**Effects of water restriction following feeding on nutrient digestibilities, milk yield and composition and blood hormones in lactating Holstein cows under heat stress conditions**

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**Abstract**

The effects of water restriction following feeding under heat stress conditions on nutrient digestibilities, milk yield and composition and some blood hormones in lactating Holstein cows were evaluated. The design was completely randomized with 30 high producing lactating Holstein cows (80.8±40.5 DIM) which were assigned to two treatment groups (15 cows per treatment). Treatments were free access to water (FAW) and 2 h water restriction (2hWR) following feeding. Average temperature-humidity index (THI) in the farm was over 80 throughout the experiment which defines heat stress conditions. Neutral detergent fibre, organic matter and ether extract digestibilities increased by water restriction (P<0.05); however, crude protein, acid detergent fibre, nitrogen free extract and dry matter digestibilities were not affected (P>0.05). Water intake was recorded daily during the digestibility period and was not different between FAW and 2hWR group (P>0.05). Fat corrected milk was higher in 2hWR group than FAW group (P<0.05). Milk fat was higher (P<0.05) in 2hWR group than the FAW group whereas milk protein, urea nitrogen, and solids not fat were not different among the treatment groups (P>0.05). Somatic cell counts were greater in 2hWR than FAW group (P<0.05). Free fatty acid concentration in blood was not affected by treatments (P>0.05). Blood prolactin and growth hormone were higher in 2hWR group than the FAW group (P<0.05). It is concluded that water restriction for 2 hours following feeding improved nutrient digestibility of some dietary components and increased milk fat percentage in lactating Holstein cows under heat stress conditions.

**Introduction**

Heat stress causes major losses ($2.4$ billion) in animal production, including dairy cattle, beef cattle, swine and poultry in the USA (St-Pierre *et al.*, 2003). Heat stress affects feed intake, body temperature, maintenance requirements and metabolic processes, feed efficiency, milk yield, reproductive efficiency, behavior and disease incidence (Cook *et al.*, 2007; Tucker *et al.*, 2007; Rhoaead *et al.*, 2009). Water is necessary for a proper rumination and digestion (Casamassima *et al.*, 2008). In addition, water loss from the body occurs via urine, faeces and milk; through sweating and by evaporation from body surfaces and the respiratory tract (West, 2003). However, the amount of water loss from a cow’s body is influenced by activity, air temperature, humidity, respiratory rate, water intake, feed consumption, milk yield and other factors. Water restriction could intensify the effect of heat stress related to performance including digesta flow rate, saliva excretion and nutrient digestibility or blood parameters and decreased performance of animals (Marai *et al.*, 2007). Furthermore, water is consumed several times per day and generally is associated with feeding or milking. Cows may consume about 50% of their daily water intake within 1 h following milking (Beede, 1992; NRC, 2001). Peak of water intake for cows occurs during the hours when feed intake is greatest (Beede, 1992). When given the opportunity, cows tend to alternate consume feed and drink water (Nocek and Braund, 1985). Water restriction in sheep increased nutrient digestibilities (Ghassemi Nejad *et al.*, 2014a) and blood cortisol levels (Ghassemi Nejad *et al.*, 2014a). High yielding dairy cows are more sensitive to water restriction than sheep or other small ruminants (Marai *et al.*, 2007; Casamassima *et al.*, 2008). Moreover, during hot weather nutrient digestibility in cows may improve (West, 1997, 2003). Restriction water intake of cattle caused an increase in the apparent digestibility of dry matter, nitrogen and other nutrients (Thorton and Yates, 1968). Microbial protein synthesis depends on the availability of nitrogen (or amino acids) and on the degradable carbohydrate content, or on the content of organic matter, and the synchronization in rumen degrada-
cows in each pen, according to a completely randomized design for 74 days. The cows were housed in a roofed shelter equipped with concrete pens confined with metal and bedded with dry manure for comfort (4.5 m² per cow). Metal automatic roofs covered the barn and were open when there was no rain. The cows were fed in mangers and each pen was equipped with separate water through. The experimental procedure and methods were approved by the animal welfare and ethics authority of Kangwon National University, Chuncheon, Korea. Data from two cows (one from each treatment groups) were not considered and withdrawn from the experiments due to serious mastitis and right abomasum displacement. Treatments were free access to water (FAW) and 2 h water restriction (2hWR) following feeding. Feed was provided as total restriction time, cows were given free access to dry feed and weighed at 07:00 h in the morning. To serious mastitis and right abomasum displacement.

Collection of residual feed (1 kg) from manger and faeces (1 kg) directly from rectum of each cow was daily at 16:00 h for 8 d at the beginning of experiment, then at one month and 10 days prior to completing the experiment, and were stored for analysis. Approximately 1 kg of faeces collected from each cow daily were sub-sampled to a plastic bag, dried (65°C for 3 days), finely ground (1 mm) and analysed for nutrients (AIA method) as described by Van Keulen and Young (1977) including ether extract (method 920.39; AOAC, 1990) and ash (method 927.02; AOAC, 1990). Five high yielding cows (3rd parity; <40 lit milk/d 53.5±30.4 DIM; 650±53 kg BW) from each group (2 cows from 1st two pens and 1 from the 3rd pen, separated by wire within pens) were chosen for individual feeding (10 days) and to collect residual feed every morning prior to milking at 0630 h. Each pen facilitated with water trough, however, single marked water bucket for each 5 cows during individual feeding was used to measure water intake. Water intake was measured during digestibility periods from the cows. The average water consumption for each cow was then recorded for each group. The N was determined by Kjeldahl procedure using Cu2+ as a catalyst, and CP was calculated as N×6.25 (method 990.03; AOAC, 1990). Neutral detergent fibre and ADF were expressed with residual ash.

Cows were milked twice daily at 06:30 and 18:00 h with milk yields recorded electrically at each milking. Milk samples (15 mL) from each cow were taken manually into 45 mL sterile tubes (Wisd, Seoul, Republic of Korea) at each milking every 15 days during the experimental period. The pooled composited milk samples were analysed for concentrations of fat, protein, and urea using a Foss 4000 milko Scan (Foss electronic, France).

**Temperature-humidity index**

Temperature and relative humidity (THI) were monitored at hourly intervals throughout the trial by using a temperature-humidity data logger device (CEM-DT-172, No. 11048007, Shenzhen, China). Average temperature-humidity index (THI) in the farm was over 80 throughout the experiment calculated using the equation of Ravagnolo et al. (2000) which defines heat stress conditions (Berman et al. 1985) (Figure 1):

\[
\text{THI} = (1.8t + 32) - (0.55 - 0.0055RH)(1.8t - 26)
\]

where \(t\) is the air temperature (°C) and RH is the relative humidity (%). The THI values obtained indicate the following: <71, absence of heat stress; 72 to <79, mild heat stress; 80 to <89, moderate heat stress; and 90 and above, severe heat stress (Armstrong, 1994).

**Statistical analysis**

Data for intake, milk yield and THI were analysed using repeated measures analysis and MIXED procedure of SAS (ver. 9.01; SAS institute Inc. Cary NC, USA) and the means were compared for significance by Tukey’s t-test (Snedecor and Cochran, 1967) at P<0.05. The statistical model used for analyses was:

\[
Y_{ijkl}=\mu+c_{i}+d_{j}+W_{k}+T_{l}+(cT)_{il}+e_{ijkl}
\]

where

\(Y_{ijkl}\) = observation for trait,
Dry matter digestibilities were not affected (P>0.05) by treatment groups (Table 4). Crude protein, ether extract, nitrogen free extract and acid detergent fibre digestibilities increased (P<0.05) between FAW and 2hWR group (Table 3).

Variance and covariance assumption structures including auto regressive (1) (AR(1)), unstructured (UN), and compound symmetry (CS) were tested, then AR(1) as the best covariance structure selected when appropriate. The interaction of treatment×time was not significant. Statistical analysis was carried out using GLM procedure of SAS except the data for intake, milk yield and THI. Initial milk yield and composition (each component) as well as DIM were used as a covariate and were included in the model when appropriate but were removed from the model when not significant. Only component which showed higher values in 2hWR group than FAW group prior to beginning the experiment was SCC. Milk variables were tested for normality and milk SCC were log-transformed to normalize their frequency distribution before performing statistical analysis. Based on this test, except for SCC, we did not transform variables to normalize their distribution before GLM analysis. Statistical differences were considered significant at P<0.05.

Results
Nutrient digestibility
Neutral detergent fibre, organic matter and ether extract digestibilities increased (P<0.05) by water restriction (Table 2). Crude protein, acid detergent fibre, nitrogen free extract and dry matter digestibilities were not affected (P>0.05) by water restriction (Table 2).

Feed and water intake, milk yield and compositions
Feed intake was not different (P>0.05) between two treatment groups (Table 3). Water intake showed no difference (P>0.05) between FAW and 2hWR group (Table 3). Actual milk yield was not different (P>0.05) between two groups (Figure 2, Table 3). Fat-corrected milk (4% FCM) was higher (P<0.05) for 2hWR than for FAW group (Table 3). Milk fat was higher (P<0.05) in 2hWR than the FAW group while milk protein, urea nitrogen and solid non fat were not different (P>0.05) among the treatment groups (Table 3). However, SCC was higher (P<0.05) in 2hWR group than FAW group (Table 3).

Plasma free fatty acids and hormones
Free fatty acid concentration in plasma was not affected (P>0.05) by treatment groups (Table 4). Prolactin and growth hormone were higher (P<0.05) in 2hWR group than the FAW group (Table 4).

Discussion
Water restriction following feeding in this study improved some nutrient digestibilities and consequently milk fat percentage and fat corrected milk (FCM). Generally, water restriction may improve nutrient digestibilities by decreasing feed intake and increasing retention time of the digesta giving more time for microbial degradation and synthesis (Ahmed Muna and El Shafei Ammar, 2001; Casamassima et al., 2008). In this study water restriction was applied following feeding and then cows had free access to water 2 hours.
post feeding, therefore, water restriction showed no effect on feed intake. However, other studies showed no increase in nutrient digestibility by water restriction (Bohra and Ghosh, 1977; Silanikove, 1987). Water is arguably the most important nutrient for dairy cows and should be available in adequate amounts especially during heat stress (West, 2003). However, restriction of water availability may negatively affect cows’ well-being and performance (West, 2003). In addition, water restriction for any reason can act as a stressor and intensify the severity of stress, particularly during heat stress (Ghassemi Nejad et al., 2014a). The effect of water restriction following feeding has on the digestibility of feed is likely to be due to changes in either the rate of breakdown of feed in the reticulo-rumen or the length of time the food remains there (Balch et al., 1953).

Water restriction does not affect feed intake (Singh et al., 1976), similar to our results, although it slows down the movement of digesta from the reticulo-rumen to the intestine (Brosh et al., 1986), which may increase apparent digestibility of feed giving more time for microbial enzymes to attach to the feed particles properly. In agreement, the present experiment showed no differences in feed intake between two treatments (Table 3). Ghassemi Nejad et al. (2014b) recently reported that water restriction in sheep for 2 and 3 h following feeding increased nutrient digestibility of feed. Water is required for proper rumination and digestion (Casamassima et al., 2008); however, water restriction increases the digestive capacity of rumen (Brosh et al., 1986) which may lead to improving nutrient digestibility of feed. It may therefore be deduced that although the digesta in the reticulo-rumen neither was more fluid nor remained for a sufficiently longer time when water intake was restricted, conditions were more favourable for breakdown of fibre. It could be postulated that during water restriction following feeding, water production of saliva was increased, and this might provide more favourable condition for fermentation in the ventral sac. Water restriction had no effect on feed intake in agreement with the study of Mengistu et al. (2004) and suggesting that ruminants are able to accumulate water in reserve to be used in periods of reduced water supply. Balch et al. (1953) reported that fistulated cows could adjust their water economy favouring the maintenance of optimum water levels in the digesta in the reticulo-rumen when water intake was restricted. Although fewer digesta of a slightly drier consistency were found in the reticulo-rumen, they mentioned it is likely that the secretion of saliva was increased and that the increased amounts of saliva favoured fermentation (Balch et al. 1953). No difference in water intake between two groups implies that water restriction for 2 h does not affect water consumption. It might be explained by no difference in feed intake which correlated with water consumption. As lactating cows in water restricted group had free access to water 2 h following feeding, they had adequate time to consume sufficient water.

Higher fat percentage in 2hWR group which led to higher FCM may be related to higher apparent digestibility of fibre and ether extract. However, in the current study, actual milk yield showed no difference between two groups. Gradual decrease in actual milk yield (Figure 2) during the experiment can be due to the effects of prolonged heat stress.

The unchanged in plasma free fatty acids indicates that there was no fat mobilization and, hence, no energy deficit during water restriction. This result is in agreement with the study of Burgos et al. (2001). Qinisa and Boomker (1999) reported several blood parameters changed in South African indigenous goats during only the first few days of a 50% water restriction period, however, those parameters returned to baseline levels thereafter. Hence, it could be possible to extend the water restriction period for a longer without additional effects (Little et al., 1976). One possible explanation for higher growth hormone and prolactin concentration in 2hWR group is higher nutrient digestibilities in this group. However, the exact reason(s) which led to higher growth hormone and prolactin concentration in water restricted group is unknown. Higher GH and prolactin values could be viewed with caution as blood sample was collected only once in a day. Regarding SCC, however, we interrogated the data to determine if there are any unintentional biases built in. We could not find any unintentional biases in SCC data set and concluded that higher values in 2hWR group at the start of the experiment continued to be higher even at the end of the experiment. Furthermore, the values for SCC are not high to decrease milk quality.

**Conclusions**

From the results of this experiment it is concluded that water restriction for 2 hours following feeding can be applied in dairy farms because it improves digestibility of some nutrients, increases 4% FCM and also milk fat percentage in lactating Holstein cows under heat stress conditions. However, more research in the area is required before this claim can be supported. Water restriction for 2 h following feeding did not reduce feed intake in the present study.

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