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Effects of concentrate supplementation during early lactation on nutrient efficiency and ruminal fermentation of herbage-fed dairy cows with differing milk production potentials

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Effects of concentrate supplementation during early lactation on nutrient efficiency and ruminal fermentation of herbage-fed dairy cows with differing milk production potentials

In Switzerland, fresh herbage is a favoured feed for dairy cows due to its high quality and availability and low production costs. We investigated the effect of concentrate supplementation on milk yield, feed intake, ruminal fermentation, and reticular pH of herbage-fed dairy cows during early lactation. Twenty-four multiparous Holstein cows were assigned to 2 groups according to the milk production in their previous lactation: high-potential (8,959 ± 984 kg) and low-potential (6,204 ± 1,000 kg) cows. Within these groups, the cows were allocated to 2 treatments receiving either only herbage ad libitum (n = 11), or herbage supplemented with concentrate (n = 13) from week 2 ante partum until week 8 of lactation. Data of a preliminary study showed that reticular pH was higher by 0.24 pH units and less variable (overall SD 0.19 vs. 0.51 pH units, resp.) than ruminal pH. Therefore, reticular pH was measured in the main study. Supplemented cows and high-potential cows produced more milk than unsupplemented cows and low-potential cows, resp. milk acetone was especially high in unsupplemented, high-potential cows. Supplementation caused a decreased herbage intake but increased total DMI. Supplementation did not affect ruminal fermentation (VFA, P = 0.105) or reticular pH (P = 0.669). In conclusion, high-potential cows depend on concentrate supplementation more than low-potential cows to minimize metabolic stress.

Keywords: concentrate supplementation, dairy cow, early lactation, herbage feeding

Technical note: A comparison of reticular and ruminal pH monitored continuously with 2 measurement systems at different weeks of early lactation

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ABSTRACT

Subacute ruminal acidosis is one of the most important digestive disorders in high yielding dairy cows fed highly fermentable diets. Monitoring of forestomach pH has been suggested as a potentially valuable tool for diagnosing subacute ruminal acidosis. The objective of the present study was to compare continuously recorded measurements of an indwelling telemetric pH sensor inserted orally in the reticulum with those obtained from a measurement system placed in the ventral sac of the rumen through a cannula. The experiment was conducted with 6 ruminally cannulated Holstein cows kept in a free-stall barn. Equal numbers of cows were assigned to 2 treatment groups based on their previous lactation milk yield. Cows in treatment CON- were offered a diet consisting of only fresh herbage cut once daily, and cows in treatment CON+ got fresh herbage plus a concentrate supplement according to the individual milk yield

of each cow to meet their predicted nutrient requirements. The experiment lasted from 2 wk before the predicted calving date until wk 8 of lactation. During the whole experiment, a pH value was recorded every 10 min in the reticulum using a wireless telemetry bolus including a pH sensor (eBolus, eCow Ltd., Exeter, Devon, UK), which had been applied orally using a balling gun. Simultaneously, in wk 2, before the estimated calving date and in wk 2, 4, 6, and 8 of lactation, the ruminal pH was measured every 30 sec for 48 h with the LRCpH measurement system (DASCOR Inc., Escondido, CA) placed in the ventral sac of the rumen through the cannula. The readings of the LRCpH measurement system were summarized as an average over 10 min for statistical analysis. The recorded pH values were on average 0.24 pH units higher in the reticulum than in the rumen. The reticular pH also showed less fluctuation (overall SD 0.19 pH units) than pH profiles recorded in the rumen (overall SD 0.51 pH units). Regardless of measurement system, pH was not influenced by treatment, but varied across wk of lactation and decreased with advancing lactation. The difference between ruminal and reticular pH varied across wk of lactation. Due to this variation, no fixed conversion factor can be provided to make pH measurements in the reticulum comparable with those in the rumen.

Key words*:* dairy cow, reticular pH, ruminal pH, herbage

Technical Note

Subacute ruminal acidosis, is a disorder of ruminal fermentation that is characterized by extended periods of depressed ruminal pH (Plaizier et al., 2008). This disease is a widespread problem of high yielding dairy cows that receive highly rumen fermentable diets (Duffield et al., 2004; Plaizier et al., 2008). Cows suffering from SARA produce less milk and milk fat because of irregular feed intake and lower ruminal fermentation efficiency and they show increased incidences of further diseases such as caudal vena cava syndrome and laminitis

(Kleen et al., 2003; Krause and Oetzel, 2006). Therefore, SARA can cause important economic losses for dairy farmers due to decreased efficiency of milk production, impaired cow health and cow welfare, and high rates of involuntary culling (Krause and Oetzel, 2005).

The symptoms of SARA are difficult to detect (Enemark, 2008); nevertheless, an early diagnosis of SARA is very important for the therapy and adjustment of feeding (Enemark, 2008). Monitoring of the ruminal pH is suggested as a potentially useful tool for the diagnosis of SARA because it would be the most meaningful and direct response parameter relating to variation in ruminal fermentation (Kleen et al., 2003). On a herd level, methods such as stomach tubing and rumenocentesis are available for obtaining spot measurements of pH obtained from rumen fluid samples. However, these methods have limitations (e.g. pH variations according to intra-ruminal localization of the stomach tube and saliva contamination (Enemark 2008); health problems and injuries caused by the invasive sampling procedure (Sato et al., (2012a)) and usually do not account for diurnal pH fluctuations. This aspect has been considered thus far by continuous measurements using indwelling probes in ruminally cannulated dairy cows (e.g., Graf et al., 2005; Penner et al., 2006).

Recently, various measurement systems have become available for continuous monitoring of the ruminal and reticular pH in non-cannulated cows. One of these is a wireless telemetry bolus that includes a pH sensor (eBolus, eCow Ltd, Exeter, Devon, UK); it is applied orally into the reticulum by using a balling gun. The objective of this study was to compare continuously recorded pH profiles of the eBolus with those obtained using a pH measurement system (Lethbridge Research Center ruminal pH measurement system, LRCpH, Dascor Inc., Escondido, CA) placed in the rumen through a cannula at different weeks of lactation (2 wk prepartum until 8 wk postpartum). Because this comparison was part of a larger study in which different diets were fed, the effect of diet was taken into account. The variation in pH differences (ΔpH) between measurement systems was also evaluated across week of lactation.

The experiment was carried out with 6 ruminally cannulated multiparous Holstein dairy cows kept in a freestall barn at Agroscope, Institute for Livestock Sciences ILS, Posieux Switzerland. All procedures were in accordance with the Swiss guidelines for animal welfare and were approved (No. 2012_12_FR) by the Animal Care Committee of the Canton Fribourg. The cows were divided into 2 homogeneous groups based on their previous lactation milk yield (CON-, 7347 (SD 420) kg; CON+, 7083 (SD 107) kg). Cows in group **CON-** received fresh herbage cut once daily ad libitum without concentrate. Cows in group **CON+** were also offered fresh herbage ad libitum and were supplemented with a cereal/corn gluten-based concentrate to meet their predicted nutrient requirements. All cows received a mineral mix and water was available at all times. The experiment was started individually for each cow at 2 wk (14 (SD 5) d) before the predicted calving date **(LW-2)** and lasted until wk 8 of lactation **(LW8)**. Each cow was equipped with the 2 measurement systems, as detailed below. Herbage samples were taken daily and pooled across week of lactation. Concentrate was sampled once per production batch. The analysis of chemical composition was carried out as described by Thanner et al. (2014). The herbage offered to group CON- contained per kilogram of DM: ADF, 285 (SD 18.9) g; NDF, 451 (SD 32.0) g; NFC, 280 (SD 37.1) g; NE_L, 5.7 (SD 0.4) MJ; absorbable protein in the small intestine (APD), 95.2 (SD 8.5) g. The herbage fed to group CON+ contained per kilogram of DM: ADF, 290 (SD 16.3) g; NDF, 461 (SD 27.6) g; NFC, 270 (SD 34.5) g; NE_L, 5.6 (SD 0.3) MJ; APD, 93.3 (SD 7.4) g. The concentrate contained per kilogram of DM: ADF, 62 (SD 4) g; NDF, 137 (SD 17); starch, 500 (SD 21), NE_L, 8.1 MJ; APD, 202 g).

The eBolus, described functionally by Mottram et al. (2008), is a wireless telemetry device that records pH and temperature continuously for up to 6 mo, according to the manufacturer's manual. The eBolus is 115 mm long, has a diameter of 27 mm, and weighs 200 g (Mottram et al. 2013). It has a perforated stainless steel end cap that contains the sensors and protects them from damage in the reticulum, but allows the free flow of reticular fluid. The remaining part of the body is composed mostly of resin surrounding the electronics. This construction ensures

that the device stays in an essentially upright position in the reticulum. The eBolus provides a real-time monitoring of the reticular pH. It is factory set to determine pH every min and record averaged data at selectable intervals (set to 10 min in the present experiment). Setup of the eBolus, as well as transfer of logged data, is made by telemetric communication. The eBoluses are factory calibrated. Nevertheless, before use in the present study they were checked and adjusted, if necessary, with calibration solution of pH 4 and pH 7. For setup and data download, the manufacturer provides a tablet computer (Samsung NP-Q1, Samsung, Seoul, South Korea) running the Windows XP operating system and an antenna that plugs into the USB port. The included software allows data downloads from the bolus and storage as .csv-type files.

The eBolus automatically turns off if the ambient temperature is below 32ºC. Therefore, in the present study, the eBolus was activated by warming up to 39°C in a water bath for about 10 min. Subsequently, the eBolus was placed in the reticulum orally using a balling gun in LW-2. Data were downloaded once per week using the provided tablet computer. The quality and stability of the radio connection depended on the cow's position and presumably on the position and orientation of the eBolus in the reticulum. The best signal reception was achieved at a distance of 10 to 30 cm from the cow, ventral to the breastbone or on the cow's left side caudal to the elbow. In a few cases, the signals could be better received on the right side of the cow. If the cow was lying down, the signal reception was more difficult, regardless of the side of the cow. The download time varied considerably, from 2 min up to 30 min, depending on the intensity of the signal. After LW8, the eBoluses still located in the reticula were removed through the ruminal cannula.

The LRCpH was used to record the ruminal pH. The system which was described in detail by Penner et al. (2006), was equipped with 2 weights fastened to the bottom of the electrode shroud to maintain the LRCpH in the ventral sac of the rumen. The system was connected with a cord to the stopper of the ruminal cannula to help maintain the electrode in a vertical position. Before inserting into and after removing from the rumen, the electrode of the LRCpH system

was calibrated in pH 4 and pH 7 buffer solutions. The drift occurring between start and end calibration was assumed to be linear over time and was used in the conversion of the recorded readings measured in millivolt to pH units (Penner et al., 2006).

The LRCpH system was inserted into the ventral sac of the rumen of each cow at LW-2, 2, 4, 6 and 8. Ruminal pH was monitored continuously for 48 h after each insertion. Readings were taken every 30 s. After 48 h, the LRCpH system was removed from the rumen. In doing this, the system was noted several times to no longer be situated in the ventral sac of the rumen. After downloading and transformation of the readings, the ruminal pH data were averaged over 10 min.

The measurements of the eBolus were compared with those of the LRCpH by taking into account the corresponding 48 h within the respective week of lactation (LW-2, 2, 4, 6 and 8). The statistical procedures were conducted with Systat 13 (Systat Software, Chicago, IL) and R version 3.1.0 (The R Foundation for Statistical Computing, Vienna, Austria) using Package 'metRology' (version 0.9-17). The pH and ΔpH [= pH (LRCpH) - pH (eBolus)] were analyzed by linear mixed models with autoregressive AR(1) error variance-covariance matrix (Verbeke and Molenberghs, 2000; Davis, 2002). The error correlation (AR(1)) was 0.676 for pH as response variable, and 0.720 for ΔpH, respectively. Autocorrelation estimates of the full set of > 21,000 data points revealed very highly correlated observations. This indicated considerable redundancy within the pH data as well as within the ΔpH differences. Redundancy reduction was applied by a random sampling of approximately 5% of the observations.

The pH data were analyzed according to the following model:

$$pH = \mu + s_i + g_j + w_k + ßt_{(k)} + A_m + T_q + e_{ijkmqr}$$

where μ = general mean; s_i = fixed effect of measurement system i, i = {LRCpH, eBolus}; g_i = fixed effect of treatment group j, j = {CON+, CON-}; w_k = fixed effect of wk of lactation k, k = {LW-2, 2, 4, 6, 8}; ß = regression coefficient for time $t_{(k)}$ within lactation wk k, $t_{(k)}$ = [0, 3.12639 d]; A_m = random effect of animal m, m = {Cow1, Cow2 Cow3, Cow4, Cow5, Cow6},

with variance σ_A^2; T_q = random effect of time difference q between measurements, q = {-16, -15, -14, -13, -11, -8, -6, -3, -1, 0, 1, 2 s}, with variance σ_T^2; e_{ijkmqr} = random (residual) error with variance-covariance $\rho^{|v-u|}\sigma^2$, autoregressive AR(1) within animals, measurement system, and wk of lactation, with error correlation ρ and time indices *v* and *u*.

The ΔpH data were analyzed with the same model used for the pH data but without the fixed effect of the measurement system.

Reduced models (without random factors with near-zero variance components) of the random subsets were finally considered for inference on the difference between measurement systems and time dependence of pH and ΔpH. The model reduction was based on the numeric values of the variance components for the random effects (s_A^2, and s_T^2). A robust estimation of the overall difference between the measurement systems was obtained for the trimmed ΔpH means of the 25 combinations of measurement system, animal, and time week of lactation using Huber`s proposal 2 robust estimator of location and scale (Huber, 1981). A one sample t-test (H_o:Δ-pH = 0) was computed from the robust estimate of the standard error, assuming (approximate) independence between the robust means. Effects were considered significant at $P < 0.05$. The results are presented as LSM together with the SEM.

The measured pH was higher ($P < 0.001$) with the eBolus in the reticulum (pH 6.35) than with the LRCpH in the rumen (pH 6.11). The mean difference was 0.24 (± 0.08) pH units. The higher reticular pH could be caused by the dilution of the reticulum content with fresh and less fermented feed or with saliva (Duffield et al., 2004; Sato et al., 2012$_b$), or both. The averaged pH profiles for all 6 cows at all weeks of lactation of measurement (Figure 1) also showed less fluctuation over 48 h and fewer variations within the reading time points for the reticular pH compared to the ruminal pH. The overall SD for the reticular and ruminal pH was 0.19 and 0.51 pH units, respectively. A reason for the more stable reticular pH might be the smaller volume of the reticulum with a more homogenous content compared with the rumen, thereby reducing

the dislocation of eBolus and the variation in the measurements. In contrast, the LRCpH seems to have moved within the rumen in the present study, resulting in varying pH readings (Duffield et al., 2004). In this context, Enemark et al. (2003) decided to use the reticular pH to follow the pH fluctuations occurring when diets with varying forage to concentrate ratio were fed and the daily distribution of concentrate changed. However, the treatment had no effect (P = 0.29, data not shown) on reticular or ruminal pH, so the lower fluctuation of the reticular pH over the 48-h measurement period compared to the ruminal pH might also indicate a lower sensitivity to changes in feeding.

Diagnosis of SARA in the past has been made using defined thresholds of ruminal pH (e.g. 5.5 by Duffield et al. (2004)). Therefore, the variation in the difference between the reticular and ruminal pH hinders the detection of SARA based on measurements in the reticulum. Sato et al. (2012b) previously suggested a higher threshold of reticular pH for the diagnosis of SARA. They found differences between reticular and ruminal pH of up to 0.7 pH units in acidotic cows; this was much higher than the mean difference in the present study (0.24 ± 0.08 pH units). However, the variations in ΔpH among week of lactation (Table 1) indicate that the difference between reticular and ruminal pH might depend on diet composition or DMI, or both. Therefore, no fixed conversion factor can be offered to correct the pH measurements from the reticulum in order to predict ruminal pH.

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Table 1. Variations in the difference between ruminal[1] and reticular[2] pH (Δ-pH) at different weeks of lactation[1]

week of lactation[3]	LW2		LW4		LW6		LW8	
	ΔpH	SEM	ΔpH	SEM	ΔpH	SEM	ΔpH	SEM
LW-2	-0.145	0.1509	0.324	0.1377	0.092	0.1370	0.299	0.1407
LW2			0.469[*4]	0.1318	0.237	0.1318	0.444[*]	0.1339
LW4					-0.232	0.1149	-0.025	0.1171
LW6							0.207	0.1170

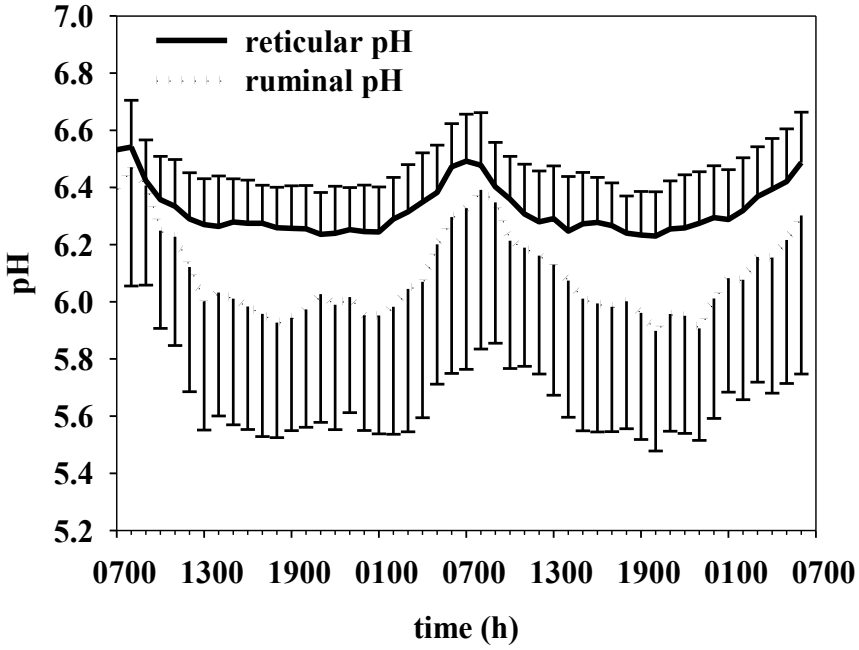
[1] pH recorded with the LRCpH (DASCOR Inc., Escondido, CA)

[2] pH recorded with the eBolus (eCow Ltd., Exeter, Devon, UK)

[3] LW-2, 2 wk before predicted calving; LW2, 2 wk postpartum; LW4, 4 wk postpartum; LW6, 6 wk postpartum; LW8, 8 wk postpartum

[4] *, $P < 0.01$

Falk, **Figure 1**



JDS-15-97-25, figure 1

Figure captions

Figure 1. Averaged 48-h pH profiles for all 6 cows at all weeks of lactation recorded simultaneously in the reticulum (eBolus, eCow Ltd., Exeter, Devon, UK) and in the rumen (LRCpH, DASCOR Inc., Escondido, CA). The bars represent SD of the arithmetic means.

Effects of concentrate supplementation in early lactation on nutrient efficiency, ruminal fermentation, and reticular pH of zero-grazing dairy cows with differing milk production potentials[1]

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Summary

In Switzerland, fresh herbage is a favoured feed for dairy cows due to its high quality and availability and low production costs. However, transition and early lactation are periods characterized by an increased nutrient demand that may not be covered by herbage alone. To compare the effects of concentrate supplementation in early lactation on nutrient efficiency and ruminal fermentation, twenty-four multiparous Holstein cows were assigned to two performance groups according to their previous lactation milk yield: high- (8959±984 kg) and low- (6204±1000 kg) potential cows. Within this group, cows were allocated to two treatment groups receiving either herbage ad libitum (n=11) or herbage supplemented with concentrate (n=13). The experiment started for each cow 2 weeks (wk) before the predicted calving date (LW-2) and lasted until lactation wk (LW) 8. Milk yield and dry matter intake (DMI) were recorded daily. The reticular pH was measured continuously using a telemetric pH bolus. Milk components and ruminal fermentation traits were analysed in LW-2, LW2, LW4, LW6, and LW8. Supplemented cows ($P<0.001$) and high-potential cows ($P=0.015$) produced more milk than unsupplemented cows and low-potential cows, respectively. Milk acetone was affected by supplementation ($P<0.001$) and milk potential ($P=0.002$) and was especially high in unsupplemented, high-potential cows until LW6. Supplementation caused a decrease in herbage DMI ($P<0.001$) but resulted in an increased total DMI ($P<0.001$), whereas milk potential had no effect on DMI. Associated with an increasing DMI ($P<0.001$), ruminal volatile fatty acid concentration ($P=0.024$) increased and reticular pH ($P<0.001$) decreased from LW2 until LW6. Apart from that, effects on ruminal fermentation and reticular pH were minor. In conclusion, even though apparent nutrient efficiency was high, high-potential cows without supplementation seem to struggle more with reduced nutrient availability than other cows; therefore, they appear to be more prone to metabolic stress and consequently to production diseases.

Keywords: concentrate supplementation, dairy cow, early lactation, reticular pH, zero-grazing,

1. INTRODUCTION

Livestock production was once based on locally available feed resources, and the distribution of ruminants was almost completely determined by the availability of natural pasture and crop residues (Steinfeld et al., 2006). This situation changed with the intensification of livestock production, resulting in an increasing use of domestically and internationally traded concentrate feeds (Steinfeld et al., 2006). However, from an economic point of view, it is still beneficial to graze dairy cows because herbage is a cost-efficient feed resource (Taweel et al., 2006) and highly digestible (Taweel et al. 2005). A shift to more forage-based diets in ruminant nutrition can also contribute to reducing the competition for food among ruminants, humans, and monogastric animals.

Nevertheless, in certain physiological situations, the nutrient and energy demands of dairy cows may not be met by herbage feeding alone (Taweel et al. 2005). In late pregnancy and early lactation, the requirements increase considerably, and dry matter intake (DMI) is relatively low, so that the cows' energy intake is particularly insufficient, resulting in a negative energy balance and the loss of body weight (Gross & Bruckmaier, 2015). However, there are large differences between individual cows in terms of the extent of the negative energy balance (de Vries & Veerkamp, 2000). Changes in the nutrition of dairy cows during the transition period influence health status, fertility, and productivity and are key determinants of the welfare and producer profitability of dairy cows (Mulligan & Doherty, 2008). Feeding of herbage only is often not sufficient to maintain the milk production of cows with high genetic potential (Sairanen, Khalili, Nousiainen, Ahvenjärvi, & Huhtanen, 2005). These cows seem less able to compensate for the reduced nutrient availability in a grazing system without concentrate supplementation than cows with lower genetic potential (Thanner et al., 2014a). Bargo, Muller, Kolver, & Delahoy (2003) concluded from their review on grazing dairy cows and Schwarz,

Haffner, & Kirchgessner (1995) observed in their study with zero-grazing cows that milk yield is lower in unsupplemented than in supplemented cows, indicating that high-producing cows need to be supplemented with concentrate to achieve their genetic potential for milk production.

On the other hand, concentrate supplementation reduces herbage intake in grazing (Heublein et al. 2017) and zero-grazing dairy cows (Schwarz et al. 1995). Therefore, in efficient grazing systems, energy and nutrient intake from herbage should be maximized, which requires intensively managed pastures. These feedstuffs are generally highly digestible and low in fibre (Reis & Combs, 2000), which may cause decreased ruminal pH and accompanying risk of occasionally or permanently low values. Accordingly, a survey of O'Grady, Doherty, & Mulligan (2008) in grazing Irish dairy cows indicated a prevalence of subacute ruminal acidosis, which may be even more pronounced when cows are supplemented with concentrate (Enemark, Peters, & Jorgensen, 2003).

Therefore, the objective of the present study was to compare the effects of concentrate supplementation in early lactation on nutrient efficiency and ruminal fermentation traits in herbage-fed Holstein-Friesian dairy cows with different milk production potentials. We hypothesized that, due to higher feed intake in cows with higher genetic potential, (i) concentrate supplementation has a more positive effect on milk performance and (ii) a more negative impact on reticular pH in cows with higher genetic potential for milk production than in those with lower genetic potential for milk production.

2. MATERIALS AND METHODS

2.1. Animals, experimental design, and feeding

The experiment was carried out with 24 multiparous Holstein dairy cows (previous lactation yield: 4,679 to 10,808 kg) at Agroscope, Posieux, Switzerland from mid-April until the end of August 2013. The experimental procedure started for each cow individually at 2 wk (14 ± 6 d) before the predicted calving date **(LW-2)** and lasted until wk 8 of lactation **(LW8)**.

In order to record the daily feed intake of each cow precisely, cows were not grazed. They were fed fresh herbage in a free stall barn equipped with automatic weighing troughs (RIC System, Insentec B.V., Marknesse, The Netherlands). In preparation for calving, cows were moved to straw-bedded calving pens. All procedures were in accordance with the Swiss guidelines for animal welfare and were approved (No. 2012_12_FR) by the Animal Care Committee of the Canton Fribourg.

Before selecting cows for the experiment, a medical check was performed, including vital parameters, as well as udder and claw health. Based on their previous lactation performance, cows were divided into a high-potential group **(H;** 8959 ± 984 kg) and a low-potential group **(L;** 6204 ± 1000 kg). Both groups were again split equally according to their predicted calving date into two feeding groups (**CON- and CON+)** yielding four treatment groups **(CON-H** [n = 6], **CON-L** [n = 5], **CON+H** [n = 6] and **CON+L** [n = 7]) in a 2 × 2 factorial design. Cows in CON- groups received fresh cut herbage ad libitum without concentrate. Cows in group CON+ were also offered herbage ad libitum, and were supplemented with a barley-maize-wheat mixture (mixing ratio = 1:1:1) and a protein-enhanced concentrate containing maize gluten (Table 1) independent of the actual milk production according to a fixed allocation scheme from 1.0 kg dry matter (DM)/d before calving to a maximum of 7.0 kg DM/d in LW4. From LW4 to LW8 the allocated amounted remained the same. The concentrate intake (kg DM/d) in LW-2, LW2, LW4, LW6 and LW8 was on average 1.0 ±0.03, 4.6 ± 0.68, 6.9 ± 0.22, 7.0 ± 0.06, 6.8 ± 0.54 for CON+H cows and 0.94 ± 0.16, 4.64 ± 0.73, 6.52 ± 0.64, 5.82 ± 1.56 , 5.19 ± 2.30 for CON+L cows. All cows were fed a mineral mixture containing (g/kg) for dry cows: Ca: 3.13 ± 0.63, P: 7.91 ± 0.25, Mg: 14.89 ± 1.73, K: 10.43 ± 0.48, Na: 47.54 ± 5.28 and for lactating cows: Ca: 107.67 ± 4.81, P: 52.12 ± 5.22, Mg: 19.53 ± 3.42, K: 3.28 ± 0.28, Na: 63.93 ± 6.23. Fresh water was available at all times. Herbage was harvested daily between 0800 and 0900 at a cutting height of 5 cm from a mixed sward. The sward was managed in such a way as to simulate as closely as possible a rotational grazing

scheme. The Concentrate was offered in an automatic feeding station (Insentec B.V.). The experiment was embedded in a larger study, in which further traits related to animal well-being were investigated (Zbinden et al., 2017).

2.2. Sample collection

Milk yield and BW after milking were determined twice daily in the milking parlour. Milk samples were taken from each cow during each milking on two consecutive days in the 2nd (LW 2), 4th (LW4), 6th (LW6), and 8th (LW8) wk of lactation. Samples were pooled per day. One subsample was subsequently preserved with Broad-Spectrum Microtabs II (Gerber Instruments AG, Effretikon, Switzerland) and stored at 5 °C for later analysis of milk components, and another subsample was frozen at -20 ºC for urea and acetone content determination. Body condition score (BCS; 1 = thin, 5 = fat) was assessed biweekly.

Each day in the morning and evening, a sample of the herbage offered was taken and dried for 24 hours at 60 ºC to determine the DM content. Furthermore, daily herbage samples were immediately frozen at -20 °C and later pooled over 3 and 4 days and lyophilized (Christ Delta 1–24 LSC, Osterode, Germany). The concentrates were sampled three times during the experiment.

Ruminal fluid was sampled from each cow at 5 time points during the experiment, namely 2 wk before the expected calving date (LW-2) and in LW 2, LW4, LW6, and LW8. Samples were taken at 0600 using a collector tube (Selekt Collector Set, Virbac, Kolding Denmark), which was inserted through the oesophagus into the cranioventral part of the rumen. After collection, the rumen fluid was filtered through a kitchen sieve, and the samples were cooled on ice. For later analysis of volatile fatty acids (VFA) and ammonia (NH_3) contents, 10 mL of ruminal fluid were mixed with 0.2 mL of 50% (vol/vol) sulphuric acid or with 0.2 mL of 50% (vol/vol) trichloroacetic acid and stored at -20 °C. For the analysis of bicarbonate, 50 mL of ruminal fluid were put in a falcon tube and stored at -20 ºC. For the later determination

of protozoal counts, 0.5 mL of ruminal fluid was mixed with 0.5 mL of 6% (vol/vol) formalin. The pH measurements were carried out continuously using a wireless telemetry bolus that includes a pH sensor (eBolus, eCow Ltd., Exeter, Devon, UK). The bolus was applied orally into the reticulum of each cow in LW-2 using a balling gun. Data were downloaded once per week using a tablet computer (Samsung NP-Q1, Samsung, Seoul, South Korea) and processed as described in detail by Falk, Münger, & Dohme-Meier (2016). The individual reticular pH data were summarized daily as minimum pH, maximum pH, and mean pH. The duration (min/d) and total area (pH × h, AUC) when pH was below 6.0 were calculated. This threshold has been recommended by Sato et al. (2012) for the diagnosis of sub-acute ruminal acidosis.

2.3. Laboratory analysis

Milk samples were analysed for fat, protein, and lactose content via mid-infrared spectrometry (Combifoss FT+, Foss, Hillerød, Denmark) (FIL-IDF, 2000; method number 141C). Energy corrected milk (ECM) was calculated based on a 4% fat, 3.2% protein, and 4.8% lactose standard (Agroscope, 2016). The urea content in the milk was determined with a differential pH analyser (Eurochem, Ardea, Italy), which compares the pH before and after hydrolysis of the urea with urease (FIL-IDF, 2000; method number 145). The acetone content in the milk was determined by transferring acetone and an internal standard (2-butanone) via the static headspace directly from the milk into the gas phase. Afterwards, the composition of the gas phase was determined by gas chromatography using a flame ionization detector (HP 5890 Series II, Agilent Technologies, Santa Clara, USA).

The lyophilized grass samples and the concentrate samples were milled through a 1.0 mm screen (Brabender mill with titanium blades, Brabender, Duisburg, Germany) and analysed for nutrient, energy, and mineral content as described in detail by Heublein et al. (2017). The content of ethanol soluble carbohydrates was determined as described by Hall, Hoover, Jennings, & Webster (1999).

The ruminal VFA profile was analysed by HPLC (Dionex, Dionex Corporation, Sunnyvale, CA) with a Shodex detector (Showa Denko K.K, Minato, Japan). Ruminal NH_3 N was determined colorimetrically with a commercial test kit (S 180, BioMerieux, Geneva, Switzerland). The analysis of bicarbonate was conducted according to the manual of Mettler-Toledo AG (Zürich, Switzerland), using a CO_2-ion electrode (No. 152 323 000) with a reference electrode (InLab 3200).

2.4. Statistical analysis

One cow in the CON-H treatment group was diagnosed with a nervous form of ketosis during LW3 and was removed from the experiment. Thus, only the data from LW-2 and LW2 for that cow were included in the analysis. Data collected over several days were averaged per cow and per LW before statistical analysis. Inferential statistics were performed via the MIXED procedure of SYSTAT 13 (Systat Software Inc., San Jose, USA), applied to the averages over wk of the variables measured. The following mixed model with "cow" as a random factor was considered appropriate for this type of repeated measures design:

$$Y_{ijkl} = \mu + t_i + p_j + z_k + A_l + (tp)_{ij} + (tz)_{ik} + (pz)_{jk} + \varepsilon_{ijkl}$$

Y_{ijkl} Observation ijkl of variable Y; random

t_i Treatment i, i = K0, KF; fixed effects

p_j Potential j, j = L, H; fixed effects

z_k Time k in lactation, k = LW-2, LW2, LW4, LW6, LW8; fixed effects

A_l Cow nr l, l = 1590, 1599, …, 1944 (24 cows); random effects

$(tp)_{ij}$ Interaction Treatment i × Potential j

$(tz)_{ik}$ Interaction Treatment i × Time point k

$(pz)_{jk}$ Interaction Potential j × Time point k

ε_{ijkl} Random error in observation ijkl

The random effects A_l and ε_{ijkl} were assumed to be normally distributed with a mean of 0 and variances of σ_A^2, and σ^2, respectively. The variance-covariance structure was modelled as "variance component" for both random terms because there was no benefit to using other modelling choices (such as compound symmetry or AR for the error term). The variables "milk urea" and "milk acetone" were log transformed before analysis. Residual diagnostics (normal plots and formal tests) were applied to decide on the applicability of the parametric model and the detection of outliers (threshold set at z-score of ±3), which were eliminated for the final analyses. There was no need to apply nonparametric or robust procedures. Least-square (LS) means and their standard errors for each combination of the fixed factors were obtained by adding the respective three-way interaction term to the model described above. For simplicity's sake, in Tables 2 to 5, only LS means and maximum standard errors of LS means are reported. If interactions occurred, data were additionally represented graphically (figures 1, 2 and 3). Factors are considered statistically significant for $P < 0.05$.

3. RESULTS

The chemical composition of the herbage varied considerably across the time of the experiment, especially with respect to crude protein (CP) and soluble carbohydrate contents (Table 1). Concentrate supplementation resulted in a higher ($P \leq 0.001$) yield of milk and of ECM and lower milk fat percentage ($P = 0.004$) and acetone content ($P < 0.001$) (Table 2). Protein and lactose percentage, the urea content of the milk, and BCS and BW were not affected ($P > 0.05$) by concentrate supplementation. The yield of ECM related to metabolic BW was higher ($P = 0.012$) in supplemented than in unsupplemented cows. High-potential cows

produced more milk (P = 0.014) and ECM (P = 0.005), had a higher milk acetone content (P = 0.002), and were heavier (P = 0.005) than low-potential cows. Milk potential had no effect (P > 0.05) on the other traits presented in Table 2. The treatment × potential interaction (P < 0.001) observed for milk acetone content (Figure 1) was a result of the extremely high values found for CON-H cows, whereas those of the other three groups were quite similar. Apart from milk urea content and BCS, all traits were influenced (P ≤ 0.004) by time in lactation, such that ECM, BW, and milk fat and protein percentage decreased with increasing days in milk. Milk yield and lactose percentage, and especially acetone content, were highest in LW4.

Concentrate supplementation caused a decrease (P < 0.001) in herbage intake and an increase (P < 0.001) in the intake of total DM, CP, and, net energy for lactation (NE_L) (Table 3). The NDF intake was unaffected by supplementation ((P > 0.05). Energy corrected milk produced per kg DM ingested was higher (P < 0.001) and milk energy efficiency (MEE) was lower (P < 0.001) in supplemented cows than in unsupplemented cows. The other production efficiency measures were not affected (P > 0.05) by supplementation. Potential had no effect (P > 0.05) on nutrient and energy intake but influenced production efficiency such that high-potential cows produced more ECM per kg of herbage (P = 0.031) and total DM (P < 0.001) ingested and had better milk protein efficiency (MPE; P = 0.008) and MEE (P = 0.001) than low-potential cows. Nutrient and energy intake increased (P ≤ 0.005) from pre-partum until LW6 and subsequently decreased in LW8. All production efficiency measures (P < 0.001) were highest in LW2 and decreased continuously up to LW8 (except for MPP, which was higher in LW8 than in LW6). Treatment × potential interaction occurred for herbage intake (P = 0.019) and NDF intake (P = 0.007). Apart from LW-2 and LW8 CON-L cows had the highest intake and CON+L cows had always the lowest intake (Figure 2). There were treatment × potential interactions for total DM intake (P = 0.023) and NE_L intake (P =0.030), mainly due to the high-potential cows, who had the highest intake with and the lowest intake without concentrate supplementation compared to low-potential cows (Figure 2). However, the difference in total

DM and NE_L intake between CON-L and CON-H cows became negligible in LW6 and LW8, which was the main reason for the treatment × time interaction (total DM, $P = 0.006$; NE_L, $P < 0.001$). Treatment × potential interaction for ECM produced per kg DM ingested ($P = 0.010$) was accounted for by the different reaction of cows to concentrate supplementation because unsupplemented high-potential cows had the highest and unsupplemented low-potential cows the lowest production (Figure 1). An interaction of treatment × potential was observed for MPE ($P = 0.002$) because CON-H cows had by far the best MPE compared to cows of the other groups, which all showed similar MPE. As the MPE of CON-H cows decreased from LW2 to LW8 and those of CON-L and CON+L cows increased in LW8, a potential × time interaction ($P = 0.033$) was observed (Figure 1).

Total ruminal VFA was not influenced ($P > 0.05$) by treatment, but molar percentage of ruminal acetate decreased in favour of propionate and butyrate ($P \leq 0.013$), and ruminal NH_3 N content and bicarbonate content were lower ($P \leq 0.048$) in supplemented cows than in unsupplemented cows (Table 4). High-potential cows had a lower ($P = 0.017$) content of total VFA and a higher ($P = 0.007$) content of bicarbonate, but the VFA profile and the content of NH_3 N remained unaffected ($P > 0.05$) by potential. The content of total VFA and the molar percentage of propionate continuously increased ($P \leq 0.024$) and molar percentage of acetate decreased ($P < 0.001$) with progressing lactation, whereas molar percentage of butyrate was unaffected ($P > 0.05$). Time in lactation influenced ($P \leq 0.010$) NH_3 N and bicarbonate content, but the effect was inconsistent. Treatment × potential and treatment × time interactions were observed for percentage of acetate ($P \leq 0.016$) and propionate ($P \leq 0.007$) because, independent of supplementation, the percentage of acetate and propionate of the low-potential cows were found to be between the values for the high-potential cows (Figure 3); however, this was not consistent across lactation weeks. There was a treatment × potential interaction ($P = 0.005$) for content of bicarbonate, mainly due to higher content in CON-H cows than in the other three treatment groups (Figure 3).

Reticular pH was neither influenced by treatment nor by potential ($P > 0.05$; Table 5), but some traits were affected ($P \leq 0.007$) by time in lactation. Daily mean, maximum, and minimum pH, as well as the time span during which pH was below 6.0, decreased continuously up to LW6.

4. DISCUSSION

4.1. Herbage quality

The average nutrient and energy content of the herbage was lower compared to other Swiss grazing and zero grazing studies (Dohme-Meier et al. 2014; Thanner et al. 2014a; Heublein et al. 2017). However, other studies with similar herbage quality exist (Müller et al. 2018; Thanner, Schori, Bruckmaier & Dohme-Meier, 2014b) indicating the difficulties of providing herbage from a mixed sward with consistently good quality over a longer period of time. The study lasted from mid-April to the end of August. During this time the herbage quality but also the composition of the sward varied in the course of the growing season. Contents of CP and WSC are often high in early spring and decline during late spring and early summer. In the present study, cows calved within two months so that cows in the same wk of lactation did not always get herbage of the same quality. Dietary effects might therefore be masked by the varying herbage quality.

4.2. Effects on Milk Production and Feed Intake

In late pregnancy and early lactation, cows struggle to meet their nutrient requirements. This is even more pronounced in feeding systems with little or no input of concentrate. Sairanen et al. (2005) showed that cows in mid-lactation produced 25.1 kg/d milk when ingesting 17.2 kg DM/d of only herbage. Furthermore, they concluded that it is difficult to achieve a milk production of more than 30 kg/d with a diet of herbage alone, possibly due more to the limited nutrient supply resulting from a combination of lower feed intake and an imbalanced diet than

to the genetic potential of the cows. The concentrate supplementation caused an increase in milk yield of 7.2 kg/d, whereas the supposed difference in milk production potential was only 3.3 kg/d. Unlike in the work of Sairanen et al. (2005), a DMI of 14.8 kg/d resulted in a milk yield of 28.4 kg/d in early lactating cows in our study. According to Kertz, Reutzel, & Thomson (1991), feed intake is a major factor limiting milk production in early lactation. In line with Ingvartsen & Andersen (2000), who found that the lowest DMI occurred at calving and the maximum DMI was reached 8 to 22 wk postpartum, the DMI of cows in this study was lowest before calving and increased with increasing days in milk, independent of treatment and milk production potential. Similar to what Thanner et al. (2014a) found, the genetic potential for milk production had no effect on DMI in the present study.

In contrast, Buckley, Dillon, Crosse, Flynn, & Rath (2000) found higher herbage DM and total DMI in cows with a high potential for milk production than in cows with average potential. Likewise, Heublein et al. (2017) found by trend a higher herbage DMI and total DMI in Swiss Holstein cows with higher potential than New Zealand Holstein cows. In contrast to other studies (e.g., Heublein et al. 2017) we found for DMI interactions between concentrate supplementation and milk production potential. Across LW, herbage DMI was lowest for CON+L, followed by CON+H and CON-H, and it was highest for CON-L. Total DMI was higher for supplemented cows; CON+H cows had the highest DMI across LW and CON-H cows had the lowest DMI until LW4, with a DMI subsequently similar to that of CON-L cows. This observation confirms previous findings that supplementation causes a decrease in herbage DMI but increases total DMI (Heublein et al. 2017; Bargo, Muller, Delahoy, & Cassidy, 2002). Furthermore, it seems that high genetic potential cows on an herbage only diet struggle to eat enough DM, at least in early lactation. Authors of a study (McCarthy et al., 2007) that compared the feeding behaviour of high-producing Holstein cows of North American origin with that of lighter and lower producing New Zealand (NZ) Holstein cows concluded that NZ Holstein cows which were genetically selected on predominantly herbage-based diets have a more aggressive

feeding drive in the pasture in order to increase their DM intake and to optimize their productivity. However, it is unclear whether cows in a zero-grazing system display the same feeding behaviour as cows on pasture.

A comparison of grazing cows and cows offered herbage of the same quality in the barn showed that grazing cows needed more time to ingest the same amount of DM than zero-grazing cows (Dohme-Meier et al., 2014). The difference in DMI between CON-H and CON-L cows was only pronounced in the first LW and might be explainable by other factors. Milk acetone content as well as the energy balance data and concentrations of blood metabolites as published by Zbinden et al. (2017), indicate that CON-H cows were especially challenged metabolically during this time, and metabolic factors such as metabolite and hormone levels may play an important role in the intake regulation of periparturient cows (Ingvartsen & Andersen, 2000). Furthermore, high-potential cows seem less able than low-potential cows to adapt to the reduced nutrient availability in an herbage-based system without concentrate supplementation (Thanner et al., 2014).

As previously shown in cows in mid-lactation (Heublein et al., 2017; Bargo et al., 2002), concentrate supplementation increased milk yield, and as hypothesized, this positive effect was stronger in cows with a high genetic potential for milk production. High-potential cows have a greater milk yield response to concentrate supplementation (Heublein et al., 2017), and this phenomenon may be attributed to greater nutrient partition to milk production in these cows than in low-potential cows (Dillon, Berry, Evans, Buckley, & Horan, 2006). Similarly, production efficiency measures as ECM per kg DMI, MEE, and MPE were better in high- than in low-potential cows. Interaction found for MEE and ECM per kg DMI showed that CON-H cows especially were highly efficient, whereas CON-L cows had similar (MEE) and the lowest (ECM/kg DMI) efficiency, respectively, compared to supplemented cows. The differences between CON-H and the other cows decreased with increasing days in milk. Concomitantly, the negative energy balance and the reduction in body weight was most pronounced in CON-H

cows compared to the other cows (Zbinden et al. 2017) and the differences were especially distinctive until LW6. This indicates that the priority of milk production after parturition is high in high-potential cows and remains so even if nutrient availability is clearly reduced.

In addition to the efficiency measures investigated in the present study, fertility and overall survival are important factors to evaluate efficiency. Although long-term on-farm studies carried out on organic dairy farms suggest that concentrate reduction has no effect on fertility (Leiber et al., 2017) or health status (Leiber et al., 2017; Sehested, Kristensen, & Søegaard, 2003), further studies are needed to check the long-term effects of herbage-only feeding, fresh or conserved, with no concentrate supplementation in high-potential dairy cows.

4.2. Effects on ruminal fermentation

According to Bargo et al. (2003), the most consistent effect of concentrate supplementation on ruminal fermentation is a decrease of NH_3 concentration in ruminal fluid, which aligns with our results. Herbage often contains more rumen-degradable protein than can be utilized by ruminal microbes. The absorption and metabolisation of the resulting surplus NH_3 leads to increased urea levels in the blood and milk (Heublein et al., 2017). However, milk urea levels in our study were similar with and without concentrate supplementation and lower compared to Heublein et al. (2017), indicating that the diet of the cows fed herbage only was not only limited in energy but also in CP. Bruinenberg, Zom, & Valk (2002) reported a frequently high excretion of urinary N in herbage-fed cows, resulting in high N losses and adverse environmental effects (Dijkstra et al., 2013). Furthermore, a reduced flux of (microbial) protein to the duodenum, due to low dietary supply of fermentable energy, is supposed to be a limiting factor for milk production in cows consuming herbage (Bruinenberg et al., 2002).

In contrast to other studies, in which milk protein was lower in unsupplemented than in supplemented cows (Sehested et al., 2003) or higher in low-potential cows than in high-potential cows (Heublein et al., 2017; Thanner et al., 2014a), no effect of supplementation or

milk production potential was found in the present study. Furthermore, milk fat percentage was, in opposition to Thanner et al. (2014), not affected by milk production potential but by supplementation. Similar to other studies (Heublein et al., 2017; Bargo et al., 2002), in the present study, although the NDF intake was unaffected, supplemented cows were found to have lower milk fat percentages, consistent with a lower proportion of ruminal acetate and a higher proportion of propionate, than their unsupplemented peers. In general, the amount of carbohydrates in the diet and their fermentability affect ruminal function (Krause & Combs, 2003). However, increasing concentrate supplementation to a diet of fresh herbage resulted in no effect on total VFA concentration, in a decrease in ruminal acetate, and in an increase in ruminal butyrate proportion (Sairanen et al., 2005). In agreement with this, in the present study, concentrate supplementation had no effect on total VFA concentration. In contrast, Bargo et al. (2002) found increased total ruminal VFA concentration and increased proportion of propionate and butyrate but no effect on acetate in grazing cows due to concentrate supplementation. These inconsistent findings might be explainable by differing compositions of the concentrate used and consequently by the varying fermentability of the carbohydrate source. Furthermore, high-quality herbage itself can contain high amounts of water soluble carbohydrates and highly digestible fibre, which may explain why moderate amounts of concentrate only have minor effects on ruminal fermentation (Sairanen et al., 2005).

Against our expectations and despite a higher DMI, high-potential cows had a lower concentration of total ruminal VFA, accompanied by a higher concentration of ruminal bicarbonate, than low-potential cows. Potential reasons for this could be found in differing rates of ruminal passage and exchange at the rumen wall. Another interesting feature are the interactions found between supplementation and potential in terms of the ratio of ruminal acetate to propionate. They showed that from LW4 onwards, high-potential cows in both treatment groups had the highest (without concentrate) and the lowest (with concentrate) acetate

to propionate ratio, whereas the low-potential cows were between the two other groups. An explanation might be the interaction in NDF, herbage and total DM intake as discussed above.

Total ruminal VFA concentration has a strong relationship with ruminal pH, with a low ruminal pH being associated with a high VFA concentration (Kolver & de Veth, 2002). However, in the present study, supplementation had no effect on total VFA concentration, or consequently on reticular pH, and differences in VFA concentration between milk production potential levels were probably too small to cause differences in reticular pH. Furthermore, measurements in the reticulum might be less sensitive than measurements in the rumen (Falk et al., 2016). However, Reis & Combs (2000) similarly found no changes in ruminal pH when concentrate was supplemented. According to Bargo et al. (2003), interactions between quantity and type of concentrate, DMI, and quality of herbage play an important role in the regulation of ruminal pH. Therefore, the influence of concentrate supplementation on ruminal pH in grazing cows is often inconsistent, and no simple relationship can be established. In our study, reticular pH decreased continuously from LW-2 until LW6, which was in line with an increasing DMI and consequently higher ruminal fermentation activity, indicated by an increasing concentration of ruminal VFA.

5. CONCLUSION

Regardless of the genetic potential for milk production, supplemented cows produced more milk than unsupplemented cows, although effects on ruminal fermentation and reticular pH remained small. High-potential cows without supplementation seemed to be less able to compensate for the reduced nutrient availability on an herbage-only diet than low-potential cows, which in turn could not use this advantage for better nutrient efficiency. The continuously high milk acetone content until LW6 and the removal of one cow diagnosed with a nervous form of ketosis from the group of high-potential cows without supplementation indicate that

these cows are particularly prone to metabolic stress and consequently to production diseases in early lactation.

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Table 1. Chemical composition and nutrient values of herbage (n = 36) and concentrates (n = 3, each) samples (means ± SD)*

	Herbage		Protein rich concentrate		Energy rich concentrate	
	Mean	SD	Mean	SD	Mean	SD
DM (g/kg of wet weight)	172	27.6	886	19.8	875	27.7
Analysed nutrients and mineral composition (g/kg of DM)						
OM	904	11.6	947	15.7	954	2.1
CP	145	30.9	248	29.4	123	14.4
NDF	457	32.7	216	139	251	170
ADF	289	20.6	61.6	4.79	51.2	2.36
Lignin	43.5	12.0	nd		nd	
ESC	94.6	18.4	nd		nd	
WSC	179	45.5	nd		nd	
Starch	nd		500	26.2	626	8.2
Ca	6.59	2.532	10.19	4.449	8.26	0.427
P	4.23	0.805	5.25	2.337	3.48	0.292
Mg	1.76	0.521	5.25	0.375	1.18	0.087
K	32.9	3.93	5.07	1.10	5.77	0.53
Calculated energy and protein supply# per kg of DM						
NE_L (MJ)	5.57	0.547	8.10	0.00	8.08	0.00
APDE (g)	94	9.5	202	0.00	111	0.00

*SD, standard deviation; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; Ethanol soluble carbohydrates; WSC, Water soluble carbohydrates; NE_L, net energy for lactation; APDE, absorbable protein in the small intestine when rumen fermentable energy is limiting microbial protein synthesis in the rumen; nd, not determined. Means taken from Zbinden et al. (2017).
#according to Agroscope (2016)

Table 2. Effects of treatment, milk production potential and time point of measurement on nutrient and milk performance, body condition score and body weight*

	Milk yield (kg/d)	ECM (kg/d)	Fat (%)	Protein (%)	Lactose (%)	Urea† (mg/kg)	Acetone† (mg/kg)	BCS	BW	ECM (kg/ kg of $BW^{0.75}$)
CON-	27.3	28.7	4.41	3.00	4.66	172	9.61	3.03	667	0.225
CON+	34.5	33.4	3.74	3.07	4.78	152	3.05	3.03	703	0.250
H	32.6	33.1	4.23	3.03	4.66	165	9.10	3.12	729	0.242
L	29.2	29.0	3.93	3.04	4.78	159	3.56	2.94	640	0.233
LW-2								3.12	753	
LW2	29.2	33.4	4.75	3.42	4.63	188	7.31	3.11	678	0.251
LW4	34.3	31.7	4.17	3.01	4.78	145	10.22	3.03	667	0.243
LW6	32.4	30.2	3.77	2.86	4.76	159	4.76	2.99	664	0.232
LW8	31.9	28.9	3.62	2.85	4.72	155	3.04	2.91	660	0.223
SEM#	2.31	1.04	0.164	0.047	0.061	34.3	4.520	0.086	22.2	0.0071
P - values										
TREATMENT (TR)	<0.001	0.001	0.004	0.271	0.126	0.389	<0.001	0.940	0.235	0.012
POTENTIAL (PO)	0.015	0.005	0.177	0.829	0.134	0.707	0.002	0.053	0.005	0.347
TIME IN LACTATION (TI)	0.029	<0.001	<0.001	<0.001	0.004	0.275	0.004	0.141	<0.001	<0.001
TR × PO	0.419	0.887	0.104	0.268	0.055	0.535	<0.001	0.632	0.070	0.065
TR × TI	0.163	0.435	0.748	0.260	0.937	0.958	0.244	0.080	0.266	0.539
PO × TI	0.157	0.252	0.194	0.159	0.754	0.879	0.478	0.862	0.162	0.449

*CON-, unsupplemented cows; CON+, supplemented cows; H, high-potential cows based on the previous lactation performance; L, low-potential cows based on the previous lactation performance; LW-2, two wk before predicted calving; LW2, two wk postpartum; LW4, four wk postpartum; LW6, six wk postpartum; LW8, eight wk postpartum; ECM, energy corrected milk; BCS, body condition score; BW, body weight; $BW^{0.75}$, metabolic body weight; SEM, standard error of the means.

#Greatest SEM is shown

†Log-transformed for statistical analysis.

Table 3. Effects of treatment, milk production potential and time point of measurement on nutrient and energy intake and production efficiency measures*

	Herbage intake (kg of DM/d)	Total DMI (kg DM/d)	CP intake (kg/d)	NDF intake (kg/d)	NE_L intake (MJ/d)	ECM (kg/kg of herbage DMI)	ECM (kg/kg of DMI)	MPE	MEE
CON-	14.8	15.0	2.11	6.78	85.3	1.94	1.91	0.420	1.09
CON+	12.9	17.5	2.53	6.53	109.7	2.61	1.86	0.414	0.93
H	14.2	16.6	2.36	6.83	99.7	2.39	2.02	0.437	1.07
L	13.5	15.9	2.27	6.47	95.3	2.15	1.76	0.396	0.94
LW-2	11.8	12.8	2.03	5.48	76.5				
LW2	13.1	15.3	2.29	6.42	90.3	2.60	2.23	0.464	1.21
LW4	14.0	16.9	2.33	6.77	103.1	2.37	1.98	0.426	1.03
LW6	15.5	18.3	2.55	7.39	110.6	2.06	1.69	0.377	0.90
LW8	14.8	18.0	2.39	7.21	106.9	2.05	1.66	0.399	0.88
SEM#	0.79	0.85	0.215	0.187	3.39	0.079	0.577	0.0142	0.027
P - values									
TREATMENT (TR)	<0.001	<0.001	<0.001	0.240	<0.001	<0.001	0.448	0.684	<0.001
POTENTIAL (PO)	0.160	0.196	0.381	0.093	0.174	0.031	<0.001	0.008	0.001
TIME IN LACTATION (TI)	<0.001	<0.001	0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TR × PO	0.019	0.023	0.347	0.007	0.030	0.087	0.010	0.156	0.002
TR × TI	0.161	0.006	0.119	0.962	<0.001	0.480	0.958	0.267	0.635
PO × TI	0.472	0.202	0.962	0.208	0.719	0.166	0.062	0.870	0.033

*CON-, unsupplemented cows; CON+, supplemented cows; H, high-potential cows based on the previous lactation performance; L, low-potential cows based on the previous lactation performance; LW-2, two wk before predicted calving; LW2, two wk postpartum; LW4, four wk postpartum; LW6, six wk postpartum; LW8, eight wk postpartum; CP, crude protein DM, dry matter; DMI, dry matter intake; ECM, energy corrected milk; MEE, milk energy efficiency was calculated as the ratio of milk energy yield to dietary energy (NE_L) intake; MPE, Milk protein efficiency was calculated as the ratio of milk protein yield to dietary CP intake; NDF, neutral detergent fibre; NE_L, net energy for lactation; SEM, standard error of the means.

#Greatest SEM is shown

Table 4. Effect of treatment[1], potential[2] and time point of measurement[3] on ruminal volatile fatty acids, ammonia and bicarbonate[*]

	Total VFA (mmol/L)	Acetate (molar %)	Propionate (molar %)	n-Butyrate (molar %)	Ammonia N (mmol/L)	Bicarbonate (mmol/L)
CON-	81.9	69.3	17.6	10.8	3.14	48.8
CON+	89.8	66.1	19.5	11.6	2.48	40.4
H	80.0	67.7	18.6	11.2	2.86	50.4
L	91.7	67.7	18.5	11.2	2.76	38.8
LW-2	79.2	69.7	16.6	10.9	4.58	55.7
LW2	81.2	68.5	18.3	11.0	2.54	47.2
LW4	87.6	67.9	18.9	10.8	1.76	39.1
LW6	88.9	66.6	19.4	11.6	2.49	40.0
LW8	92.3	66.0	19.6	11.5	2.70	41.1
SEM[#]	7.90	1.30	1.01	0.68	0.79	9.04
P-Value						
TREATMENT (TR)	0.105	<0.001	<0.001	0.013	0.046	0.048
POTENTIAL (PO)	0.017	0.999	0.819	0.931	0.748	0.007
TIME IN LACTATION (TI)	0.024	<0.001	<0.001	0.227	<0.001	0.010
TR × PO	0.105	0.016	0.007	0.530	0.495	0.005
TR × TI	0.806	0.004	0.001	0.578	0.774	0.828
PO × TI	0.644	0.521	0.79	0.286	0.705	0.396

[*]CON-, unsupplemented cows; CON+, supplemented cows; H, high-potential cows based on the previous lactation performance; L, low-potential cows based on the previous lactation performance;; LW-2, two wk before predicted calving; LW2, two wk postpartum; LW4, four wk postpartum; LW6, six wk postpartum; LW8, eight wk postpartum; SEM, standard error of the means; VFA, volatile fatty acids.
[#]Greatest SEM is shown

0

Table 5. Effect of treatment[1], potential[2] and time point of measurement[3] on the pH in the reticulum[*]

	pH mean	pH maximum	pH minimum	AUC > pH6 (pH units × h/d)	time below pH6 (min /d)
CON-	6.26	6.59	5.96	31.3	203
CON+	6.22	6.54	5.95	32.0	263
H	6.22	6.56	5.92	43.1	305
L	6.26	6.57	5.99	20.3	161
LW-2	6.44	6.81	6.08	2.6	74
LW2	6.25	6.61	5.96	17.3	164
LW4	6.20	6.53	5.92	44.6	316
LW6	6.14	6.44	5.87	55.6	347
LW8	6.18	6.44	5.95	38.3	264
SEM[#]	0.18	0.18	0.20	41.6	238.7
P-Value					
TREATMENT (TR)	0.669	0.489	0.916	0.978	0.688
POTENTIAL (PO)	0.651	0.858	0.473	0.396	0.341
TIME IN LACTATION (TI)	<0.001	<0.001	<0.001	0.396	0.007
TR × PO	0.592	0.690	0.660	0.631	0.846
TR × TI	0.451	0.605	0.376	0.896	0.729
PO × TI	0.142	0.260	0.059	0.510	0.142

[*]CON-, unsupplemented cows; CON+, supplemented cows; H, high-potential cows based on the previous lactation performance; L, low-potential cows based on the previous lactation performance; ; LW-2, two wk before predicted calving; LW2, two wk postpartum; LW4, four wk postpartum; LW6, six wk postpartum; LW8, eight wk postpartum; SEM, standard error of the means; AUC, area und the curve.
[#]Greatest SEM is shown.

Figure captions

Figure 1

Milk acetone (A), energy corrected milk per dry matter intake (B), and milk energy efficiency calculated as the ratio of milk energy yield to dietary energy (NE_L) intake (C) in high-potential cows (based on the previous lactation performance) without concentrate supplementation (●), low-potential cows without concentrate supplementation (○), high-potential cows with concentrate supplementation (■), low-potential cows with concentrate supplementation (□) in lactation week (LW) -2, two wk before predicted calving; 2, two wk postpartum; 4, four wk postpartum; 6, six wk postpartum; 8, eight wk postpartum. Data are given as mean values ± SEM.

Figure 2

Herbage intake (A), total dry matter intake (B), neutral detergent fiber (NDF) intake (C) ,and energy (NE_L) intake (D) in high-potential cows (based on the previous lactation performance) without concentrate supplementation (●), low-potential cows without concentrate supplementation (○), high-potential cows with concentrate supplementation (■), low-potential cows with concentrate supplementation (□) in lactation week (LW) -2, two wk before predicted calving; 2, two wk postpartum; 4, four wk postpartum; 6, six wk postpartum; 8, eight wk postpartum. Data are given as mean values ± SEM.

Figure 3

Ruminal acetate (A), ruminal propionate (B), and ruminal bicarbonate (C) in high-potential cows (based on the previous lactation performance) without concentrate supplementation (●), low-potential cows without concentrate supplementation (○), in high-potential cows with concentrate supplementation (■), low-potential cows with concentrate supplementation (□) in lactation week (LW) -2, two wk before predicted calving; 2, two wk postpartum; 4, four wk postpartum; 6, six wk postpartum; 8, eight wk postpartum. Data are given as mean values ± SEM.

Figure 1

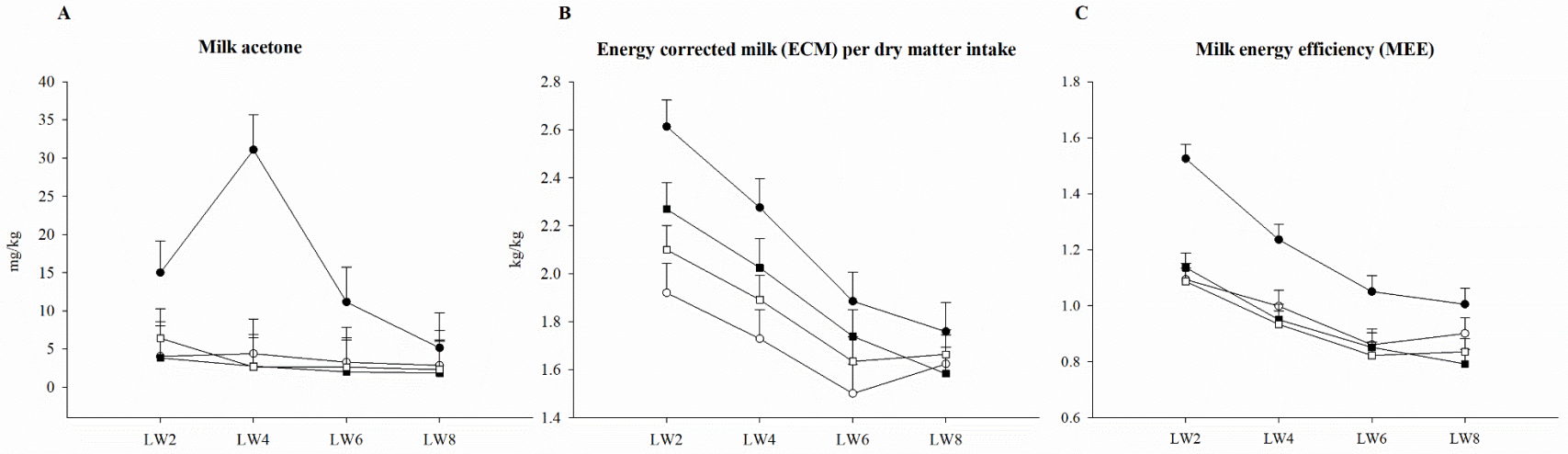

A
Milk acetone
mg/kg
40
35
30
25
20
15
10
5
0
LW2
LW4
LW6
LW8
B
Energy corrected milk (ECM) per dry matter intake
kg/kg
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
LW2
LW4
LW6
LW8
C
Milk energy efficiency (MEE)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
LW2
LW4
LW6
LW8

Figure 2

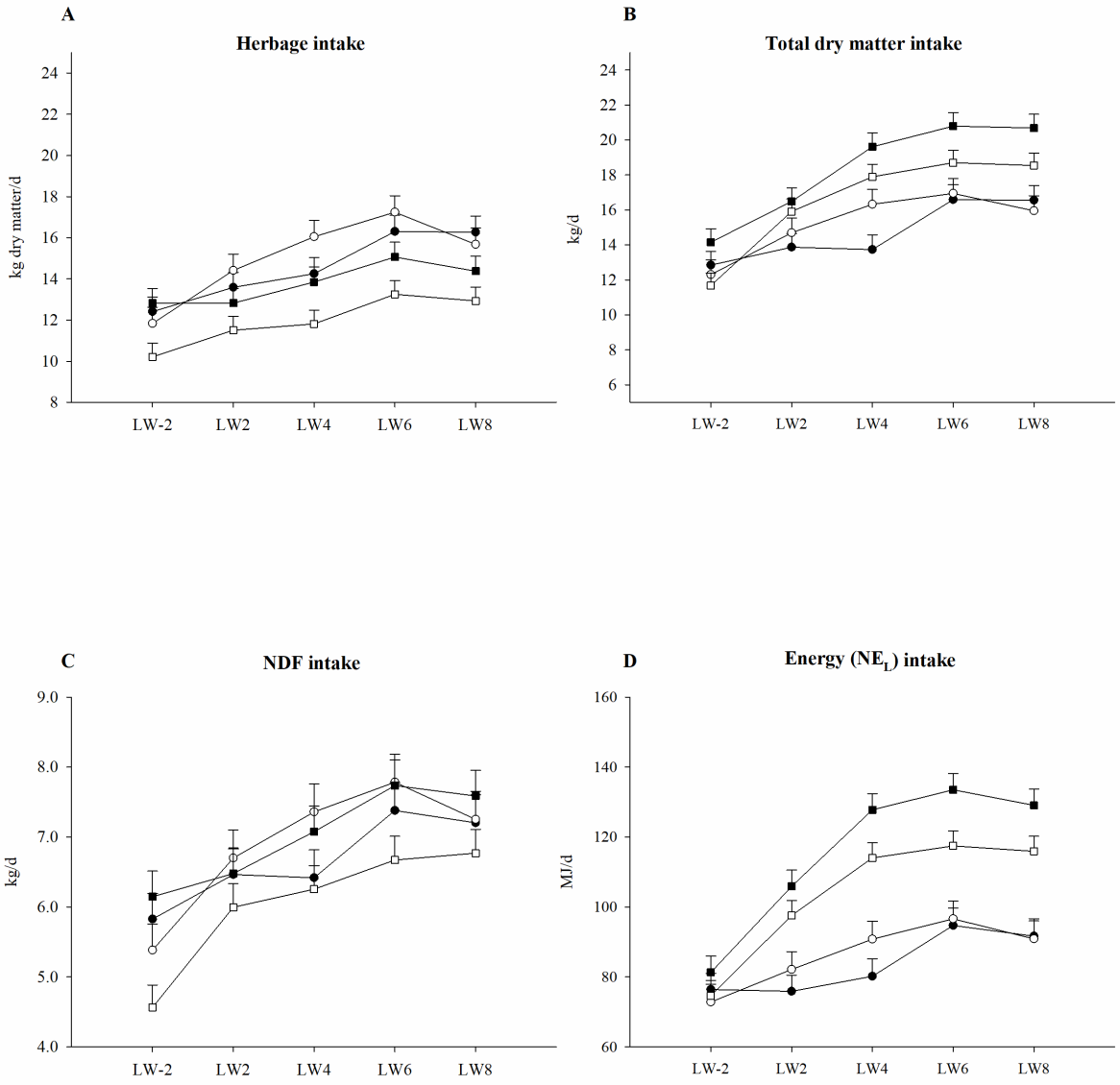
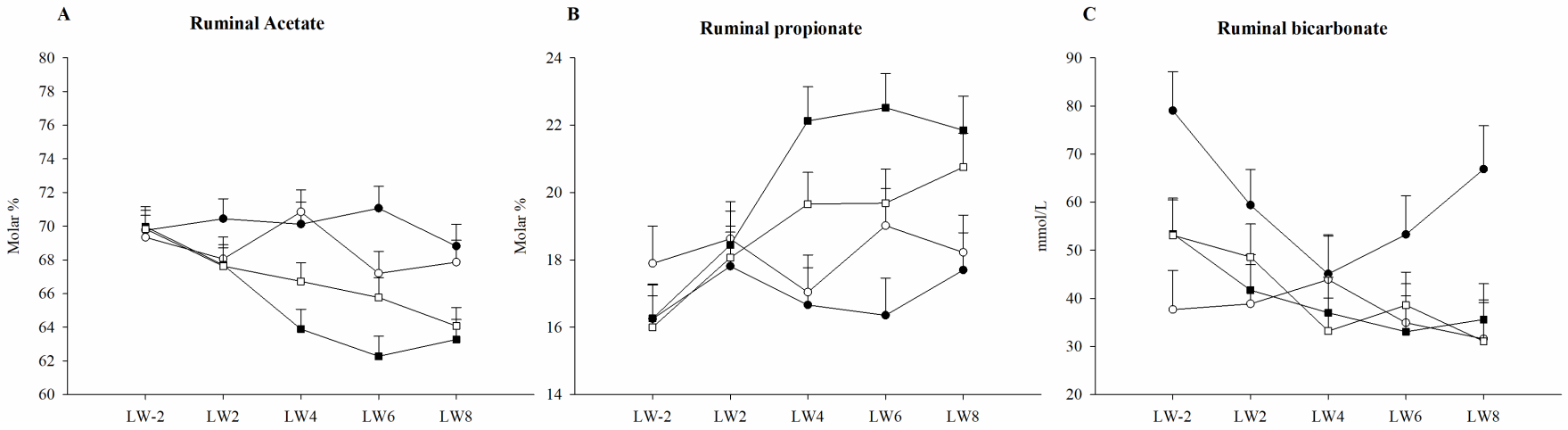

A
Herbage intake
kg dry matter/d
24
22
20
18
16
14
12
10
8
LW-2
LW2
LW4
LW6
LW8
B
Total dry matter intake
kg/d
24
22
20
18
16
14
12
10
8
6
LW-2
LW2
LW4
LW6
LW8
C
NDF intake
kg/d
9.0
8.0
7.0
6.0
5.0
4.0
LW-2
LW2
LW4
LW6
LW8
D
Energy (NEL) intake
MJ/d
160
140
120
100
80
60
LW-2
LW2
LW4
LW6
LW8

Figure 3


A
Ruminal Acetate
Molar %
80
78
76
74
72
70
68
66
64
62
60
LW-2
LW2
LW4
LW6
LW8
B
Ruminal propionate
Molar %
24
22
20
18
16
14
LW-2
LW2
LW4
LW6
LW8
C
Ruminal bicarbonate
mmol/L
90
80
70
60
50
40
30
20
LW-2
LW2
LW4
LW6
LW8

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