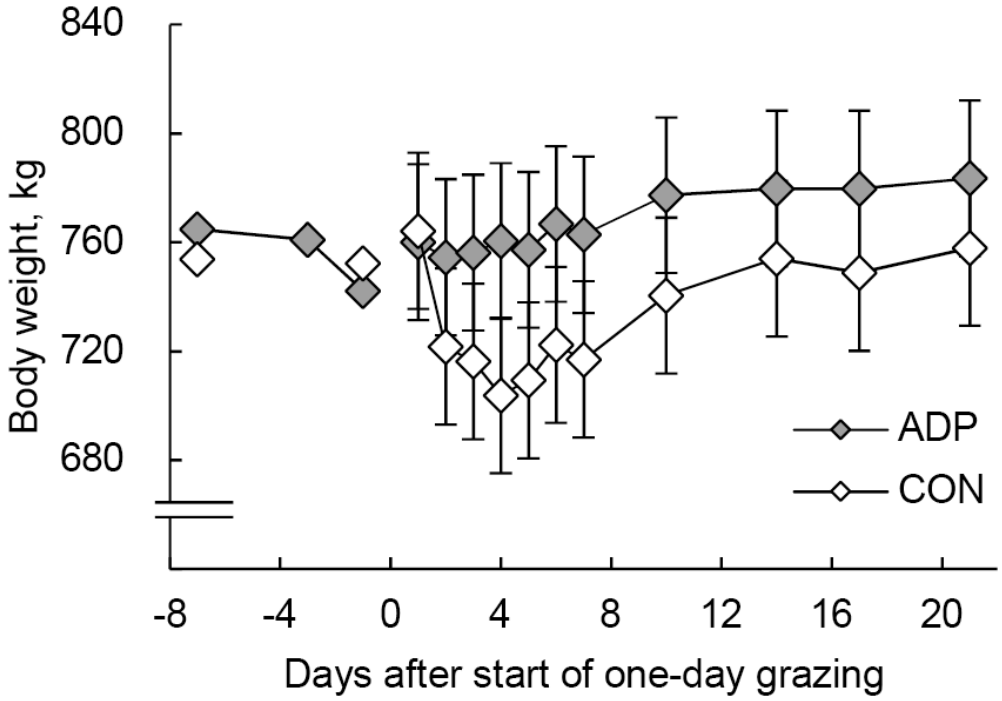
Wetherburn, M. W. (1967). Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry*, 39(8), 971–974. https://doi.org/10.1021/ac60252a045

Figure legends

Figure 1. Change in body weight in grazing cows subject to one-week adaptation to grazing (ADP) and non-adaptation (CON) during the trial.
Probability of the effects in the experimental period (*P* =): Treatment, 0.42; Day, < 0.01; Interaction, < 0.01.
Plots at day – 8: data measured on the day before starting the experiment (basal data).
Error bar: standard error of means.

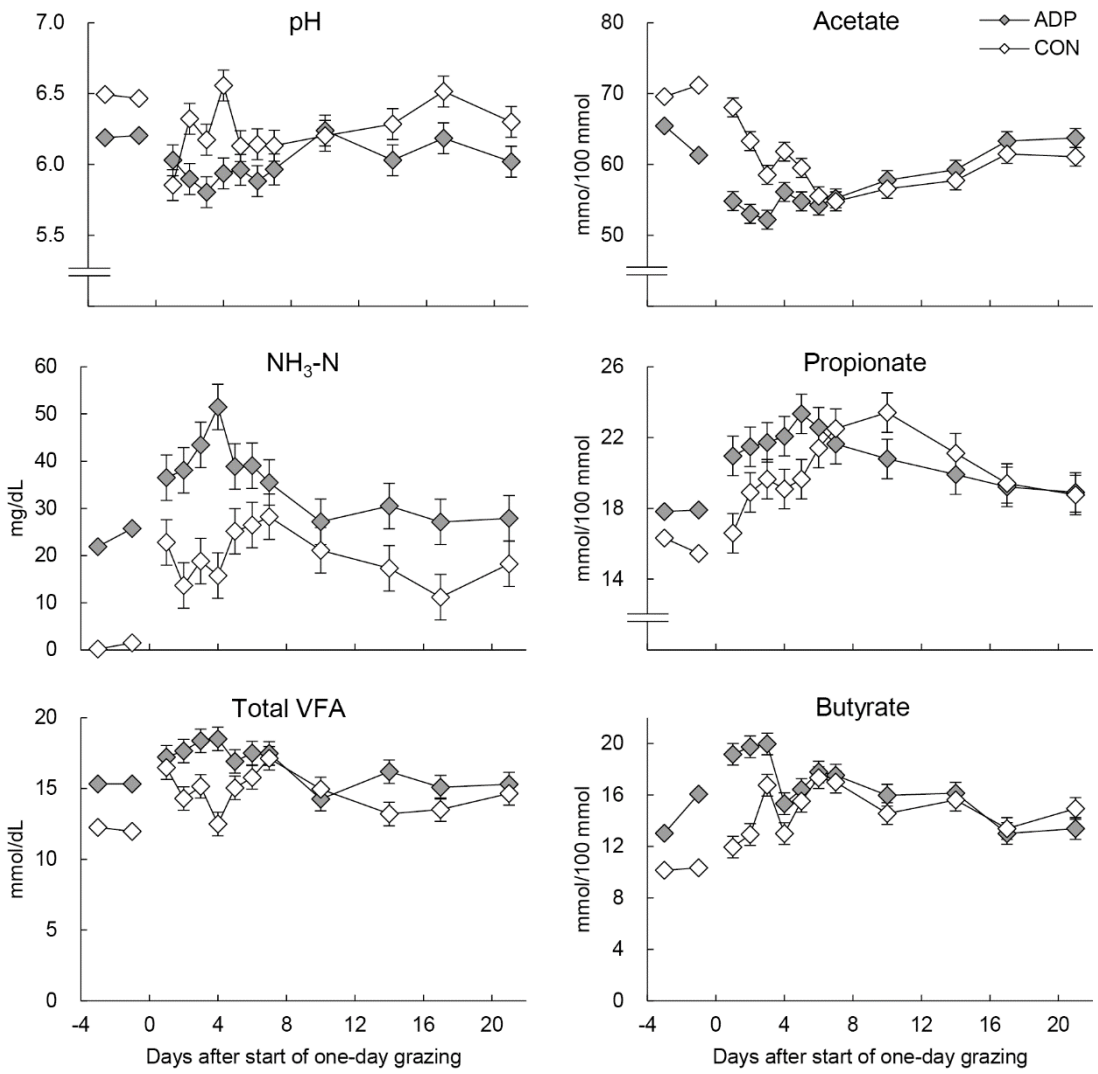
Figure 2. Changes in the ruminal pH, NH_3-N, and total volatile fatty acid (VFA) concentrations, and proportion of each VFA of grazing cows subject to one-week adaptation to grazing (ADP) and non-adaptation (CON) during the trial.
Probability of the effects in the experimental period (*P* =): Treatment, Day, and Interaction; 0.09, < 0.01, and < 0.01 in pH, 0.03, < 0.01, and < 0.01 in NH3-N, 0.05, < 0.01, and < 0.01 in total VFA, 0.06, < 0.01, and < 0.01 in acetate, 0.36, < 0.01, and 0.01 in propionate, 0.04, < 0.01, and < 0.01 in butyrate, respectively.
Error bar: standard error of means.

Figure 3. Changes in urea-N (BUN), non-esterified fatty acid (NEFA), and glucose concentrations in the blood of grazing cows subject to one-week adaptation to grazing (ADP) and non-adaptation (CON) during the trial.
Probability of the effects in the experimental period (P =): Treatment, Day, and Interaction; < 0.01, < 0.01, and < 0.01 in BUN, 0.23, < 0.01, and < 0.01 in NEFA, 0.72, < 0.01, and 0.02 in glucose, respectively.
Error bar: standard error of means.


840
800
760
720
680
Body weight, kg
ADP
CON
-8
-4
0
4
8
12
16
20
Days after start of one-day grazing

1

2


pH
Acetate
ADP
CON
NH3-N
Propionate
Total VFA
Butyrate
mg/dL
mmol/dL
mmol/100 mmol
Days after start of one-day grazing
Days after start of one-day grazing

1

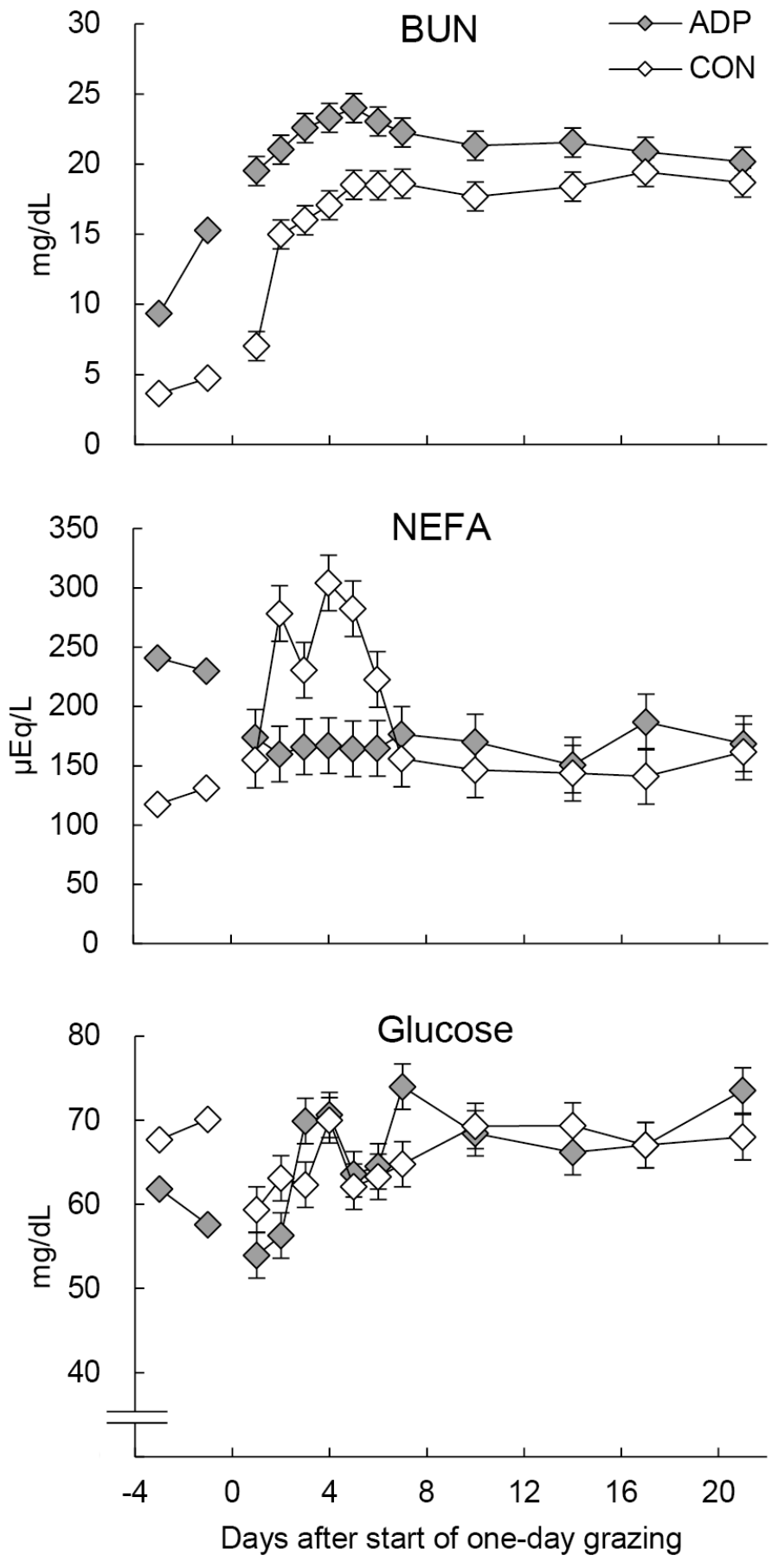
2

3

4

5

6


BUN
ADP
CON
mg/dL
30
25
20
15
10
5
0
NEFA
μEq/L
350
300
250
200
150
100
50
0
Glucose
mg/dL
80
70
60
50
40
-4
0
4
8
12
16
20
Days after start of one-day grazing

1

1

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.
Hebage 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.

1