



# Manure contamination of drinking water influences dairy cattle water intake and preference

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## ABSTRACT

Water intake is closely related to feed intake and its reduction associated with negative welfare and production implications. This study aimed to assess the intake and preference for drinking water of different quality; clean (tap water), and water contaminated with either 0.05 mg (Low) or 1 mg (High) fresh manure/g water. Water and feed intake of 18 non-lactating, pregnant cows were monitored in individual, indoor pens. Each cow was given one of the three water treatments for 5 days, then a different treatment for a second 5-day period (no choice), and finally access to both in a choice test (5 days). Data were analysed using REML and paired t-tests. Contamination of water significantly affected intake in the no choice phase ( $P < 0.001$ ). Cows provided with Low or High contaminated water had 10 and 28%, respectively, lower water intake than those provide with clean water (Clean: 37.0 L/day, range: 28.4–53.6; Low: 33.2 L/day, range: 26.0–44.9; High: 26.6 L/day, range: 13.6–37.8). Feed intake was not influenced by water treatment. During the choice test, cows showed a clear preference for clean water over water contaminated with manure (Clean vs. Low: 19.8 vs. 6.5 L,  $P = 0.014$ , SED: 3.60 L; Clean vs. High: 29.8 vs. 0.21 L,  $P < 0.001$ , SED: 1.82; and Low vs. High: 27.2 L vs. 0.29 L,  $P < 0.001$ , SED: 1.70). Cows only consumed 1% of their daily water intake from the High treatment when they had another option. In conclusion, dairy cattle can detect low levels of manure contamination in their drinking water, and avoid drinking it if possible. The preference to drink clean water was particularly clear. This study highlights the importance of providing cattle with clean drinking water.

## 1. Introduction

Water is essential to life and an important nutritional component to animals. It is a medium for transportation of nutrients, waste products, hormones, and other chemical messengers, and aids in the movement of feed through the gastrointestinal tract (McAllister et al., 1994). The water intake of ruminants consists of voluntarily consumed water and water in the feed and is positively associated with feed intake in both beef (Brew et al., 2011) and dairy cattle (Stockdale and King, 1983). Factors that affect voluntary water intake include animal factors, such as milk yield and body weight (Meyer et al., 2004), as well as external factors, such as weather conditions (Blackshaw and Blackshaw, 1994), dry matter content of the feed (Stockdale and King, 1983), and trough design (Pinheiro Machado Filho et al., 2004).

Water intake restriction has negative effects on animal production and welfare. Severe water restriction (50–60% of voluntary intake) decreased feed intake (Utley et al., 1970; Little et al., 1978), milk yield, body weight (Little et al., 1978, 1980), and caused a change in behaviour, such as increased aggressiveness and time spent around the water

trough, and less lying (Little et al., 1980). These changes in behaviour were also evident when cows were provided 90% of voluntary water intake in a second study by Little et al. (1980) thus indicating that having free access to drinking water is important to dairy cattle.

Factors which limit the quality and palatability of drinking water, have the potential to not only reduce welfare, but limit growth and production. The most studied influences on the quality and palatability of water include high concentrations of dissolved minerals (Weeth and Hunter, 1971; Grout et al., 2006), microbial contamination, particularly from faecal matter (Willms et al., 2002; Lardner et al., 2005), and temperature (Lofgreen et al., 1975; Wilks et al., 1990).

The few studies that have investigated contamination of manure of drinking water have been undertaken on beef cattle in free ranging systems. Pastured cattle may be exposed to drinking water contaminated with faecal matter by having direct access to ponds and other types of waterways, thereby contaminating the water themselves. Access to waterways is often restricted in pastured dairy systems for environmental reasons. Nevertheless, other sources of contaminants, such as wild birds utilising the water source, or the spreading of effluent

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for pasture growth can impact water quality. Analysis of trough water from three pasture-based farms (two dairy, one beef), reported faecal coliform (including *E. coli*) contamination between < 10 to 30,000 faecal coliform units (cfu)/100 ml water (unpublished data). Dairy cattle may therefore at times have to consume water that has been contaminated with faecal matter and coliform bacteria. As a comparison, guidelines for bacterial limits for adult cattle in the literature range from < 10 to 1000 cfu per 100 ml water (Smith et al., 1993; MUE, 1995; Grant, 1996; Looper and Waldner, 2002). To our knowledge, no studies have described dairy cattle preference and consumption of drinking water contaminated with manure. Willms et al. (2002) demonstrated that free ranging beef cattle avoid drinking water containing 0.005% manure, however, potential breed differences, management systems, and selection pressures may result in different levels of sensitivity of dairy cattle and warrant further investigation. Therefore, our aim was to study the effects of water quality (different levels of manure contamination) on dairy cattle feed and water intake and preference. It was hypothesised that manure contamination of drinking water would affect intake and animal preferences. We predicted that cows would drink less of the water that had higher levels of contamination, and when given a choice, show a preference for clean water over contaminated water.

## 2. Materials and methods

### 2.1. Animals and study design

All procedures involving animals in this study were approved by the Ruakura Animal Ethics Committee under the New Zealand Animal Welfare Act 1999. The study was undertaken at the DairyNZ Lye Farm, Hamilton, New Zealand (37°76'S 175°37'E) during May 2018 (Southern Hemisphere winter). The study utilised 18 individually housed, non-lactating, pregnant, Friesian and Friesian-cross dairy cows who were either 3 or 4 years of age with an average body weight of  $452 \pm 12.6$  kg and an average body condition score of  $4.5 \pm 0.21$  (SD) on a 10-point scale (Roche et al., 2004). The cows were selected based on calving date (late calving) and similar live weight and body condition score. They were then randomly assigned into the different treatments. The study consisted of two no-choice phases ( $2 \times 5$  days) where each cow had access to two water treatments (Clean and Low, Clean and High, or Low and High), sequentially (order of treatment exposure balanced between cows), followed by a choice phase (5 days) where the cows had simultaneous access to the two water treatments they had previously experienced (Table 1). Between each phase all cows were provided clean water for 1 day to minimise any carry-over effects. The cows had *ad libitum* access to drinking water at all times. The treatments (both during the no-choice and choice phase), order of treatments, and the location of treatment troughs (left or right) during the choice phase were balanced throughout the barn and between animals.

**Table 1**

Experimental design of the trial investigating the effects of three water treatments (Clean, Low and High) on water intake and animal preferences. The initial phase of the experiment exposed cattle to each water treatment exclusively with a period of habituation beforehand ( $n = 12$  per treatment in total). Cattle were then asked to choose between the two water treatments they had previously experienced ( $n = 6$  per pairwise choice).

| Sample  | 7 days <sup>a</sup> | 5 days | 1 day <sup>a</sup> | 5 days | 1 day <sup>a</sup> | 5 days                |
|---------|---------------------|--------|--------------------|--------|--------------------|-----------------------|
| $n = 3$ | Habituation         | Clean  | Recovery           | Low    | Recovery           | Choice Clean vs. Low  |
| $n = 3$ | Habituation         | Low    | Recovery           | Clean  | Recovery           | Choice Clean vs. Low  |
| $n = 3$ | Habituation         | Clean  | Recovery           | High   | Recovery           | Choice Clean vs. High |
| $n = 3$ | Habituation         | High   | Recovery           | Clean  | Recovery           | Choice Clean vs. High |
| $n = 3$ | Habituation         | Low    | Recovery           | High   | Recovery           | Choice Low vs. High   |
| $n = 3$ | Habituation         | High   | Recovery           | Low    | Recovery           | Choice Low vs. High   |

<sup>a</sup> All cows had *ad libitum* access to clean drinking water during the habituation and recovery periods.

### 2.2. Experimental facility

The experimental facility was a free-stall barn ( $26 \times 23$  m) with partly open ends with two rows of sand bedded free-stalls on either side. In the middle of the barn was a central lane (width: 5.5 m) where feed was provided. A smaller lane (width: 1.0 m) ran behind the free-stalls on each side of the barn allowing for collection of drinking water residuals and refilling of water bins. Ten individual pens were created by dividing the free-stall area with conventional farm gates to existing pipework. Each pen ( $7.0 \times 2.2$  m) consisted of a feeding area (0.7 m wide in the central alley with an access slot 200 mm wide), a grooved loitering concrete area ( $4.5 \times 2.2$  m), and two lying areas (two free-stalls with deep bedded sand), each measuring  $1.1 \times 2.5$  m. Two water troughs ( $2 \times 60$  L, each measuring 600 mm (length)  $\times$  400 mm (width)  $\times$  320 mm (depth), Payless Products, Hamilton, New Zealand) at a height of 65 cm were mounted into a 20 mm thick plywood frame and placed under the shoulder rail of the right hand free-stall. The 2 troughs were located next to each other, 10 cm apart. In the back corner of each trough, 20 mm Guyco valves (Norma Group, Melbourne, Australia) were mounted to enable the troughs to be emptied in an easy and consistent manner. Plywood divisions next to the water troughs ensured that a cow could not gain access to her neighbour's water. Nine cows were housed in each row of the barn ( $2 \times 9$  cows in total).

All cows were habituated to the experimental facility for 7 days, being offered *ad libitum* access to clean drinking water during this time. At approximately 08:00 h on 3 days each week the cows were taken and stood outside the barn on a concrete yard for approximately 1 h whilst the pens were cleaned. The cows had no access to drinking water during this time.

### 2.3. Water treatments

There were three drinking water treatments consisting of clean (tap) water with different levels of manure contamination (mg refrigerated manure/g water): 0 mg/g water (Clean), 0.05 mg/g water (Low), or 1.0 mg/g water (High). To maintain consistency of treatments throughout each 5-day period, manure was collected from cows who had previously received clean water. All pens were cleaned 3 times per week and fresh looking manure was collected off the floor and then refrigerated. The treatments were created by adding the required amount of manure to either 70 ml (Low) or 250 ml (High) specimen containers (Export Lodge Veterinary Supplies Ltd., Palmerston North, New Zealand) using calibrated scales (Mettler Toledo model PG-503-S, Mettler Toledo, Columbus, OH, USA). The samples were stored in a chiller at 1 °C and all water treatments were made fresh each day. Samples from all three batches of manure ( $3 \times 5$  days throughout the whole trial period) were dried for 72 h at 65 °C in an oven and the percentage dry matter (DM) calculated as dry weight/wet weight  $\times$  100. The DM content of the collected manure throughout the trial period was, on average,  $12.8 \pm 0.33\%$  (SD).

Water treatment application commenced at approximately 08:45 h each day. Treatments were made daily, the required volume of water

was dispensed into the trough using an automatic dispenser (Pistola K500 LT/BSP, Piusi Spa, Suzzara, Italy). A sample from this water volume was added to the refrigerated manure sample and shaken vigorously to form a slurry which was then poured and stirred thoroughly through the water in the trough. After treatment allocation no additional stirring was undertaken. During the no-choice phases a total of 30 L of water was supplied per trough (60 L in total per cow), this amount was increased to 40 L during the choice phase to enable a free choice between the two water treatments (80 L in total per cow). If necessary, these were topped up with additional treated water in the afternoon to maintain an *ad libitum* supply of all treatments during the night.

Four control troughs were located in the empty pen on each side of the barn to measure water temperature and consistency of the applied treatments over time (changes in bacteria concentration after 5 days of refrigeration). The troughs were treated in the same manner as troughs used by cows. Treatment water of days one and five of each period were analysed for *E. coli* concentrations. The method used for analysis was Membrane Filtration, count on mFC agar, incubated at 44.5 °C for 22 h, the default detection limit was 1 cfu/100 mL. *E. coli* concentrations for the two contaminated water treatments on the first day of application were, on average, for Low: < 1 - < 10 cfu/100 ml (range only) and for High: 717 cfu/100 ml (range: < 10 - 1900 cfu/100 ml). On day five the concentrations were, on average, for Low: < 1 - 5 cfu/100 ml (range only) and for High: 1153 cfu/100 ml (range: < 10 - 3000 cfu/100 ml).

The water temperature was measured every 30 min using ibuttons (Embedded Data Systems, DS1921H- F5#, resolution: 0.125 °C, accuracy: 1 °C, Lawrenceburg, KY, USA) located at the bottom of each control trough. Average water temperature was 13.0 °C (range: 2.6–18.7 °C) excluding times between 09:00 and 11:30 h as troughs were emptied during this period.

## 2.4. Water and feed intake

Residues of drinking water were measured at approximately 08:45 h each day before re-application of the water treatment. Water residues were drained from each trough into a container on calibrated scales (Model WS207, Wedderburn, Hamilton, New Zealand). Sponges were used to ensure all water was retrieved and then the troughs were wiped clean.

Cows were offered a daily average ration of  $22.3 \pm 1.89$  kg grass silage (DM: 43%) and  $11.8 \pm 2.5$  kg maize silage (DM: 32.6%) at approximately 09:00 h. Individual feed intake was recorded by weighing refusals of maize and grass silage combined using a scale (Smartscale 500, Gallagher Group Ltd., Hamilton, New Zealand).

## 2.5. Environmental measures

Air temperature (°C) and relative humidity (%) were monitored in the barn every 30 min using two Lascar data loggers (EL-USB-2-LCD+, resolution: 0.5 °C and 0.5%, accuracy: 0.45 °C and 2.05%, Lascar Electronics Ltd., Salisbury, UK) which were placed centrally in the feed area of the barn at a height of 110 cm. The average temperature within the barn during the study was 12.3 °C (range: 0.5–22.5 °C) and average humidity was 84.5% (range: 63–98.5%). The average outside environmental conditions were recorded every 30 min throughout the duration of the trial using a portable weather station (Vantage Pro2 Plus, Davis Instruments Corp., CA, USA) located in an unsheltered location outside the facility. Average temperature during the duration of the trial was 10.1 °C (range: –2.4–20.6 °C) and average humidity was 89% (range: 62–97%).

## 2.6. Statistical analysis

In the no-choice phase, the average water consumption of each cow over the 5 days of each period was analysed using a repeated measures

analysis (REML). The model included treatment as fixed factor. Random terms were, cow, carry-over effects, period effects and all relevant interactions. There was no evidence of any carry-over effects. In addition, a REML using splines was undertaken to investigate whether the treatment effects changed over the 5 days within each period. This model included fixed factors for the treatment and day of trial and their interaction, day spline terms overall, by treatment and by cow, the last term modelling the serial correlation. Random effects were cow, period, and any carry-over effects. One animal with extreme outliers, however otherwise seemingly normal, was removed from the analysis; the treatment effect was significant at  $P < 0.001$  with and without this animal, however, the standard error of the differences between the means was substantially smaller when the animal was omitted. Paired comparisons were done using the *t*-test. The feed residuals during the no-choice phase (averaged per cow over the 5 days) were analysed using the same model as for water consumption.

In the choice phase, the average water consumption of each cow over the 5 days of the period was analysed using REML. The model included treatment as fixed factor and cow as random term. The water consumption of cows with the High treatment was very nearly zero and the variability within this treatment was consequently very low. Therefore, the model also allowed for a different variance for each treatment and a *t*-test on within cow differences was used to test each pair of treatments. A REML was also undertaken to investigate whether the treatment effects changed over the 5 days within each period; there were no such significant effects. All statistical analyses were conducted using the statistical package Genstat, version 19 (VSN International, Hemel Hempstead, UK). Mean values and the standard error of the means (SEM) or difference (SED) are presented. Statistical significance was set at  $P < 0.05$ .

## 3. Results

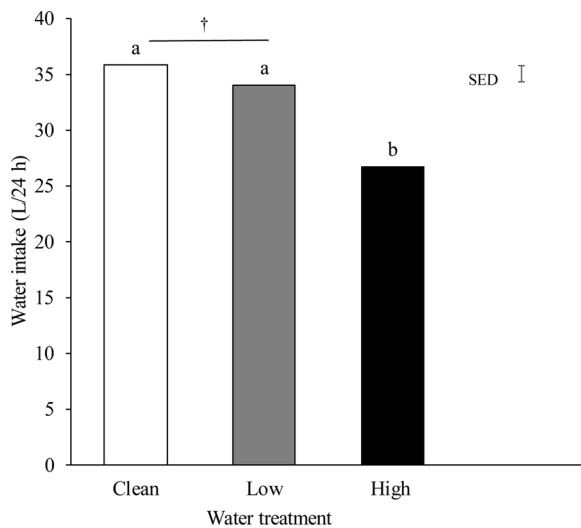
### 3.1. No-Choice phase

Water treatment significantly affected water intake ( $P < 0.001$ ,  $F_{2,15} = 24.27$ ). Cows reduced their water intake by 10% when they only had access to the Low treatment ( $P = 0.061$ ,  $t_5 = 2.41$ ), and by 28% when they received the High treatment ( $P = 0.012$ ,  $t_5 = 4.39$ ), compared to clean water (Fig. 1). Cows also consumed less water when they had access to the High treatment compared to the Low treatment ( $P = 0.012$ ,  $t_5 = 3.82$ ; Fig. 1). The water intake was for Clean: 37.0 L/day (range: 28.4–53.6), Low: 33.2 L/day (range: 26.0–44.9) and High: 26.6 L/day (range: 13.6–37.8).

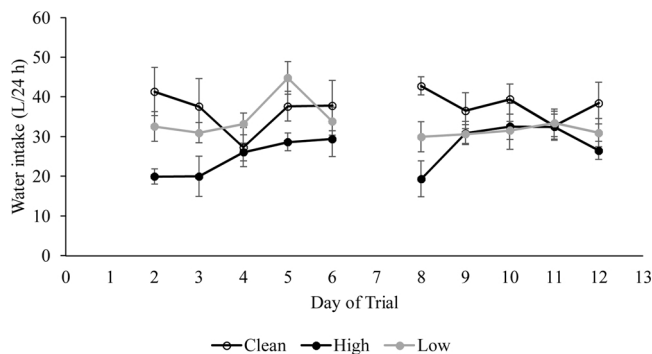
Water consumption did not change significantly over the 5 days of exposure ( $P = 0.171$ ,  $F_{1,2} = 1.88$ ; Fig. 2). Drinking water treatment did not influence feed intake; the feed residuals were similar between treatments ( $P = 0.969$ ,  $t_5 = 0.04$ ; Clean: 1.1 kg/cow/day, Low: 1.1 kg/cow/day, and for High: 1.2 kg/cow/day, SED: 0.48 kg).

### 3.2. Free choice phase

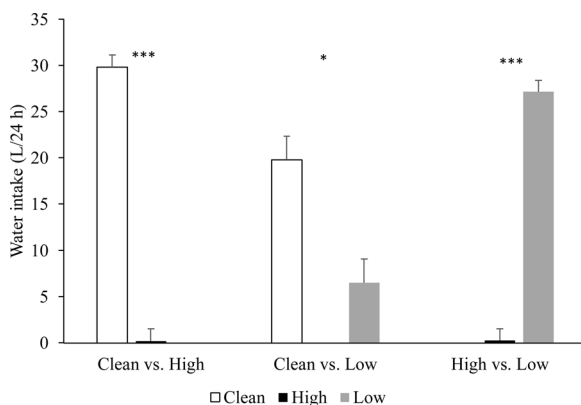
When cows had a pairwise choice between the water treatments they showed a clear preference for drinking clean water; 75 and 99% of the water intake was from the clean water when the other choice was Low and High contaminated water, respectively (Clean vs. Low: 19.8 vs. 6.5 L, SED: 3.60 L,  $P = 0.014$ ,  $t_5 = 3.69$ , Clean vs. High: 29.8 vs. 0.21 L, SED: 1.82,  $P < 0.001$ ,  $t_5 = 16.28$ ; Fig. 3). When the options were Low and High contamination, cows preferred to drink the water with less contamination (Low: 27.2 L, High: 0.29 L, SED: 1.70,  $P < 0.001$ ,  $t_5 = 15.79$ ; Fig. 3). Cows consumed only 1% of their daily water intake from the High water treatment when they had another option (either the Clean or the Low treatment).



**Fig. 1.** Water intake of pregnant, non-lactating dairy cattle ( $n = 12$  cows/treatment) that received drinking water with different manure contamination; Clean, Low (0.05 mg/g water), or High (1 mg/g water) for 5 consecutive days. Values are daily averages and the Standard Error of the Difference (SED). Different letters indicate  $P < 0.05$  based on pair-wise comparisons. † indicates  $P = 0.061$ ,  $t_5 = 2.41$ .



**Fig. 2.** Water intake of pregnant, non-lactating dairy cattle ( $n = 12$  cows/treatment) that received drinking water with different manure contamination; Clean, Low (0.05 mg/g water), or High (1 mg/g water). Each cow had access to one water treatment for 5 consecutive days and then a different water treatment for another 5 consecutive days with one day of recovery in between. Values are daily averages and the Standard Error of the Means.



**Fig. 3.** Water intake of pregnant, non-lactating dairy cattle when offered a simultaneous choice between two drinking water treatments. The treatments were either clean water (Clean) or water contaminated with manure (Low: 0.05 mg/g water, or High: 1 mg/g water,  $n = 6$  cows per pairwise choice). Values are daily averages and the Standard Error of the Means. \*\*\* $P < 0.001$ , \* $P < 0.05$  (from paired t-tests with 5 degrees of freedom).

#### 4. Discussion

Pregnant, non-lactating dairy cattle that received drinking water contaminated with 0.05 and 1 mg fresh manure/g water, reduced their daily water intake by 10 and 28%, respectively, compared to cows provided with clean drinking water. When given a free choice, cows showed a clear preference for the clean drinking water.

There is a close relationship between feed and water intake (Stockdale and King, 1983; Brew et al., 2011) and the quality and palatability of water affects productivity. For example, beef cattle spent more time grazing and gained more weight when they had access to clean drinking water rather than pond water (Willms et al., 2002), which the authors speculated could be due to increased water and feed intake associated with the provision of clean water. Indeed, conditions that lead to depressed water intake are likely to result in decreased production (milk and weight gain) as has been shown by others (Little et al., 1978, 1980; Solomon et al., 1995).

Water consumption of beef cattle was depressed at manure amounts above 2.5 mg/g water in Willms et al. (2002). In the present study, water consumption was reduced at both 0.05 mg and 1 mg manure/g water contamination. We speculate that the differences between studies could be due to factors, such as feeding conditions, dry matter content of the manure used in the treatments, or breed differences.

Feed intake was depressed when beef cattle received 5 mg fresh manure/g water in Willms et al. (2002), whereas there was no effect of water contamination on feed intake in the present study. The cows reduced their water intake by almost 30% when they were only offered water with 1 mg manure/g water compared to clean water, and it is possible that this reduction in water intake was not sufficient to depress feed intake. Restricting drinking water to 60% of free choice in steers caused a reduction in feed intake from 6.2 to 4.8 kg per day (Utley et al., 1970). It is also possible that the provided feed amount was not enough to detect a difference in residues as cows in all treatments had very low residues. The cows were fed according to their pregnancy status and to ensure appropriate body condition to avoid calving issues and could therefore not be provided unlimited feed. Further research should investigate the effects of water quality on feed intake of cows that are provided *ad libitum* feed.

When cows had a free choice between the different water treatments they showed a clear preference to drink the clean drinking water; 75 and 99% of the water intake was from the clean water source when the other option was the low and high contaminated water, respectively. Yearling steers that had a free choice between drinking water containing 0, 0.05, and 0.25 mg fresh manure/g water consumed 75% of their daily intake from the clean water source, and only 6.2% from the water source contaminated with 0.25 mg/g water (Willms et al., 2002). Similar to the study by Willms and collaborators, the cows in our study always preferred to drink the water with the least contamination levels, showing that cattle are sensitive to manure contamination of their drinking water. The preference for clean water was evident from the first day of testing, which is in contrast with findings of Willms et al. (2002) where beef cattle showed no preference on the first day of testing when offered clean water and water contaminated with 0.05 mg manure/g water. Even though similar contamination amounts were used in the two studies, we speculate that there were differences in some characteristics of the applied manure that contributed to the different response; for example, in the dry matter content of the manure. The mechanisms for how cows decide on the palatability of water are unclear. We observed that the cows sniffed the water before drinking or refusing it and we speculate that there is a certain amount of contamination required for cows to be able to detect faecal matter in the water. The specific compounds responsible for reducing water palatability are unknown although Dohi et al. (1999) identified organic fractions in cattle faeces that appear to be responsible for causing avoidance. To our knowledge, this is the first study to demonstrate that dairy cattle can detect very small amounts of manure in their drinking



water and that they avoid drinking contaminated water, if possible. We encourage further research investigating the effects of water quality and palatability on feed intake and productivity in lactating dairy cattle.

Avoiding contamination with manure and maintaining hygiene is likely biologically relevant from an evolutionary perspective to reduce the risk of pathogen exposure and disease. Dairy cattle avoid contamination of their bodies with manure while defaecating (Whistance et al., 2011), and by actively avoiding lying down on surfaces contaminated with manure (Schütz et al., 2019). Environments contaminated with manure influence the cleanliness and hygiene of cows and increase the risk of infection, high somatic cell count (Barkema et al., 1999; Reneau et al., 2005) and clinical mastitis (Schreiner and Ruegg, 2003). Cattle avoid forage contaminated with manure (Forbes and Hodgson, 1985; Bao et al., 1998) and the avoidance of contaminated drinking water, both in beef (Willms et al., 2002; Lardner et al., 2005) and dairy cattle in the present study, demonstrates the importance of providing cattle with a clean environment as well as feed and water that do not contain contamination with manure.

## 5. Conclusions

Dairy cattle that received drinking water contaminated with manure consumed less water compared to animals with clean drinking water. When given a free choice, cows showed a clear preference for the clean drinking water. The aversion against the water with the highest contamination levels was particularly marked. This is the first study to demonstrate that dairy cattle avoid drinking water that has been contaminated with manure and highlights the importance of providing cattle with clean drinking water.

## Conflict of interest

The authors declare there was no conflict of interest.

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