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Drinking behavior of dairy cows under commercial farm conditions differs depending on water trough design and cleanliness

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ABSTRACT

In dairy cows, an adequate water supply is necessary for optimal feed consumption, productivity, health, and animal welfare. In the present study, we tested the hypothesis that dairy cows' drinking behavior is altered by the design and cleanliness of their drinking troughs. The study was conducted at a commercial dairy farm with a herd of 135 lactating cows held in a free-range barn. Drinking behavior at two identical tank troughs (length: 2.00 m; width, 0.43 m; depth, 0.15 m; volume, 70 L) and two identical double-valve troughs (length, 0.73 m; width, 0.32 m; depth, 0.10 m; variable volume, 5–15 L), each made of stainless steel, was video-recorded daily in the first 2 h after feeding for 15 d in each of two study periods (December 2019 and February 2020). To determine the effects of trough cleanliness on drinking behavior, one trough of each design was cleaned daily, and the other trough of each design was not cleaned (2 × 2 Latin square study design), allowing the cows to choose between clean and unclean troughs of each design. Drinking episodes were analyzed and characterized using Behavioral Observation Research Interactive Software. Drinking water quality was analyzed at the start and end of the study periods and monitored daily using rapid tests. At tank troughs relative to double-valve troughs, the following were observed: a shorter total duration of drinking episodes ($P < 0.001$), higher odds for smelling behavior while tasting ($P = 0.01$), lower odds for drinking episodes consisting only of tasting behavior ($P = 0.002$), shorter ($P = 0.03$) and fewer drinking breaks ($P < 0.001$), lower odds for swallowing difficulties ($P = 0.001$), and higher odds for interruptions due to agonistic behavior ($P = 0.001$). The water quality at the start and end of the study periods did not differ significantly. Nevertheless, the cows' drinking behavior changed according to trough cleanliness. At unclean troughs relative to clean troughs, the following were observed: more ($P = 0.02$) and longer ($P = 0.03$) drinking breaks, a higher number of sips per drinking episode ($P < 0.0001$), tendentially higher odds for drinking episodes consisting only of tasting behavior ($P = 0.08$), and lower odds for swallowing difficulties ($P = 0.001$). In total, daily cleaning of the troughs altered 7 and trough design 8 out of 13 drinking behavior variables, giving additional insights in dairy cows drinking behavior.

1. Introduction

In dairy farming, a sufficient quantity of high-quality drinking water is essential for animal health, welfare, and performance (LeJeune and Gay, 2002). For example, the absence of prolonged thirst is a major indicator of the Welfare Quality® protocol in dairy cows (Welfare Quality®, 2009) and one of the most powerful indicators used to classify animal welfare at the farm level (Heath et al., 2014). In addition to the availability of water, the method of water supply also affects animal welfare. Filho et al. (2004) showed that cows prefer to drink from large-volume troughs, although the trough volumes were both high (189 vs. 568 L) compared to the troughs used in European regions, such as Western German dairy farms, where small-volume (< 5 – 49 L) or mid-volume troughs (50 – 119 L) are common (Hayer et al., 2022).

Abbreviations: BORIS, Behavioral Observation Research Interactive Software; CC, Coliform count; NRC, National Research Council; RLU, Relative light units; TMR, Total mixed ration; TVC, Total viable count.

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Research on how different trough volumes, especially small-volumes (e.g., < 50 L), and different trough designs (e.g., open troughs vs. valve troughs) affect drinking behavior is lacking.

Contaminated water troughs can be a source of infections caused by pathogens such as *E. coli* O157 (LeJeune et al., 2001a). In addition, microbiological and fecal contamination of drinking water, even at relatively low contamination levels (e.g., 0.05 mg fresh manure/g water), is associated with its rejection by livestock (Schütz et al., 2019). Quality thresholds for livestock drinking water have been suggested in several countries including Germany, the Netherlands, the United States and Canada to prevent the negative effects of low water quality (NRC, 2001; Kamphues et al., 2007; Waldner and Looper, 2007); however, these thresholds are heterogeneous and rarely based on scientific data (LeJeune et al., 2001b; van Eenige et al., 2013). Studies on drinking behavior have evaluated the effects of various fecal contamination levels (Schütz et al., 2019), treated and untreated water (Lardner et al., 2013), and different trough volumes (Filho et al., 2004) as well as the acceptability of different water types (e.g., standing or flowing water) (Willms et al., 2002). However, to the best of our knowledge, no study has evaluated the effects of trough cleaning on the quality of livestock drinking water and dairy cows' drinking behavior. Moreover, previous studies focused only on the amount of water consumed and total drinking time (Filho et al., 2004; Meyer et al., 2004; Teixera et al., 2017; Schütz et al., 2019). Thus, analyzing and characterizing specific drinking behavior parameters might produce important insights that could help improve the water supply of dairy cows (Melin et al., 2005).

Hence, in the present study the effects of different trough designs (tank troughs vs. valve toughs) and cleaning status (daily cleaning vs. no cleaning) on the quality of livestock drinking water and dairy cows' drinking behavior were assessed under working farm conditions. We hypothesized that cows prefer tank troughs over valve troughs and that uncleaned troughs lead to reduced water quality and changes in dairy cows' drinking behavior, such as fewer drinking visits.

2. Material and methods

This study was conducted following the principles stated in the Directive 2010/63/EU on the protection of animals used for scientific purposes. We adhered to the ethical standards and data privacy agreements of the University of Bonn (University of Bonn, 38/2018) and the federal and institutional animal use guidelines (FF AZ 01 K 1901 201912).

2.1. Experimental facility

The study was conducted on a commercial dairy farm in North-Rhine-Westphalia, Germany, at 195 m above sea level. The average annual rainfall and annual temperature in the area are 987 mm and 10.7 °C (min: 5.1 °C max: 17.4 °C), respectively (Wetterdienst, 2022). The experiment was performed between December 2019 and February 2020, corresponding to winter in the Northern Hemisphere.

On the commercial dairy farm used for this experiment, a dairy herd of 135 lactating Holstein-Friesian cows continuously held in a

Table 1
Characteristics of the experimental lactating dairy cow herd.

Variable	Mean	Minimum	Maximum
Days in milk	170	19	470
Lactation number	2.2	1	8
Milk production (kg/day)	32.8	13.7	57.0
Milk fat (kg)	4.32	2.10	6.3
Milk protein (%)	3.6	2.76	4.44

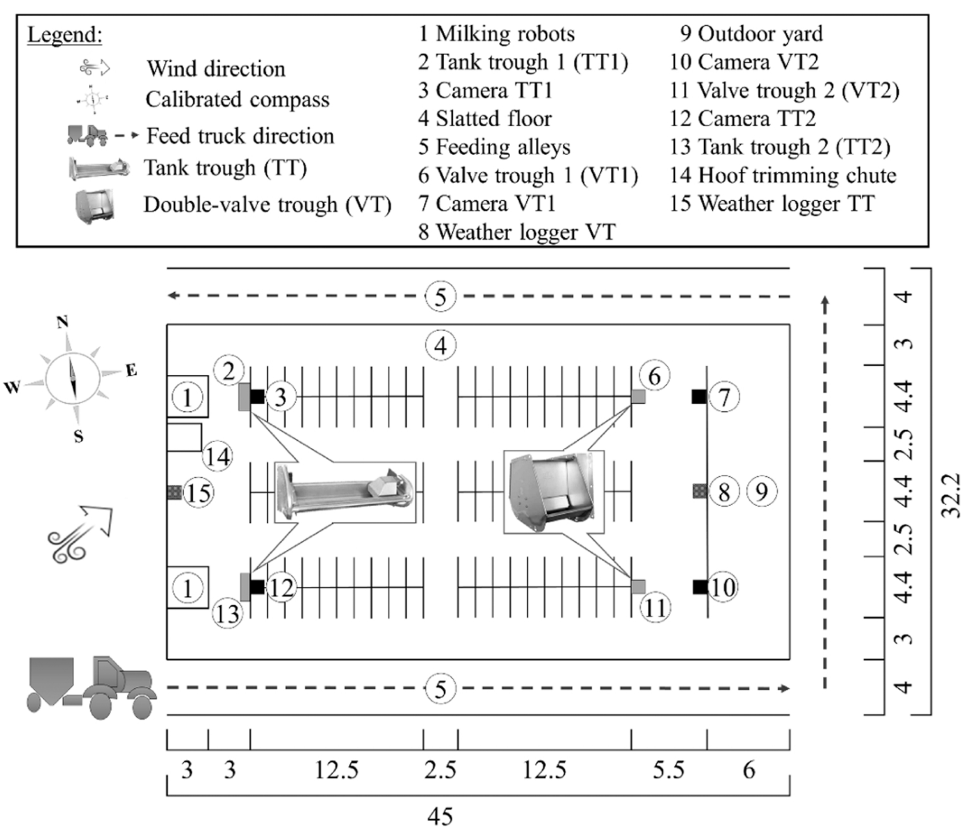

Fig. 1. Commercial dairy cow facility used in the drinking behavior study. Distances are shown in meters. Farm characteristics and experimental elements are marked with a number and are not drawn to scale for visualization purposes. The route of the feeding trucks is highlighted by dashed lines, and the wind direction and a calibrated compass are shown on the left.

symmetrical free-range barn (Fig. 1, Table 1). They were milked with two automatic milking system and fed once a day in the morning (at approximately 09:00). A total mixed ration (**TMR**) was offered in two 45-m-long feeding alleys. The TMR per animal contained corn silage [5.8 kg dry matter (**DM**)], grass silage (8 kg DM), and concentrate (3.5 kg DM). Additional concentrate was fed in two automatic milking systems (up to 6.2 kg DM per cow per day according to milk yield). The drinking water fed into the water supply system was well water from the farm that complied with standards for human drinking water quality (Supplementary table 1).

2.2. Experimental procedure

The drinking behavior of the dairy cows was video-recorded daily in the first 2 h after feeding at two identical open troughs (length, 2.00 m; width, 0.43 m; depth, 0.15 m; volume, 70 L) and two identical double-valve troughs (length, 0.73 m; width, 0.32 m; depth, 0.10 m; variable volume, 5–15 L), each made of stainless steel (Fig. 1). The trials took place for 15 d at the beginning of December 2019 and for another 15 d in February 2020. Two troughs (one open trough and one double-valve-trough) were randomly assigned (by flipping a coin) to be cleaned daily across the barn, and the other two troughs were not cleaned during the experimental period (a 2 × 2 Latin square design). Thus, cows were free to choose a trough for drinking: clean open trough, uncleaned open trough, clean double-valve trough, or uncleaned double-valve trough. On day one of each trial, all troughs were cleaned. Water samples were taken from each water trough at the beginning (day 1) and end (day 15) of each study period. In addition, water quality was monitored daily using rapid water-free ATP test and a visual scoring system. During a 10-day pre-trial period we took water samples every day and installed cameras to familiarize the animals to the experimental procedures.

2.3. Analysis of drinking behavior

A detailed description of each drinking behavior parameter recorded can be seen in Table 2. A drinking episode began with the cows' head crossing the edge of the water trough, which simultaneously was defined as the beginning of the tasting period. The tasting period was either terminated by the cow stepping away from the trough or the cow taking more than 5 continuous sips during the water intake. This period can also include the expression of "smelling", "tasting by tongue play" or "looking around". If a drinking episode ended after tasting, it was scored as "tasting only". The entire drinking behavior was subdivided into periods of water intake and drinking breaks. During water intake, the number of sips were additionally counted. Additionally, swallowing difficulties, agonistic behaviors, and interruptions due to agonistic behaviors were recorded. Four time-lapse cameras (TLC 200, Brinno, Taipei City, Taiwan) were used to record the dairy cows' behavior. These cameras were installed above the troughs to capture images of the entire drinking trough as well as a 2-m radius around the trough (Fig. 1). Videos were analyzed using Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba, 2016).

2.4. Laboratory analysis

To analyze the biological quality of the drinking water, water samples (1 L) were collected from each trough on the first and last day of the study period by lowering a sterile bottle into the water to a depth of 2–5 cm, imitating the drinking depth of cows. These samples were immediately cooled and processed within 24 h in a laboratory at the Institute of Animal Science, University of Bonn. Specifically, they were diluted in series (1:10) using sterile saline solution (Oxoid, Basingstoke, UK) containing 1% tryptone (VWR, Leuven, Belgium). The *Escherichia coli* and total coliform count (**CC**) were determined by plating the dilution steps onto Chromocult Coliform agar (Merck KGaA, Darmstadt, Germany) using a dual approach. After 24 h of incubation at 36 °C under

Table 2
Characterization of dairy cows' drinking behavior used in the analysis of video-recorded behaviors.

Variable	Data type	Unit	Description
Total duration of drinking	continuous	s	Time elapsed from the moment a cow crosses the edge of the trough until the cow leaves the trough area (ca. 1 m radius around the trough), including the beginning of the tasting period.
Duration of tasting period	continuous	s	Timespan, beginning with the cow crossing the edge of the trough and ending with water intake, including short periods of water intake < 5 sips with < 3 s between sips and drinking breaks, or the cow leaving the trough.
Tasting combined with other motions[b]	dichotomized	%	**Smelling:** *Planum nasolabiale* remaining slightly above the surface of the water trough or the surface of the water without direct contact with the water. **Tasting using the tongue:** Tongue is visible outside the *planum nasolabiale. Planum nasolabiale* is above the trough area or in contact with water, but no water consumption is observed. **Looking around:** Cow's head is above the trough and it does not immediately leave the trough area or initiate other tasting behaviors.
Only tasting	dichotomized	%	Begins with a cow crossing the edge of the trough and ending with the cows stepping away from the trough without water intake or including short periods of water intake < 5 sips excluding water intake periods > 5 continuous sips with < 3 s between sips. Cows leave the trough after the tasting period.
Duration of water intake[a]	continuous	s	Total time the *planum nasolabiale* is under the water surface or in contact with the water surface during a total drinking event, including both a water intake of > 5 continuous sips or during a water contact with drinking breaks lasting < 3 s and water intake periods of < 5 sips or during a water contact with drinking breaks lasting > 3 s
Number of water intake periods	continuous	s	"Total number of "water intake" periods during "total drinking episode", including both, water intake periods of > 5 continuous sips or during water contact with drinking breaks lasting 3 s"
Number of sips per drinking episode[a]	continuous	count	Number of sips measured as counts per "drinking episode." Total number of sips while tasting and sips per period of water intake. Sips are "a movement of the animal's throat swallowing water, while its mouth is submerged" (Filho et al., 2004) and visible by contraction of the cheek

(*continued on next page*)

Table 2 (*continued*)

Variable	Data type	Unit	Description
			muscle and/or marked contraction of the throat and/or clear water movement while *planum nasolabiale* is in contact with the water surface.
Duration of drinking breaks	continuous	s	Total time during a drinking episode the *planum nasolabiale* is above the water surface, i.e. not in contact with the water, including the time before, between and after water intake periods.
Drinking breaks	continuous	s	Total number of "drinking breaks" during a "the total duration of drinking."
Swallowing difficulties	dichotomized	%	Coughing with throat extended, usually combined with a visible tongue.
Agonistic behaviors	dichotomized	%	Disturbance of the drinking animal by other animals or the corresponding behavior against other animals by the drinking animal. Including displacement, head bump, pushing with core body.
Interruption of the drinking episode due to agonistic behavior	dichotomized	%	Interruption or termination of a drinking episode resulting from agonistic behavior.

[a] Based on Kamphues et al. (2007).
[b] "Smelling," "tasting using the tongue," and "looking around" after the tasting phase were not included in the evaluation.

aerobic conditions, plates containing 10–300 colonies were analyzed. Dark blue colonies were counted as *E. coli*, whereas salmon-colored colonies were counted as other coliform bacteria. Using a dual approach, aerobic total viable count (**TVC**) (20 °C and 36 °C incubation temperature) was determined via pour plating with nonselective plate count agar (Merck). Poured plates were incubated for 72 h at 20 °C and 36 °C, respectively. All visible colonies were counted from plates containing 10–300 colonies.

Physicochemical analysis of livestock drinking water quality was performed according to DIN EN ISO 10523 (pH), DIN EN 27888 (C 8), 1993–11 (electrical conductivity and salinity), DIN ISO 15923–1 (D 49), 2014–07 (ammonium, chloride, nitrate, nitrite, and sulfate), DIN EN ISO 11885 (E 22), and 2009–09 (phosphate, phosphorus, and iron) (Supplementary table 1).

2.5. On-farm water trough evaluation

The soiling of livestock drinking water was assessed prior to daily sampling by the same trained researcher for each trough. First, troughs cleanliness was categorized using a three-tiered visual scoring system (1 = no visible soiling, clear water; 2 = minor soiling at the bottom of troughs, water is slightly turbid; and 3 = heavy soiling at the bottom of troughs, water is heavily turbid). Subsequently, the free adenosine triphosphate content of the drinking water was measured using a rapid water-free ATP test (3 M™ Clean-Trace™ Water-Free ATP Test Swab AQF100; 3 M, Neuss, Germany). The ATP test is based on a bioluminescence reaction with ATP as a cofactor and has been applied in previous livestock research to analyze surfaces (Renaud et al., 2017; Barry et al., 2019; Heinemann et al., 2020, 2021). Specifically, 100 μL of livestock drinking water was sampled by dipping the sampling swab of the test system into the water, after which the test was activated by pushing down the stick handle to remove the membrane and start the enzymatic reaction as the chemical solutions were combined. After 10 s of shaking, the amount of emitted light was measured in relative light units (RLU) using a luminometer (NG, 3 M). The RLU values were log-transformed and are shown as $\log_{10}$(RLU/mL).

Water temperature and pH were measured using a pH meter (Testo 206-pH1 pH Measuring Instrument, Testo AG), which was dipped into the water to a depth of 2–5 cm. Light irradiation was measured 10 cm above the water surface using a lux meter (D-LUX meter 10244, GrandBeing).

2.6. Environmental measures

Climate data were recorded to determine climatic changes during the experimental period. At each barn site, two weather loggers (DROP D2AG Livestock Heat Stress Weather Meter, Kestrel, Boothwyn, USA) were positioned between the drinking troughs at a height of 2 m (Fig. 1). Ambient temperature and relative humidity were measured every 10 min, and the temperature–humidity index was calculated automatically.

2.7. Statistical analysis

The obtained data were analyzed using the FREQ and MEANS procedures in SAS version 9.4 (SAS Institute Inc., Cary, NC) for categorial variables (shown as distributions) and continuous variables (shown as means ± standard error), respectively. Three separate statistical analyses were conducted to evaluate the effects of trough design and cleaning status on (*i*) each drinking episode in detail, (*ii*) the average drinking episodes per day and the results of rapid tests, and (*iii*) the livestock drinking water quality at the beginning and end of a study period.

Continuous data on detailed drinking episodes were checked for normality using a Shapiro–Wilk test via the "dplyr" package in R version 4.1.1, and outcome variables were log-transformed when this was necessary (i.e., for non-normally distributed residuals). To achieve a normal distribution, a value of 10 was added to the "total duration of drinking" data, which was then logarithmized to base 10. Similarly, the data on "number of sips per drink," "duration of tasting period," "duration of water intake," "number of water intake periods," "duration of drinking breaks," and "drinking breaks" was logarithmized to base 10 after adding a value of 1. In SAS, differences between the continuous drinking behavior variables ("total duration of drinking," "number of sips per drinking episode," "duration of tasting period," "duration of water intake," "periods of water intake," "duration of drinking breaks," and "drinking breaks") were calculated using a linear mixed-effect model with "trough design" (tank troughs vs. valve troughs) and "trough cleaning status" (cleaned vs. uncleaned) as fixed factors. The factor "trough" was included as a repeated effect to account for the repeated assessment of each trough. In addition, binary logistics regression was applied using the MASS package (version 7.3–53) in R version 4.1.1 based on Rawat (2017) to model the effects of "trough design" and "trough cleaning status" on the categorical variables. The calculated logistic odds ratios and confidence intervals were exponentiated to obtain the final odds ratios and confidence intervals.

The normality of the rapid test system data was checked, and another mixed model, including "trough design" and "trough cleaning status" as fixed factors, was combined with a post-hoc Tukey test to analyze the effects on the daily averages of drinking behaviors and the results of the rapid test systems. The factor "trough" was again included as a repeated effect to account for the repeated assessment of each trough.

Spearman's rank correlations were also conducted in SAS (PROC CORR Spearman) to determine the correlative relationship between drinking behavior and the biological quality of drinking water.

In all cases, $P < 0.05$ indicated a significant difference, whereas $P < 0.01$ was considered highly significant and $P < 0.10$ was considered a tendency.

3. Results

3.1. Characterization of drinking behavior

In total, 4103 drinking episodes were recorded (2435 and 1650 episodes at tank troughs and valve troughs, respectively), and most drinking episodes took place 30–60 min after feeding during the total observation period of two hours after feeding (Fig. 2).

Tank trough 2 (TT 2) was the most visited trough, both when being cleaned daily (48 ± 3 visits) and when being not cleaned at all over the study (48 ± 2 visits), followed by tank trough 1 (TT 1) (cleaned: 34 ± 1; uncleaned: 37 ± 3 visits), valve trough 1 (VT 1) (cleaned: 26 ± 2; uncleaned: 35 ± 3 visits), and valve trough 2 (VT 2) (cleaned: 27 ± 3; uncleaned: 22 ± 2 visits) (Fig. 2).

The total duration of drinking was 123 ± 90 s(mean ± SE; min: 2 max: 820 s) , including 33 ± 42 s of tasting behavior. Displayed behaviors during the tasting phase included smelling in 95% of cases, playing with the tongue in 7% of cases, looking around in 5% of cases, and a combination of the abovementioned behaviors in 6% of cases. Swallowing difficulties occurred in 4% of drinking episodes. During a drinking episode, water was consumed in 3 ± 3 water intake periods, lasting 27 ± 26 s and including 20 ± 17 sips. Between the water intake periods, there were 3 ± 3 drinking breaks lasting 14 ± 19 s. In 908 (22%) drinking episodes, agonistic behaviors were recorded, resulting in drink interruptions in 14% of these cases.

3.2. Effects of trough design on drinking behavior

Compared with the drinking episodes at tank troughs, drinking episodes at valve troughs were longer and included more and longer drinking breaks ($P < 0.05$) (Table 3).

The odds of a drinking episode consisting of tasting behavior only without water intake were higher at valve troughs (26% of drinking episodes consisted only of tasting behavior) than those at tank troughs (22% of drinking episodess consisted only of tasting behavior) ($P < 0.01$). However, the odds of smelling behavior and interruptions due to agonistic behavior taking place were lower at valve troughs (94% smelling; 9% interruptions) compared with those at tank troughs (95% smelling; 18% interruptions) (Fig. 3).

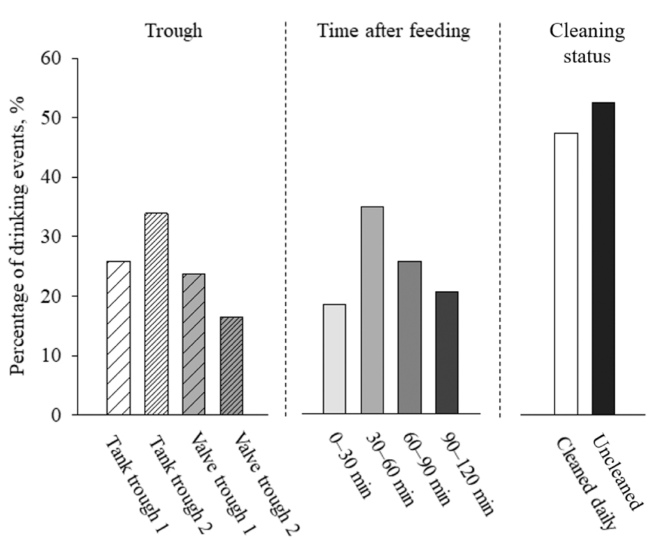

Fig. 2. Distribution of the recorded drinking episodes (n = 4103) in the first 2 h after feeding at two tank troughs and two valve troughs, which were either cleaned daily or uncleaned over 15-d study periods at a commercial farm housing 135 lactating dairy cows. Drinking episodes are shown according to trough design, time after feeding, and cleaning status.

3.3. Effects of neglected trough cleaning on drinking behavior

The number of drinking episodes per day at cleaned troughs (34 ± 2) was numerically but not statistically lower than that at uncleaned troughs (36 ± 2). Cows displayed less "looking around" at uncleaned troughs (5%) than that displayed at cleaned troughs (6%) (Fig. 4). Relative to cleaned troughs, the number and duration of drinking breaks and the number of sips were significantly higher at uncleaned troughs. There was also a tendency for more drinking episodes consisting only of tasting behavior at uncleaned troughs (26%) compared with those at cleaned troughs (22%) ($P < 0.08$), and cows showed fewer swallowing difficulties at uncleaned troughs than were shown cleaned troughs (3% and 5%, respectively; $P < 0.05$). According to the interaction between fixed variables, cows displayed less "tasting using the tongue" (5% vs. 10%) and fewer "swallowing difficulties" (0.2% vs. 1.0%) at uncleaned tank troughs than were shown at cleaned troughs. Notably, agonistic behavior at TT 2 in the northern part of the barn almost doubled with daily cleaning compared with neglected cleaning. At uncleaned valve troughs, more sips per drinking episodes were observed than those at cleaned valve troughs (22 ± 3 and 16 ± 8 sips, respectively) ($P < 0001$). Cows also displayed fewer "swallowing difficulties" (7% vs. 11%) and more agonistic behaviors (24.3% vs. 17.3%) at uncleaned valve troughs compared with those displayed at cleaned valve troughs.

3.4. Drinking water quality

3.4.1. Biological water quality

Trough water ATP content was higher in tank troughs [2.7 ± 0.3 $\log_{10}$ (RLU/mL)] compared with that in valve troughs [2.3 ± 0.5 $\log_{10}$ (RLU/mL)] ($P < 0.0001$). The water ATP content of uncleaned troughs [2.6 ± 0.5 $\log_{10}$ (RLU/mL)] was numerically but not statistically higher than that of cleaned troughs [2.5 ± 0.5 $\log_{10}$ (RLU/mL)]. Water ATP content did not increase over time in any trough (Fig. 4); however, it fluctuated in both cleaned and uncleaned troughs, and the range of water ATP content was higher in uncleaned troughs than that in clean troughs [3.6 $\log_{10}$(RLU/mL) vs. 3.3 $\log_{10}$(RLU / mL), respectively].

The water quality rating "clean" was recorded in 75% of troughs, whereas the ratings "soiled" and "heavily soiled" were recorded in 16% and 9% of troughs, respectively. In valve troughs, no visible soiling of the drinking water was recorded.

According to microbiological analysis of livestock drinking water at the start and end of the study periods, *E. coli* was not found but CC and TVC were relatively high at 36 °C (Supplementary table 1). Nevertheless, no clear increase of the bacterial load in uncleaned troughs compared to cleaned troughs were measurable.

3.4.2. Physicochemical water quality

Physicochemical analysis of drinking water at the beginning and end of the study periods showed that values were well-below thresholds for poor livestock drinking water quality (Supplementary table 1). Daily pH was not influenced significantly by trough design or trough cleaning status (7.4 ± 6.4). However, the water temperature in tank troughs (11.6 °C ± 2.5 °C) was significantly higher than that in valve troughs (10.1 °C ± 3.3 °C) ($P < 0.01$).

3.5. Influence of the biological quality of water on drinking behavior

Water ATP content was correlated with the following specific drinking behavior parameters: the " total number of drinking episodes" ($r = 0.3$, $P < 0.001$), of the "total duration of drinking" ($r = -0.2$, $P < 0.05$), duration of "water intake" ($r = -0.2$, $P < 0.05$), number of "sips per drinking episode" ($r = -0.2$, $P = 0.05$), and "swallowing difficulties" ($r = -0.4$, $P < 0.0001$).

Table 3
Characterization of dairy cows' drinking behavior (means ± standard error) observed at two identical tank troughs and two identical valve troughs that were either cleaned daily or uncleaned over a 15-d period.

	Tank troughs		Valve troughs		*P-value*		
Variable	Cleaned troughs	Uncleaned troughs	Cleaned troughs	Uncleaned troughs	Trough design	Cleaning status	Trough design ×Cleaning status
Total duration of drinking (s)	110.0 ± 2.3[B]	118.2 ± 2.3[B]	132.4 ± 3.5 [A]	136.5 ± 3.4 [A]	< 0.001	0.09	0.8
Duration of tasting period (s)	30.8 ± 1.1	32.6 ± 1.2	32.0 ± 1.4	35.2 ± 1.5	0.4	0.3	1.0
Drinking breaks	2.6 ± 0.1[B,(b)]	2.9 ± 0.1([a])	3.1 ± 0.2 [A]	3.1 ± 0.1	< 0.001	0.02	0.4
Duration of drinking breaks (s)	13.2 ± 0.4	13.9 ± 0.5[B]	14.5 ± 0.6[(b)]	15.5 ± 0.8 [A,(a)]	0.03	0.03	0.1
Duration of water intake (s)	28.0 ± 0.8 [A]	25.1 ± 0.6	26.0 ± 1.1[B,b]	28.2 ± 0.9[a]	0.06	0.1	< 0.001
Number of water intake periods	3.0 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	3.2 ± 0.1	0.1	0.2	0.9
Number of sips per drinking episode	19.7 ± 0.4 [A]	20.7 ± 0.5	16.8 ± 0.6[B, b]	22.3 ± 0.6[a]	< 0.001	< 0.001	< 0.001

[A, B] Different uppercase superscript letters indicate significant differences (P < 0.05) between trough designs (tank trough vs. valve trough).
[a, b] Different lowercase letters indicate significant differences (P < 0.05) and lowercase letters in brackets indicate trends (0.05 < P < 0.07) between cleaning statuses (daily cleaned vs. uncleaned) within the same trough design

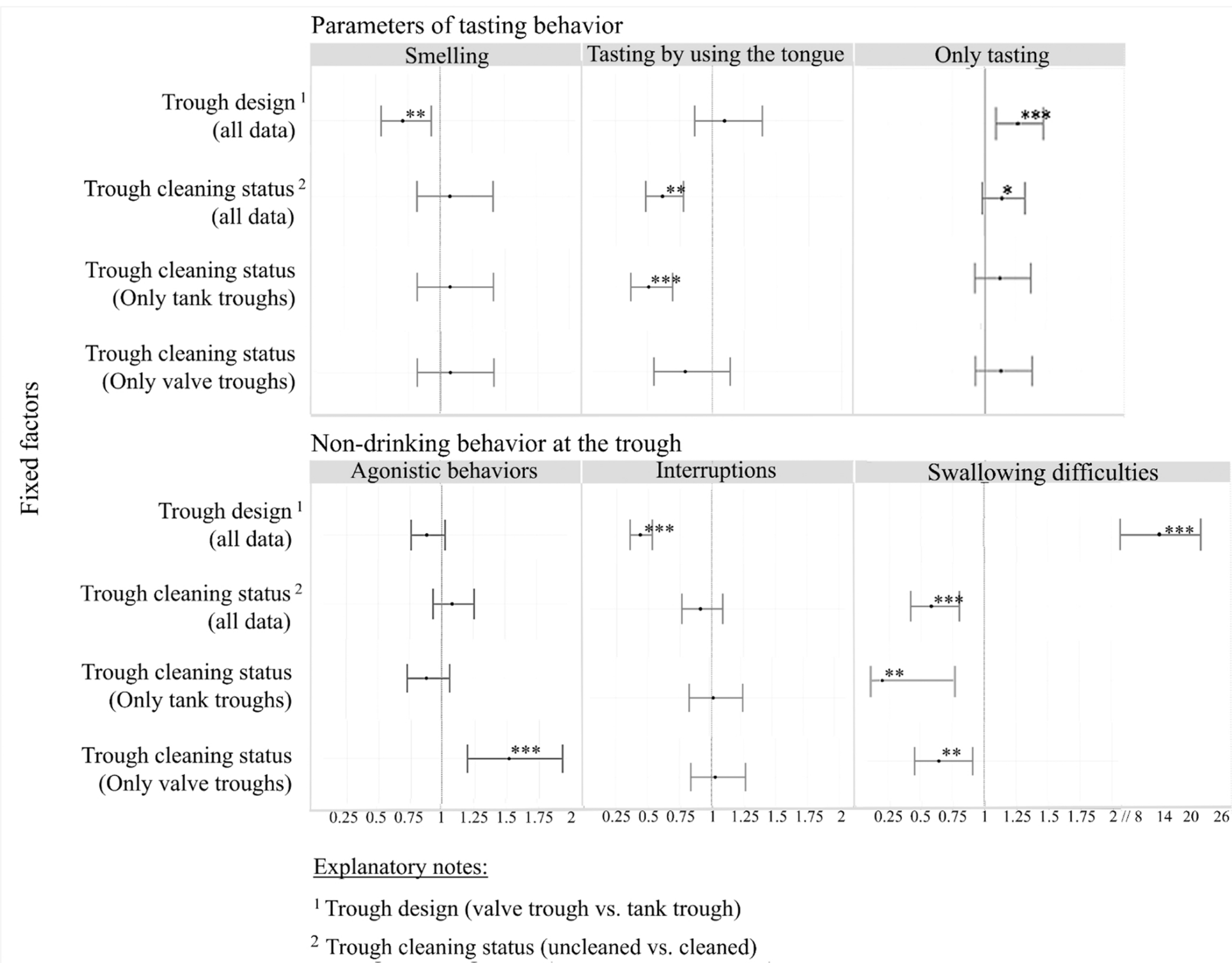

Fig. 3. Calculated odds ratios for the effect of trough design (valve trough vs. tank trough) and trough cleaning status (uncleaned vs. cleaned) on six drinking behavior parameters. The odds ratio is shown as a dot, and the whiskers show the 95% confidence intervals. * 0.05 < P < 0.1; ** P < 0.05; *** P < 0.01.

3.6. Climatic conditions during the study periods

Ambient temperature in the first and second study periods was similar at 6.5 °C ± 5.9 °C and 6.8 °C ± 2.1 °C, respectively. Relative humidity was 80.4% ± 8.9% in the first study period and 87.9% ± 5.4% in the second study period. The ambient light irradiation was 184.9 ± 107.9 lux.

4. Discussion

In the present study, a drinking episode lasted 122.4 s on average, which is comparable to the observation of Jago et al. (2005) (117 s),

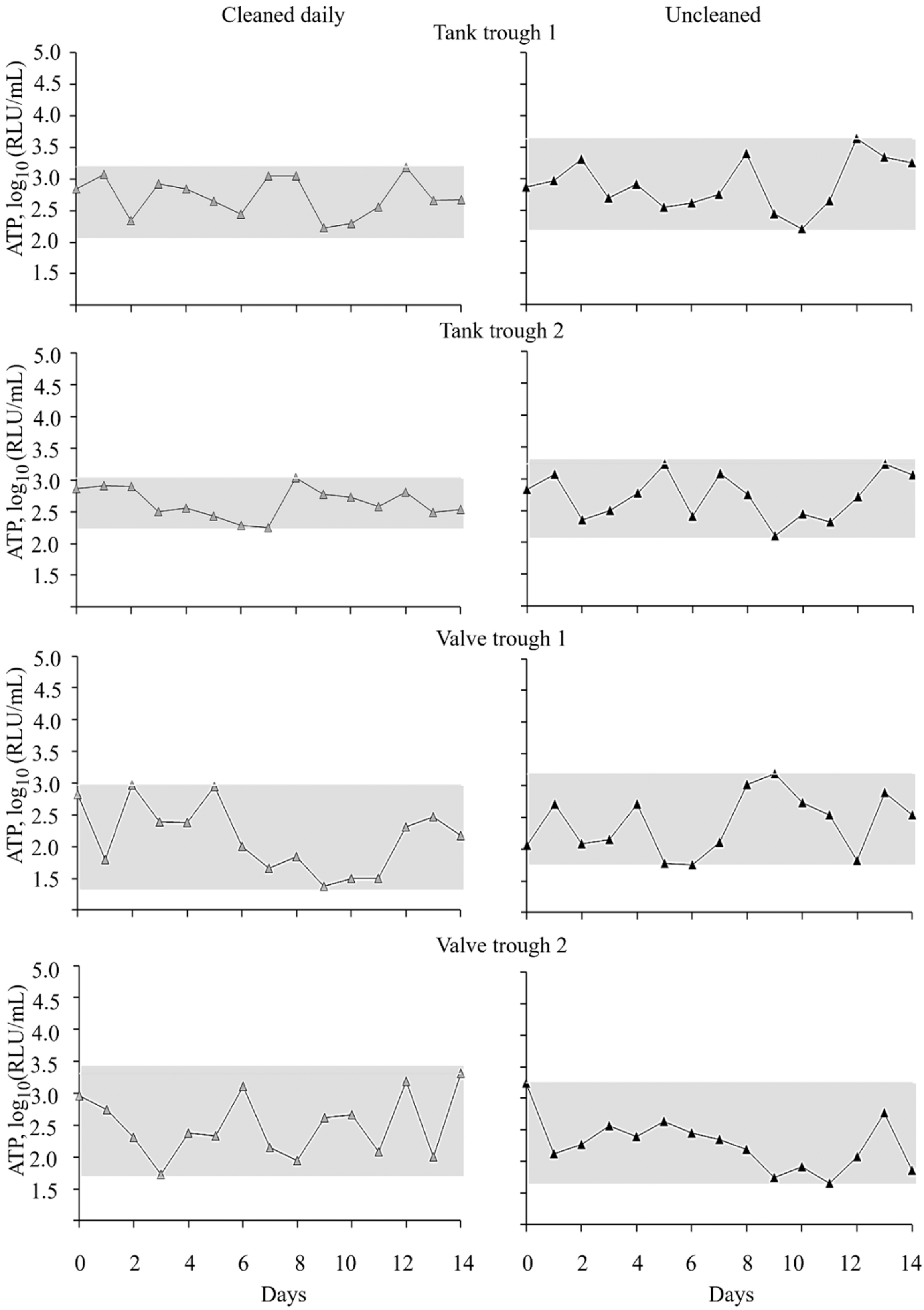

Fig. 4. Adenosine triphosphate (ATP) content of livestock drinking water in two identical tank troughs and two identical valve troughs that were either cleaned daily or uncleaned over 15-d study periods. ATP content was measured daily before feeding. The range for each trough and study period is shown in gray.

lower than that of Genther and Beede (2013) (210 s), and higher than those of Chapinal et al. (2007) (79.8 ± 3.5 s) and Willms et al. (2002) (77 s). However, the maximum duration of drinking in the present study, 17.1 min, was higher than that in other studies on dairy cows' drinking behavior, i.e., 3.4–10.0 min (Filho et al., 2004; Jago et al., 2005; Chapinal et al., 2007). Each drinking episode consisted of 20 sips. Sips were defined based on the definition of Filho et al. (2004), who reported 19 sips per drinking episode. For the other behavioral variables assessed in the current study, a lack of previously published data exists. Indeed, most studies have focused only on the number and total duration of drining episodes and amount of water consumed. Measuring the amount of water consumed was not possible in the present study due to the technical limitations of the water supply system, and such measurements might be unpractical for on-farm assessments in general. However, drinking behavior variables in dairy cows have rarely been defined or standardized, which explains the described variation among studies. Jago et al. (2005) defined a drinking episode as a "cow dipping her muzzle in the water and swallowing," whereas Filho et al. (2004) measured drinking time when the cow entered the paddock and until "she stopped drinking and turned toward the gate." A standardized protocol for evaluating dairy cows' drinking behavior would increase the comparability among studies and practical applicability of the collected data.

In the current study, more drinking episodes were observed at tank troughs (n = 2435) than at valve troughs (n = 1650), which might be attributable to the different volumes of these troughs (70 L and ~5–15 L, respectively). Filho et al. (2004) found that cows preferred drinking from "high-volume" troughs (568 L) rather than "small-volume" troughs (189 L) of the same design, i.e., cows spent more time drinking (small: 2.4 ± 2.1 s; large: 27.3 ± 6.2 s) and took more sips (small: 1.6 ± 1.5; large: 17.6 ± 4.3) from the larger troughs. In the present study, the evaluated troughs were smaller in terms of volume and differed in design, but more drinking episodes were nevertheless recorded at the larger troughs; however, the duration of water intake did not differ significantly between the two trough designs. At valve troughs, the "total duration of drinking" was higher and cows showed more and longer "drinking breaks" as well as more "swallowing difficulties." These behavioral differences might also be related to trough volume, as swallowing difficulties may arise due to interrupted vacuum formation during the process of swallowing water from low-volume valve troughs, which could lead to more and longer drinking breaks. When drinking, cows form a vacuum by pressing their lips together, allowing water to flow into their mouth (Schönholzer, 1958); thus, cows can consume 15–20 L of water per min (Andersson et al., 1984). Because of the low water level and variable volume of valve troughs, this vacuum might be interrupted, resulting in coughing, swallowing difficulties, and drinking breaks. The evaluated troughs also differed in terms of the number of cows with simultaneous access to the trough (up to around four cows at tank troughs and two cows at valve troughs). Interactions with other cows might explain the different number of visits and higher proportion of drinking interruptions due to an increased number of agonistic behavior events at tank troughs compared with that at valve troughs. Lastly, the placement of troughs in the barn differed as tank troughs were permanently installed near the automatic milking systems, whereas valve troughs were placed on the opposite side at the entrance to the yard. According to previous studies, the highest levels of water consumption in dairy cows follow milking and feeding (Jago et al., 2005; Cardot et al., 2008), so the proximity to the milking systems could have influenced the number of visits to tank troughs.

In addition to trough design, we evaluated the effect of trough cleaning status (cleaned daily vs. uncleaned) and the associated biological quality of the drinking water on dairy cows' drinking behavior. Contrary to the hypothesis that a lack of cleaning would reduce livestock drinking water quality, the initial and final water quality measurements during a study period did not differ statistically. Furthermore, daily water ATP measurements did not increase temporally. The troughs used in this study were stainless steel and the ambient temperature was low because the study was conducted in winter; these are two factors that can hinder microbial growth. For example, LeJeune et al. (2001) found that water in troughs made from steel had lower CC and *E. coli* counts than that in troughs made from other materials, such as plastic or concrete. In addition, bacteria, especially fecal bacteria, show increased proliferation as water and ambient temperatures increase (LeChevallier et al., 1996; LeJeune et al., 2001b). Despite this low-risk scenario, we observed differences in the drinking behaviors of dairy cows at clean and unclean troughs. The higher number of visits to uncleaned troughs in the present study is contrary to the observations of Schütz et al. (2019) and Willms et al. (2002), who found that cattle preferred to drink clean water and avoid manure-contaminated water. In the current study, the *E. coli* content of the drinking water was below the detection limit in all troughs, even after 15 d without cleaning; hence, fecal contamination of the water cannot be assumed. Schütz et al. (2019) observed cows sniffing water before drinking or refusing to drink. Although we did not observe differences in smelling behavior, cows displayed less tasting using the tongue at uncleaned troughs. The factors influencing the palatability of dairy cows' drinking water and the associated behaviors have not been researched in detail (Willms et al., 2002; Schütz et al., 2019). Nevertheless, differences in animal behavior have been discussed as indicators of drinking water palatability (Genther and Beede, 2013). The possible factors affecting palatability include taste (Goatcher and Church, 1970), organic fractions of cattle feces (Dohi et al., 1999), water temperature (Wilks et al., 1990), chemical components (Grout et al., 2006; Genther and Beede, 2013), and water treatment (Challis et al., 1987; Lardner et al., 2013) as well as individual- and species-specific factors, such as genetic constitution, age, sex, or disease (Goatcher and Church, 1970). Andersson (1987) found that room partitioning, social hierarchy, and performance levels all play roles in the drinking frequency and water consumption of dairy cows. McDonald et al. (2020) studied dominance behavior at troughs in association with heat stress, finding that lower-ranking animals shift drinking times on days when the occurrence of competitive behavior is increased. Although social hierarchy was not assessed in our study, observations of agonistic behavior associated with trough cleaning status indicate a possible influence of social rank on drinking behavior. The occurrence of agonistic behaviors doubled at the most visited trough (TT2), which was cleaned daily, whereas the number of drinking episodes remained relatively constant in terms of cleaning status in general. We assume that displaced cows switched to the nearest trough, which was an uncleaned tank trough. Hohenbrink and Meinecke-Tillmann (2012) found that almost half of the cows in a studied herd (42%) were rank-subordinate, whereas 22% were intermediate and 27% were dominant. Therefore, a relatively small number of dominant cows could have caused a higher number of subordinate cows to switch to uncleaned drinking troughs. The assumption that higher-ranking animals are more likely to drink at cleaned troughs and the finding that cows drinking from clean troughs took fewer sips overall are consistent with the finding of Andersson (1987). Less tasting using the tongue and a tendency for more drinking episodes consisting only of tasting at uncleaned troughs support our hypothesis that cows prefer clean water over water from uncleaned troughs. Nevertheless, as the geographical place of each trough in the current experimental barn, and thus sunlight-and wind exposition differed at the different troughs, this may per se affect the drinking behavior. We analyzed dairy cows' drinking behavior under working farm conditions in a commercial barn; therefore, our study has several limitations, e.g., no randomization of trough placement, only behavioral analysis, and a limited period of behavioral recording).

5. Conclusion

Our results indicate that trough design and cleaning status influence dairy cows' drinking behavior. At tank troughs, the "total duration of drinking" and the "number of sips per drinking episode" were higher while the “number of drinking breaks” were lower compared to valve troughs. Regarding the trough cleaning status, the number and duration of “drinking breaks” and the “number of sips per drinking episode" were lower at daily cleaned compared to uncleaned troughs. Nevertheless, this behavior is seemingly complex, with different behaviors displayed at different trough designs and placements and a potential effect of social hierarchy. In further studies, it will be interesting to evaluate differences in dairy cows’ drinking behavior under higher risk scenarios (e.g., high ambient temperatures), the effects of different trough positions, and the influence of social hierarchy on drinking behavior.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Declarations of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2022.105752.

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