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Assessment of dairy cows' water supply by using animal-related indicators

Franziska Katharina Burkhardt

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Summary

Assessment of dairy cows' water supply by using animal-related indicators

Given the high water content in milk and the related water losses through milk excretion, dairy cows have an exceptionally high demand for water. EU law requires the on-farm assessment of welfare using animal-related indicators; however, almost no specified animal-related indicators exist to assess water supply management. Legal regulation requires an adequate water trough design, construction, cleanliness, and position in the barn, minimizing competitive interactions. However, there is a lack of knowledge about how these factors influence dairy cow's drinking behavior and water consumption.

This thesis aimed to investigate the suitability of using behavioral parameters to assess water supply management in dairy cows. Therefore, in a first approach, this thesis characterized dairy cows' behavior at the trough on a herd level. In this context, the interaction of trough design (tank trough vs. valve trough) and cleanliness (daily cleaned vs. uncleaned) on dairy cows drinking behavior and the biological water quality under cold ambient temperatures (6.7 °C) were investigated. It was further expected, that warm ambient temperatures may increase the number of drinking episodes and the related behavior, as well as the bacterial burden in troughs. Therefore, in a second approach, dairy cows drinking behavior (n = 8.081 drinking episodes) and the biological water quality were comparably analyzed under moderately warm ambient temperatures (16.6 °C). To do so, a video-based behavioral analysis was performed under practical farm conditions, using 13 drinking behavior variables. Effects of the trough cleaning interval were tested using a Latin square design, allowing cows to choose between daily cleaned and uncleaned troughs of each design during four 15 days (d) trials (n = 60 d in total). The drinking water quality was analyzed at the start and end of the study trials, and monitored on a daily basis using rapid tests. Warm ambient temperatures increased the microbiological burden of livestock drinking water, especially at uncleaned troughs and increased agonistic interactions at the trough. The drinking behavior altered depending on trough design, cleanliness, and climatic conditions. Cows visited more frequently tank troughs, respectively uncleaned troughs, under both cold and warm ambient temperatures. Moreover, fewer drinking episodes were recorded under warm ambient temperatures during the 2 hours (h) observation periods after feeding. In this context, the diurnal feeding rhythm of cows, the feeding management, the trough location, and social hierarchy is assumed having a significant impact in the frequency the water troughs were used. Several authors described cows shifting their drinking times in dependence of seasonal changes in ambient temperatures, of the troughs distance to feeding, or the cows rank within the herd to avoid conflicts. However, the current thesis investigated dairy cows' behavior during the same time of the day for two hours after the morning feeding under cold and moderate warm ambient temperatures. Additionally, the feeding truck was placed during mixing in the immediate vicinity of one of the tank troughs, likely provoking a temporary accumulation of cows in the tank troughs environment and thus, an increased number of drinking episodes there. Thus, increasing agonistic interactions when daily cleaning the referred trough and resulting interruptions led to displacements to the next trough, which was an uncleaned tank trough. These factors are assumed to account for a higher number of drinking episodes under moderate warm than cold ambient temperatures, respectively at uncleaned than daily cleaned troughs.

In a third study, a more comprehensive analysis of dairy cows' behavior at the water trough was conducted to gain a better understanding of the impact of social hierarchy under consideration of body and performance characteristics on dairy cows individual drinking behavior. Dairy cows' behavior at the water trough was recorded at a milking parlor-facing drinking trough during 22 milkings under experimental conditions. A special emphasis was given to competitive interactions and the social hierarchy, resulting in a total of 33 behavioral variables being investigated. High-yielding cows consumed approximately twice as long and much compared to low-yielding cows. They were twice as often involved in agonistic interactions, highlighting the need for adequate trough availability in sufficient quantity and distance of troughs in high-yielding dairy cow herds or groups. Social hierarchy was not only determined by direct interactions, but to a large extend by indirect, non-physical interactions. Cows with a high competitive success started most frequently agonistic interactions, got fewer interruptions during drinking, and drank shorter and less than cows with a low competitive success. Cows with low competitive success had a lower ratio of drinking duration and water consumed and are potentially discriminated in their access to the drinking trough.

In summary, assessing dairy cows' water supply management using animal-related indicators seems valuable. However, influencing effects and social hierarchy should be taken into account for interpreting the observations. The number and duration of drinking episodes, water intake periods and drinking breaks, the number of sips, and agonistic interaction, including non-physical interactions, were identified as reliable indicators providing insights into dairy cows' welfare during water consumption.

Zusammenfassung

Bewertung der Wasserversorgung von Milchkühen anhand tierbezogener Indikatoren

Bedingt durch den hohen Wassergehalt in Milch und die damit verbundenen Wasserverluste durch die Milchexkretion haben Milchkühe einen besonders hohen Wasserbedarf.
Das EU-Recht verlangt die Bewertung des Tierwohls auf Betriebsebene anhand tierbezogener Indikatoren; es gibt jedoch keine spezifizierten tierbezogenen Indikatoren zur Bewertung des Wasserversorgungsmanagements. Die gesetzlichen Vorschriften verlangen eine angemessene Tränketechnik, Sauberkeit und Positionierung, um Konkurrenzsituationen zu minimieren. Es mangelt jedoch an Wissen darüber, wie diese Faktoren das Trinkverhalten und den Wasserverbrauch von Milchkühen beeinflussen.

Ziel dieser Arbeit war es, die Eignung der Nutzung von Verhaltensparametern zur Bewertung des Wasserversorgungsmanagements bei Milchkühen zu untersuchen. Daher wurde in einem ersten Ansatz das Trinkverhalten von Milchkühen auf Herdenebene charakterisiert. In diesem Zusammenhang wurde die Interaktion von Tränkedesign (Trogtränke vs. Ventil-Trogtränke) und Sauberkeit (täglich gereinigt vs. ungereinigt) auf das Trinkverhalten von Milchkühen und die biologische Wasserqualität bei kalten Umgebungstemperaturen (6,7 °C) untersucht. Weiterhin wurde erwartet, dass warme Umgebungstemperaturen zum einen mit einem Anstieg an Trinkvorgängen und andererseits einer höheren bakteriellen Belastung in den Tränken einhergehen und damit das Trinkverhalten signifikant beeinflussen. Daher wurden in einem zweiten Ansatz das Trinkverhalten von Milchkühen (n = 8.081 Trinkvorgänge) und die biologische Wasserqualität bei relativ warmen Umgebungstemperaturen (16,6 °C) vergleichend analysiert. Zu diesem Zweck wurde eine videobasierte Verhaltensanalyse unter Praxisbedingungen durchgeführt, welche die Erfassung von 13 Verhaltensvariablen umfasste. Die Auswirkungen des Reinigungsintervalls der Tränke auf das Trinkverhalten wurden mit Hilfe eines lateinischen Quadrat-Designs getestet, bei dem die Kühe während vier 15-tägigen Versuchen (insgesamt n = 60 Tage) zwischen täglich gereinigten und ungereinigten Tränken jedes Designs wählen konnten. Die Tränkwasserqualität wurde zu Beginn und am Ende der Versuche analysiert und täglich mit Schnelltests überprüft. Warme Umgebungstemperaturen verschlechterten die biologische Wasserqualität in den Tränken, insbesondere an ungereinigten Tränken, und führten zu einem Anstieg an agonistischen Interaktionen an der Tränke. Das Trinkverhalten änderte sich in Abhängigkeit des Tränkedesigns, der Sauberkeit und den klimatischen Bedingungen. Sowohl bei kalten als auch bei warmen Umgebungstemperaturen suchten die Kühe häufiger

Trogtränken als Ventil-Trogtränken und ungereinigte als gereinigte Tränken auf. Außerdem wurden bei warmen Umgebungstemperaturen während des Beobachtungszeitraums weniger Trinkvorgänge registriert. In diesem Zusammenhang spielten der diurnale Nahrungsaufnahme-Rhythmus von Kühen, das Fütterungsmanagement, der Standort der Tränke und die soziale Hierarchie eine Schlüsselrolle hinsichtlich der Häufigkeit, mit der die einzelnen Tränken genutzt wurden. Verschiedene Autoren berichten, dass Kühe ihre Trinkzeiten in Abhängigkeit von den jahreszeitlichen Veränderungen der Umgebungstemperaturen, der Entfernung der Tränke zur Futterstelle oder der Rangordnung der Kühe innerhalb der Herde verschieben, um Konflikte zu vermeiden. In der vorliegenden Arbeit wurde jedoch das Verhalten von Milchkühen bei kalten und mäßig warmen Umgebungstemperaturen zur gleichen Tageszeit für zwei Stunden nach der morgendlichen Fütterung untersucht. Zusätzlich wurde der Fütterungswagen während des Anmischens in unmittelbarer Nähe einer der Trogtränken platziert, was potentiell durch die temporäre Ansammlung von Kühen in der Umgebung dieser Tränke die Anzahl von Trinkvorgängen dort erhöhte. Die zunehmenden agonistischen Interaktionen bei der täglichen Reinigung der genannten Tränke und die daraus resultierenden Unterbrechungen führten zu einer Verdrängung der Kühe an die nächsten Tränke, welche nach dem Versuchsdesign demnach ungereinigt war. Es wird angenommen, dass diese Faktoren zu einer höheren Anzahl an Trinkvorgängen bei kalten verglichen zu warmen Umgebungstemperaturen bzw. an ungereinigten verglichen zu täglich gereinigten Tränken führte.

In einer dritten Studie wurde eine umfassendere Analyse des Verhaltens von Milchkühen an der Tränke durchgeführt, um ein besseres Verständnis der Auswirkungen der sozialen Hierarchie unter Berücksichtigung von Körper- und Leistungsmerkmalen auf das individuelle Trinkverhalten von Milchkühen zu gewinnen. Das Trinkverhalten von Milchkühen wurde an einer dem Melkstand zugewandten Tränke während 22 Melkvorgängen unter Versuchsbedingungen aufgezeichnet. Das Verhalten und insbesondere agonistische Verhaltensmuster wurden anhand von 33 Verhaltensvariablen umfassend erhoben. Hochleistende Kühe tranken nahezu doppelt so lange und viel verglichen mit niedrigleistenden Kühen. Zudem waren sie ca. doppelt so häufig in agonistische Interaktionen verwickelt, was die Notwendigkeit einer angemessenen Verfügbarkeit von Tränken in ausreichender Menge und in ausreichendem Abstand zu den Tränken in Milchkuhherden oder -gruppen mit hoher Leistung verdeutlicht.

Rangkämpfe an der Tränke waren nicht nur durch direkte, sondern zu einem großen Teil auch durch indirekte, nicht-physische Interaktionen geprägt. Dominante Kühe starteten am häufigsten agonistische Interaktionen, wurden beim Trinken seltener unterbrochen, tranken kürzer, und

weniger als rangniedere Kühe. Rangniedere Kühe hatten ein geringeres Verhältnis von Trinkdauer zu verbrauchtem Wasser und waren möglicherweise beim Zugang zur Tränke benachteiligt.

Die durchgeführten Studien zeigen, dass die Bewertung der Wasserversorgung von Milchkühen anhand tierbezogener Indikatoren praktikabel ist, jedoch sollten Interaktionseffekte und die soziale Hierarchie berücksichtigt werden. Als geeignete Verhaltensindikatoren wurden die Anzahl und Dauer der Trinkvorgänge, der Wasseraufnahmeperioden und Trinkpausen, die Anzahl an aufgenommenen Schlücken und agonistische Interaktionen, einschließlich nicht-physischer Interaktionen identifiziert. Diese können Einblicke in das Wohlergehen von Milchkühen während der Wasseraufnahme gewähren, wenn sie in Wechselwirkung miteinander, mit spezifischen Managementpraktiken, dem Tränkedesign und der Platzierung sowie der sozialen Hierarchie betrachtet werden.

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Publications associated with this thesis

Publications in peer reviewed journals

Burkhardt, F.K., Wahlen, R., Hayer, J. J., and J. Steinhoff-Wagner. 2025. Association between individual animal traits, competitive success and drinking behavior in dairy cows after milking. Animals 15(4): 534.

Burkhardt, F. K., Hayer, J. J., Heinemann, C., and J. Steinhoff-Wagner. 2024. Effect of Climatic Condition, Type of Trough and Water Cleanliness on Drinking Behavior in Dairy Cows. Animals. 14 (2): 257. https://doi.org/10.3390/ani14020257

Burkhardt, F. K., Hayer, J. J., Heinemann, C., and J. Steinhoff-Wagner. 2022. Drinking behavior of dairy cows under commercial farm conditions differs depending on water trough design and cleanliness. Appl. Anim. Behav. Sci. 256:105752. https://doi.org/10.1016/j.applanim.2022.105752.

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Burkhardt, F. K., Hayer, J. J., Steinhoff-Wagner, J.: Season and cleaning interval affect biological water quality and drinking behavior of dairy cows. 73rd Annual Meeting of the European Federation of Animal Science, Wageningen Academic Publishers, 2022.

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Wahlen, R.: Einfluss der Rangordnung auf das Trinkverhalten von Milchkühen nach dem Melken. Bachelor thesis. Technical University of Munich, TUM School of Life Sciences, Professorship for Animal Nutrition and Metabolism, 2024.

Technical journal article (non-peer reviewed, German)

Hayer, J. J.; Burkhardt, F. K.; Steinhoff-Wagner, J.: Das Trinkverhalten lesen und die Versorgung von Kühen verbessern. Milchpraxis 2, 2024.

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List of Abbreviations

AMS	automatic milking system
ATP	adenosine triphosphate
BMEL	Bundesministerium für Ernährung und Landwirtschaft
BW	body weight[1]
CC	coliform count
CD	Council Directive
d	days
D	drinking water intake[1]
DOY	day of the year
EC	European Commission
E-coli	*Escherichia coli*
EFSA	European Food Safety Authority
EP	extracellular pool
EU	European Union
F	feed water intake[1]
h	hours
IP	intracellular pool
ISO	International Organization for Standardization
MY	milk yield[1]
PMR	partial mixed ration
PRP	post-reticulorumen pool
QM-Milch	Qualitätsmanagement-Milch e.V.
R^2	coefficient of determination
RP	reticulorumen pool
SD	standard deviation

spp.	species pluralis
T	total water intake[1]
TDS	total dissolved solids
THI	temperature-humidity index
TierSchG	Tierschutzgesetz
TMR	total mixed ration
TVC	aerobic total viable count
WQ[1]	Welfare Quality® project

[1] only Figure 7

1. General Introduction

1.1. Water metabolism and demand in dairy cows

Metabolism, as defined by Murphy (1992), refers to the processes of regulating the handling, control, or management of a specific substance, such as water. As a function of oxidative phosphorylation, peptide bonds, and enzyme activity, several metabolism-related processes involve water. Hence, in the context of water, despite its passive moving within the organism through osmosis-directed diffusion, metabolism is considered an appropriate expression. An animal's body water turnover determines its water demand (Figure 1) (Jensen and Vestergaard, 2021).

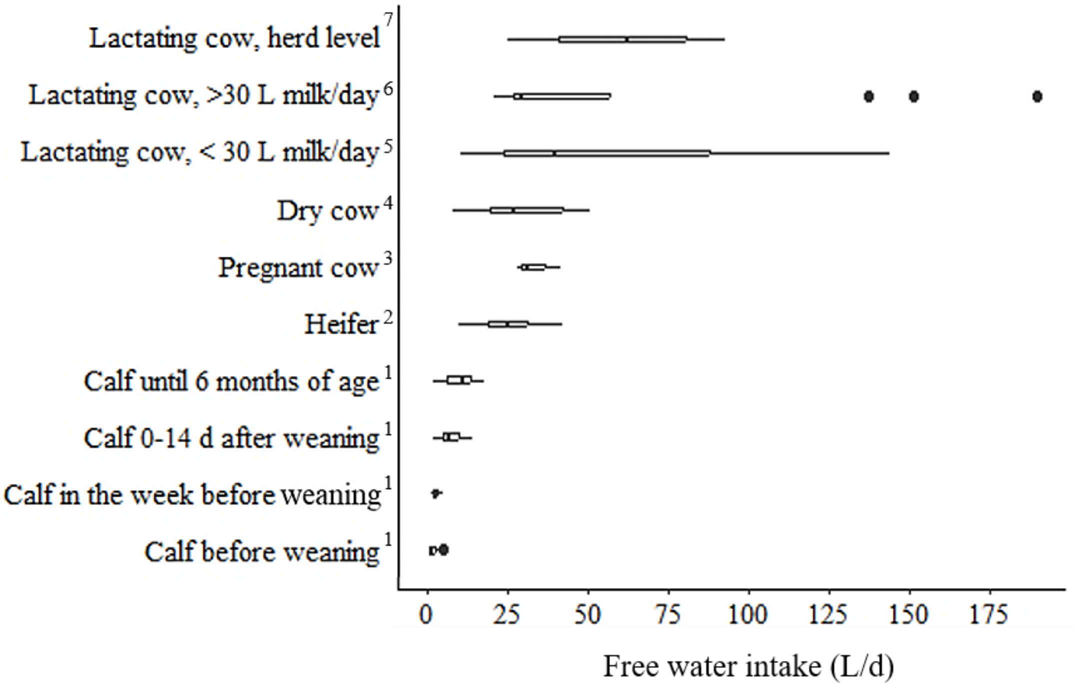

Figure 1: Water demand of dairy cows as a function of the physiological state (calf, heifer, dry cow, lactating cow). References: [1] Publications reviewed in Jensen and Vestergaard (2021); [2]Grant (1995), Grant and Albright (2001), Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (2008); [3]Parker (2003); [4] Osborne et al. (2002), Pereyra et al. (2010); [5]Castle (1972), Castle and Watson (1973), Parker (2003), Golher et al. (2015); [6]Andersson et al. (1984), Holter and Urban (1992), Parker (2003); [7]Wilks et al. (1990), Holter and Urban (1992), Spörndly and Wredle (2005), Meyer et al. (2004), Cardot et al. (2008), Ammer et al. (2018).

Dairy cows fulfill their water demand through drinking (about 83% of total demand), feed, and metabolic water, namely by lipid, protein, and carbohydrate oxidation (Murphy, 1992; Meyer et al., 2004; Golher et al., 2021) (Figure 2). Regarding thermoregulation, water plays an important role, protecting the animal from overheating and freezing by evaporative cooling or vasoconstriction (Murphy, 1992). Additionally, water is vital as a solvent for a wide range of substances, such as salts, sugars, and acids (Breynaert et al., 2020). The reason for this is its polar but non-ionic nature. The surface tension of water enables a slight dissociation into ions, combined with a particular electrical conductivity and, on the other hand, a rapid capillary uptake (Murphy, 1992). Adult dairy cattle's total body water content ranges between 56% (fat dry cows) and 81% (lactating cows) of body weight. The increased water intake in lactating cows closely mirrors water loss through milk excretion (Woodford et al., 1984). Generally, body water pools are divided into intracellular (IP), extracellular (EP), reticulorumen (RP), and post-reticulorumen (PRP) pools (Murphy, 1992; Appuhamy et al., 2014) (Figure 2). Besides the loss of water through milk excretion, cows predominantly loose water under thermoneutral conditions through feces, urine, and, to a lesser extent, trough sweating, salivating, and evaporating (Little and Shaw, 1978; Murphy, 1992; Beede, 2005; Appuhamy et al., 2014). *Bos taurus* cattle survived up to four days, losing 7 – 8% of their body water per day, reflecting their high adaptive potential (Mac Farlane et al., 1971). Assuming a body water content of 56%, cows compensated up to 44.0 – 50.4 kg of daily water losses. However, free access to ad libitum fresh water is crucial for maintenance, growth, pregnancy, and lactation throughout all stages of a dairy cow's production life (Beede, 2005) .

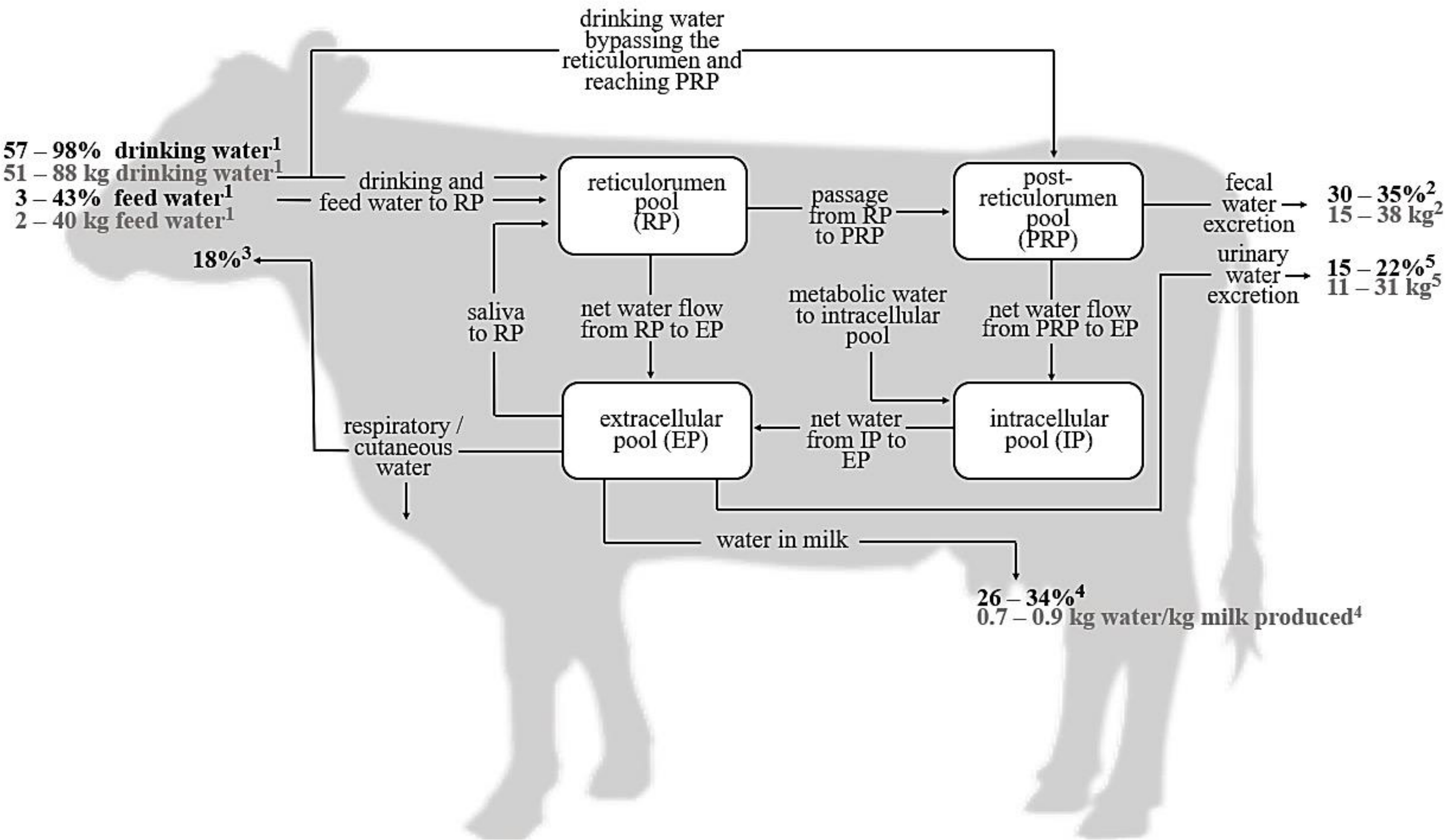

Figure 2: Schematic visualization of lactating dairy cows water balance, illustrating the water pools (extracellular pool, EP; intracellular pool, IP; reticulo-rumen pool, RP; post-reticulorumen pool, PRP), and water sources (feed, drinking and metabolic water) and losses (milk, urinary and fecal excretion, respiratory and cutaneous water) in percent (black font) and kg water (grey font) per day, adapted by Appuhamy et al. (2014). References: [1] Data calculated from studies conducted in 1972 until 2018 (n = 18); [2-5] Little and Shaw (1978), Murphy et al. (1983), Murphy (1992), Beede (2005), Appuhamy et al. (2014). Created with BioRender.com.

1.2. Legal regulations regarding dairy cow water supply

At European and national levels, many rules, regulations, and laws exist relating to the requirements for the husbandry of food-producing animals. Regulations and standards regarding water supply management are rarely included, not specified, or differentiated regarding the animal's production stage (Figure 3). At the EU level, the European Council adopted in 2002 the Regulation of the European Commission (EC) No. 178/2002 (Basic regulation) to ensure food and feed safety (EU, 2002). Although on a national level water is clearly attributed to feedstuffs in the positive list for animal feed (Kamphues et al., 2007; ZDL, 2023), the preamble in the council directive (CD) 2009/767/EC states that on EU-level, the basic regulation does not include water (EU, 2009). Regarding water hygiene management, requirements are covered by the Feed Hygiene Regulation (EC) No. 183/2005. The section on "good feeding practice" in Annex III requires that the design, construction, and placements of the drinking systems minimize the contamination of the water on a biological, chemical, and physical level, as well as competitive interactions (EU, 2005). However, the terminology is not further defined. Therefore, in this thesis, ‘design’ refers to the type of pipe system and water supply mechanisms (float-operated or self-actuated water troughs), the dimensions (depth, length, width), and the water volume. Concerning the construction, it refers to the installation, particularly in height and material, and the placement relates to the position in the barn. In Annex III, additionally the necessity of water suitability for the animals is emphasized. The latter is also laid down in the Codex Alimentarius as part of the "Code of Practice on Good Animal Feeding" (2004) (Codex Alimentarius Commission, 2004; Kamphues et al., 2007). The Codex Alimentarius aims to ensure safe food for human consumption by following good feeding and watering practices when keeping food-producing animals (Codex Alimentarius Commission, 2004). Providing information on food safety-relevant zoonotic agents, such as *Salmonella* spp., specific *E. coli, Campylobacter* spp., *Shigella* spp., certain rotaviruses, and *Cryptosporidia* spp. (Kamphues et al. 2007), the Zoonoses Directive 2003/99/EC (EU, 2003) and the Animal diseases Directive 2016/429/EC (EU, 2016) are also relevant.

As standards for the drinking water of farm animals in the European Union are not specified at a federal German level, the German Drinking Water Ordinance represents the transposition of the EU Drinking Water Directive into national law (Federal Ministry of Health, 2016). Nevertheless, following Kamphues et al. (2007), quality requirements for human consumption are not directly transferable to livestock water quality, as performance, health, or food safety are not necessarily impaired when exceeding reference values (Kamphues et al., 2007). However, in this context, the precautionary principle stated in Article 174 of the Amsterdam Treaty of the European Union, and emphasizing the importance of environmental preservation and public health may also be considered (Ahteensuu and Sandin, 2015). Food and feed safety aspects are regulated by the German Food and Feed Code (DLMBK, 2005).

Regarding the construction of the water supply technique, the Animal Welfare and Farm Animal Husbandry Ordinance (Tierschutznutztierverordnung) (TschNutztV, 2002) adopted the requirements demanded by the above-mentioned Feed Hygiene Regulation (EC) No. 183/2005. Still, minimizing conflicts at the trough is emphasized, thus addressing animal-related needs, whereas previous regulations were limited to resource and management related aspects. In this context, the Animal Welfare Act (Tierschutzgesetz, TierSchG) (TierSchG, 1972) explicitly demands the collection of suitable animal-related indicators (animal welfare indicators) to evaluate feeding management (Figure 3).

EU level

Evaluation / Gaps in requirements	Content/Requirements regarding supply of livestock drinking water	Legal Regulation
→ drinking water is a feedstuff under EC law (Kamphues et al. (2007). Ambiguous definition Preamble 2009/767/EC "Unlike food, as defined in Regulation (EC) No 178/2002, the definition of feed does **not** include water."	Feedstuffs are "substances or products, including additives, whether processed, partially processed or unprocessed, intended for oral animal feeding"	**CD 2002/178/EC Basic Regulation of Food Law**
Requirements about -water quality indicators and water sampling methods -the design,- construction, and placement -and the cleaning interval of drinking troughs to identify and eliminate risks of biological,- or physico-chemical contaminants are missing.	"Water for drinking (…) shall be of appropriate quality for the animals being produced. (…) Feeding and watering equipment must be designed, constructed and placed (…) that contamination of feed and water is minimised (…) [and] shall be cleaned and maintained regularly, where possible."	**CD 2005/183/EC Feed Hygiene Regulation**
Knowledge on how the bacterial burden of cattle drinking troughs affects animal health, welfare and food safety is missing.	"The collection of data on the occurrence of zoonoses and zoonotic agents in (…) feed (…) is necessary to determine the trends and sources of Zoonoses. "	**Zoonoses Directive 2003/99/EC**
	"Animal diseases are not only transmitted through direct contact between animals or between humans and animals. They are also spread via the water (…). Pathogens can also be found in food or other products. "	**Animal diseases Directive 2016/429/EC**

Federal German level

Legal Regulation	Content/Requirements regarding supply of livestock drinking water	Evaluation/ Gaps in requirements
Food and Feed Code (LFGB)	"For the purpose of reducing or eliminating the causes of undesirable substances in feedingstuffs (…) where an exceedance of established maximum levels of undesirable substances or action limits is detected (…) [investigate to identify (…)] the causes."	Established maximum levels of undesirable substances in livestock drinking water are missing.
German Drinking Water Ordinance (Trinkwasserverordnung)	"Microbiological requirements and requirements regarding indicator parameters for drinking water"	Represents the transposition of the EU Drinking Water Directive into national law, which is why the maximum levels specified in the Drinking Water Ordinance are regarded as reference values (Siraj Raya 2011). → No direct transferability to the quality requirements in animal husbandry (performance and health of the animal nor the quality of the food are affected (Kamphues et al., 2007).
Animal Welfare Law (Tierschutzgesetz)	"Persons who rear livestock for commercial purposes must (…) collect and evaluate suitable animal-related characteristics (animal welfare indicators)."	Animal-related indicators evaluating the water supply management are missing.
Animal Welfare and Farm Animal Husbandry Ordinance (Tierschutz-Nutztier-haltungsverordnung)	"Livestock facilities must be equipped with (…) watering systems that are designed and arranged in such a way that each animal has access to a sufficient quantity of (..) water and that contamination of (…) water as well as conflicts between the animals are minimised."	→ concrete specifications of trough construction requirements for dairy cow husbandry are missing.

Figure 3: Legal regulations associated with water supply in dairy husbandry at European (left) and federal German level (right) indicating requirements and gaps in requirements.

1.3. Animal-related indicators of water supply

Different scientists, public agencies, and assessment protocols have developed resource, management, and animal-based indicators to comply with EU and national legal requirements for achieving compliance. As summarized by Hayer (2002), resource-based indicators focus on the animal's living conditions and husbandry system, considering flooring, bedding, feed, climate, and space. They are considered easy to observe but primarily measure prerequisites for animal welfare rather than welfare itself. Management-based indicators evaluate on-farm management practices such as procedures, cleaning, disinfection, and feeding routines. Like resource-based indicators, they are easily assessed but criticized for measuring necessary conditions rather than direct indicators of good welfare. Animal-based indicators directly evaluate the animal, including locomotion characteristics, health conditions, body condition scores, cleanliness, and behavior (Hayer, 2002). They directly focus on individual animal welfare but are more time-consuming and prone to errors during assessment (Hayer, 2002; Passillé and Rushen, 2005; Lagerkvist et al., 2011; EFSA, 2012). Current water supply assessments mainly focus on resource and management-based indicators (Table 1). However, the European Food Safety Authority (EFSA) explicitly demands that animal-based indicators are implemented in herd monitoring and surveillance protocols to document time-dependent welfare changes (EFSA, 2012).

Additionally, animal-based indicators enable welfare assessment on an individual level, as they provide insights into welfare and stress levels (Guevara et al., 2022). Furthermore, they "are valuable for direct decision-making" (Hayer, 2002) if welfare impairments are severe. The Welfare Quality protocol (Welfare Quality®, 2009) is a holistic approach for assessing animal welfare at a farm level using animal-based indicators. However, the water supply assessment is limited to resource-based indicators. The time required for such an assessment is limiting in terms of practicability (approximately seven hours for a dairy farm with 125 dairy cows). Therefore, Heath et al. (2014) identified the "absence of prolonged thirst" as an iceberg indicator that can theoretically reliably predict the overall classification and, thus, transform the multidimensional assessment approach into a balanced summary. With an accuracy of correctly classified farms in 88% of the cases, the "absence of prolonged thirst" prevailed over the "positive emotional state" (67%) (Heath et al., 2014). The spectrum of animal-related indicators assessing water supply is rare (Table 1). Therefore, scientists investigated the water intake to fulfill this approach (Battini et al., 2014).

Table 1: Assessment of dairy cow water supply management using animal welfare indicators according to the Kuratorium für Technik und Bauwesen in der Landwirtschaft (KTBL, 2008), the QM-Milch e.V. (QM-Milch, 2022), and the Welfare Quality® project (WQ).

Indicator	Category	Requirements	KTBL	QM-Milch	WQ
Water surface per cow	Resource	≥ 6 cm per animal (tank troughs)	✓	✓	✓
Number of troughs Free-range cows	Resource	Tank troughs: at least two water points and troughs: max. 20 animals per trough Bowl trough: > 2 bowl troughs, max. 10 animals per trough (exception: groups < 7 animals) and >1 for 15 animals	✓[4]	✓	✓
Tied- up cows		At least one trough per two animals		✓	
Cows on pasture		At least one trough per 20 animals		✓	
Functioning of water points	Resource	Movability of levers and water flow when moving	✓		✓
Water level	Resource	≥ 80 cm water surface to ground level	✓	✓	✓
Water depth	Resource	≥ 60 mm from surface to bottom within the trough	✓		
Water flow[1]	Resource	Tank troughs: >20 L/min at refill Bowl troughs: >10 L/min at refill	✓[4]	✓	✓
Trough material[2]	Resource	Stainless steel instead of concrete for reduction of microbial growth			
Water quality[2]	Resource	Matching the thresholds for water quality (BMEL, 2007)		*	
Water cleanliness[3]	Management	> 70% of all troughs better than category 1 and no of category 2, daily trough cleaning		✓	✓
Agonistic behaviors	Animal	Max. five agonistic interactions per cow/hour; minimum distance between troughs	✓		✓

[1] 0 = optimum water flow, 1 = reduced water flow, 2 = greatly reduced water flow (KTBL, 2006).
[2] Based on Kamphues et al. (2007).
[3] Categories: 0 = The cows have access to clean troughs and clean water 1 = The water troughs are partially soiled, but the drinking water is clean
2 = The water troughs are heavily contaminated, and the animals have access only to contaminated drinking water (Welfare Quality®, 2009).
[4]Indicators included in KTBL, 2020.
*Assessed indirectly by controlling the self-control of feed stuffs.

In general, animal-related indicators regarding water supply were previously evaluated in one of the following three contexts (adapted by Burn (2020)):

1. The dairy cow in its current situation, e.g., drinking frequency and duration, water consumption, social competition

2. The individual dairy cow in interaction with the herd, e.g., drinking frequency and duration, water consumption, social competition

3. The dairy cow exposed to environmental, resource, and management-related factors.

While individual water intake has been adequately investigated under experimental conditions using water flow meters (Cardot et al., 2008; Lukas et al., 2008; Williams et al., 2020), automatic water bins (Chapinal et al., 2007; Ammer et al., 2018), or calibrated scales (Lanham et al., 1986; Schütz et al., 2019), this is hardly feasible under practical conditions. Standard free-stall barns in Western Europe commonly provide a combination of tank troughs and self-operating valve troughs (Suevia Haiges GmbH, 2023). Tank troughs allow several animals to drink simultaneously; therefore, water intake measurements in dairy farms using tank troughs can be conducted only on a herd level (Sadrzadeh and Kamyabi, 2021). However, given that such an assessment include spilled water losses in the water amount consumed, it would be inexact. Further, an exclusive quantification of water consumption does not identify the reasons for a high or low water intake. A solution orientated, multidimensional assessment approach demands the inclusion of diverse factors. Evaluating the drinking behavior may counteract these challenges and represent a reliable, feasible to implement alternative if considered in context with various factors known to influence water intake, such as climatic conditions, water trough design, or water cleanliness. However, using a specific behavior as an indicator demands knowledge of the context in which the behavior occurs (Watters, 2014), of external factors potentially influencing the behavior (Knegt et al., 2013), and of the behaviors reliability and validity in terms of the state or condition being reflected (Miller et al., 2023).

1.3.1. Drinking behavior and water consumption

Dairy cows meet most of their daily water demand after milking and feeding (Andersson, 1987; Laínez and Hsia, 2004; Cardot et al., 2008). Drinking behavior, induced by a certain drinking motivation, is the reaction to maintaining homeostasis. These mechanisms are regulated by an interaction of hypothalamus associated nerve cells, hormones, and sense organs in heart and blood vessels (McKinley and Johnson, 2004; Hogan et al., 2007; Jensen and Vestergaard, 2021). Schönholzer (1958) initially categorized a dairy cow's drinking sequence into four periods: the pre-drinking phase, drink initiation, actual water intake, and post-drinking phase.

Following Schönholzer (1958), the pre-drinking phase begins with mouth licking, signaling its onset. Extending this phase involves tongue movements that indicate water tasting without direct contact between the mouth and water. Water is solely transported into the mouth by the tongue.

Once the drinking process is initiated, the main water intake periods begins. This phase is defined by "suck drinking," wherein the animal's lips touch the water, forming a small opening for water inflow, drinking up to 24 L/min (Jensen and Vestergaard, 2021). The necessary pressure is created by enlarging the oral cavity while maintaining lip position through downward movement of the lower jaw and pressing the tongue firmly against it. Subsequent reduction of the oral cavity size and undulating tongue movements displace water into the throat, allowing the cow to swallow larger volumes of water. Maintaining a specific mouth-to-water surface relation prevents air from entering while keeping nasal openings free.

In the post-drinking phase, the animal is no longer in contact with the water surface. Specific movements are observed to remove residual water from the mouth's edges (Schönholzer, 1958; KTBL, 1976).

Recent studies subdivided a dairy cow drinking episode primarily in the three phases: accessing the trough, drink initiation, and leaving the trough. No delineation of the different periods of a drinking episode was performed. Supplementary observations have been partially included (Figure 4).

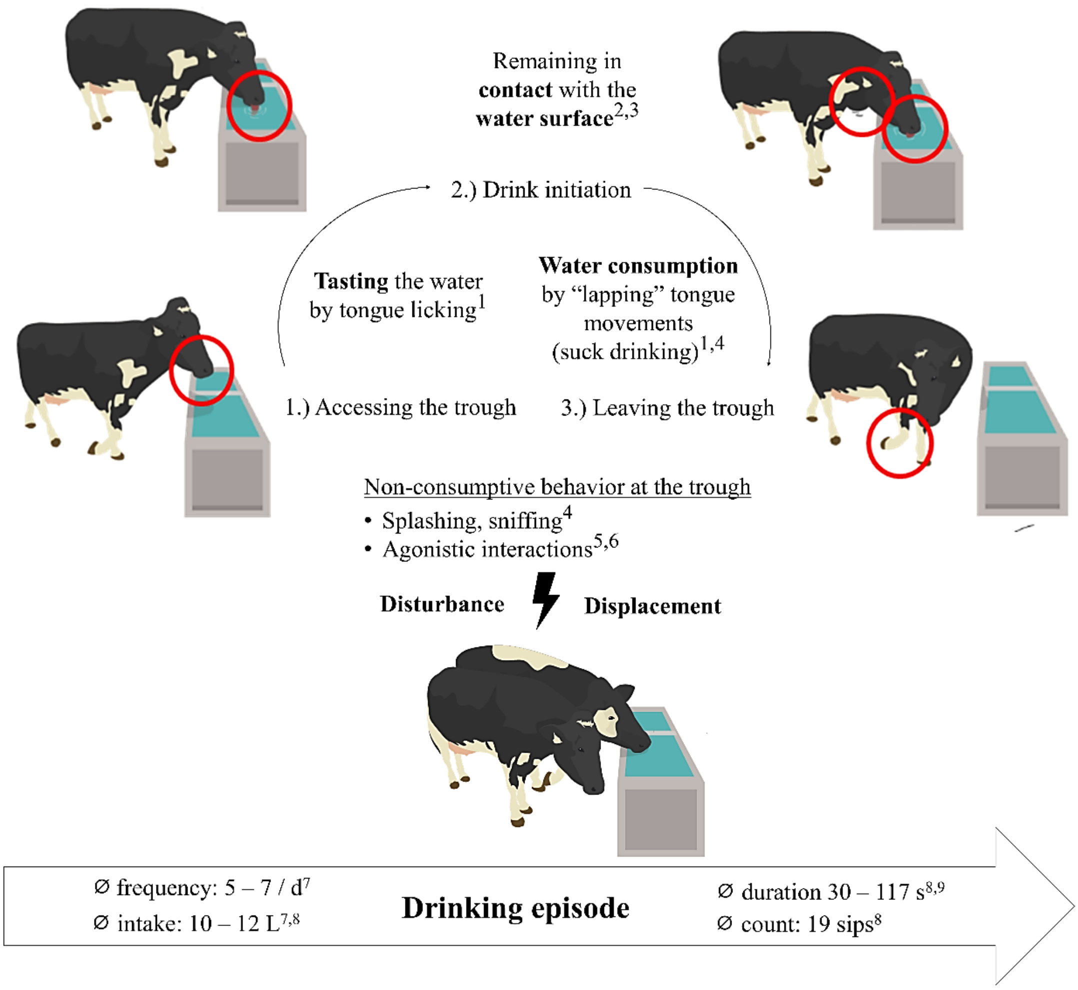

Figure 4 Sequence and species-specific aspects of a dairy cows drinking (adapted by 1: Schönholzer (1958); 2: Coimbra et al. (2012); 3: Williams et al. (2020); 4: Genther and Beede (2013); 5: Guzhva et al. (2016); 6: Foris et al. (2019) ; 7: Cardot et al. (2008) 8: Filho et al. (2004) 9: Jago et al. (2005)).

1.3.2. Integration of drinking behavior indicators in the assessment of water supply

Besides the measurement of the water volume consumed, recent studies identified several potential animal-based behavioral indicators for water supply management: the drinking frequency, the drinking duration, and agonistic interactions at the trough.

Drinking time and frequency measurements depend on the defined start and end time of drinking (Laínez and Hsia, 2004; Cardot et al., 2008; McDonald et al., 2020), although studies differ in the respective time points. Behaviors such as tasting, "lapping" (Genther and Beede, 2013), swallowing (Jago et al., 2005; Williams et al., 2020) or taking sips

(Filho et al., 2004; Teixera et al., 2017), as well as non-consumptive behaviors as sniffing or splashing were partly included (Genther and Beede, 2013). Non-consumptive behaviors also include social competition and agonistic interactions at the trough (Figure 4). Aggressive physical interactions described are displacements by body pushing or sniffing, respectively head butting and pressing (Guzhva et al., 2016). Some authors describe indirect dominance effects as non-physical, subtle threats such as head lowering, moving, swinging, or abrupt pushing towards another cow (Coimbra et al., 2012; Hohenbrink and Meinecke-Tillmann, 2012). However, besides investigations of the water volume consumed, the drinking frequency, or the drinking duration under experimental farm conditions, no studies have evaluated a broader spectrum of drinking related behaviors in context of specific influencing factors on water intake under practical farm conditions. Thus, there is a lack of knowledge about (1) how dairy cows behave at the drinking trough under practical conditions within the herd, (2) how drinking behavior is influenced by various management, environmental, and animal-related factors, and (3) which factors can be used to deduce possible animal welfare impairments on the basis of the drinking behavior.

1.4. Influencing factors on water intake and drinking behavior

Various management, environmental, and animal-related factors impact a dairy cow's water intake and drinking behavior (Figure 5).

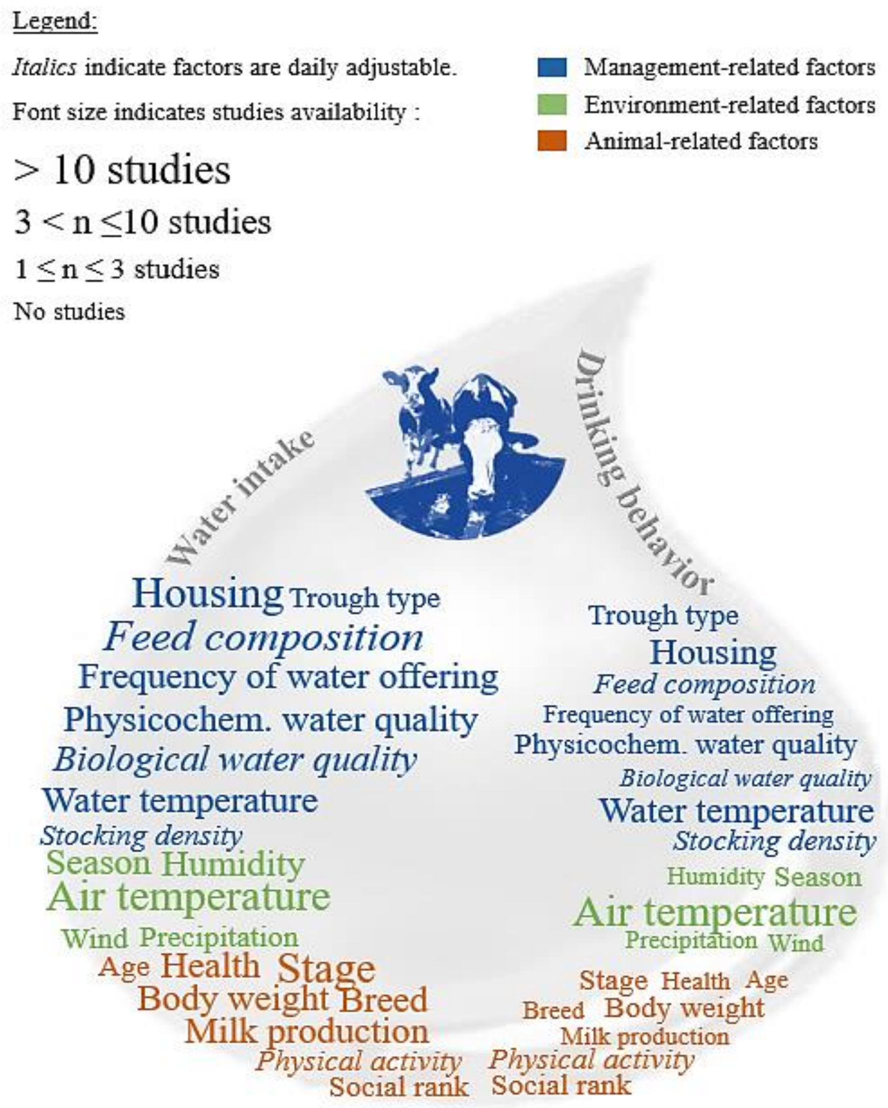

Figure 5: Management (blue), environmental (green), and animal-related (orange), factors influencing dairy cows water intake and drinking behavior. Italics indicate factors that can be adjusted daily. Font size indicates the studies availability (n = 0, n < 3, n < 10, n > 10 studies).

Some factors, such as the effect of feed composition or ambient temperature, have been studied particularly intensively (> 10 studies) and have been considered in prediction equations, even though study conditions often remain unclear (Table 2). However, other factors such as the influence of the trough type, the biological water quality, specific body characteristics, or social rank on water intake have hardly, or, concerning drinking behavior, not at all been researched (Figure 5) (Golher et al., 2021; Jensen and Vestergaard, 2021).

Table 2: Literature-reviewed regression functions of dairy cows drinking water demand (L/d) and influencing factors, indicating variable ranges.

Indicator category			**Management**		**Environment**				**Animal**				
Reference	Mean water intake (L/d)	Intercept	DM content in feed (%)	Concentrate content in feed (%)	Rainfall (mm/d)	Minimum Temperature (°C)	Mean Temperature (°C)	Day of the year (DOY)	Milk yield (kg/d)	Body weight (kg)	DM intake (kg/d)	Na intake (g/d)	R^2
Krauß et al. (2016)	91.9/ 54.4[1]	-27.93					0.49		3.15 (0.0 – 83.9)				0.67
Khelil-Arfa et al. (2012)	62.7	-77.6	0.83 (14.6 – 89.0)	-0.28 (0.0 – 77.8)					0.92 (5.6 – 45.1)	0.037 (358 – 756)	3.22 (5.3 – 27.1)		0.92
Cardot et al. (2008)	83.6	-26.65	0.89 (36.3 – 57.0)		-0.30 (0.2 – 33.2)	0.58 (-6.8 – 16.2)			1.33 (7.3 – 46.3)		1.54 (5.4 – 31.0)		0.45
Meyer et al. (2004)	82	-26.12					1.516 (-5.6 – 23.3)		1.299 (5.6 – 56.9)	0.0058 (466 – 812)	1.516 (1.8 – 36.8)	0.406 (4 – 101)	0.60
Dahlborn et al. (1998)	67.5	14.3	0.32						1.28				
Holter and Urban (1992)	36.6	-32.39	0.6205 (33.6 – 71.4)					0.0911×DOY -0.000257 ×DOY²	0.6007 (16.2 – 52.0)		2.47 (9.7 – 26.3)		0.69
Stockdale and King (1983)	35	11.34	-0.036				0.84 (7.2 – 18.9)				4.63 (6.3 – 10.1)		0.99
Little and Shaw (1978)	56.5	12.3							0.73 (13.7 – 30.0		2.15 (4.6 – 14.4)		*
Castle and Thomas (1975)	49.9	15.3	0.45 (25.4 – 88.3)						2.53 (11.4 – 25.4)				*
Murphy et al. (1983)	89.2	15.99				1.20 (-12.8 – 20.4)			0.90 (3.5 – 51.0)		1.58 (5.2 – 27.2)	0.05 (12.0 – 153.0)	0.59

*not reported

1.4.1. Water consumption in response to management-related influences

Management-related influences are, compared to environmental, and animal related influences, adjustable and, therefore, have a high potential for animal welfare improvements.

Water consumption in response to the feed ration and intake

Water intake is a function of the feeding ration, DM intake and content across all stages of a dairy cow's life (Murphy et al., 1983; Meyer et al., 2004). During calf rearing, increasing the DM content of the milk replacer (Jenny et al., 1978), and for heifers, dry cows, and lactating cows, an increase of the daily DM intake and the DM content of the ration increased the amount of water consumed. Decreasing the water content in the ration (70 to 40%) increased water intake by seven liters but simultaneously lowered total water intake (including feed water) by 15 L (Holter and Urban, 1992). Findings of an increase in water intake with rising DM contents of the ration were supported by several studies (Murphy, 1992; Kume et al., 2010). Although water intake increased, several studies also report total water intake (drinking and feeding water) to decrease with increasing DM intake (Paquay et al., 1970; Stockdale and King, 1983), as space in the gastro intestinal tract is limited. Following Murphy (1992), the urinary water loss increased as a result of increased N and K urine excretion. According to Stockdale and King (1983), dairy cows on pasture increased water intake by 2.3 kg/d per additional kg DM, respectively 0.053 kg for each g/kg. Moreover, water intake increased by 4.52 kg/cow with every extra kg feed intake (fresh matter) in early lactating cows at pasture at a DM intake ranging from 6.3 to 10.1 kg DM per cow. Nevertheless, on pasture, water intake from grass plays a key role in total water intake (Golher et al., 2021).

Only few studies addressed the relationship between feed composition and drinking behavior. Ammer et al. (2018) did not find an effect of the feeding ration on the number of drinking episodes. Comparing drinking frequencies in cows fed a total mixed ration (TMR) (49.2% DM) vs. cows on pasture (16.5% DM) showed that TMR-fed cows drank more often in 24 h (5.2 vs. 3.5 times) (Jago et al., 2005). Golher et al. (2021) concluded that a higher DM content in the TMR is the decisive factor in this context.

Besides DM content, the dietary crude protein, K, and Na content stimulated dairy cows' water intake. Increasing daily crude protein contents from 12% to 18%, or K contents from 1.6% to 2.3%, increased water intake from 20 – 25 L to 80 – 100 L (Kume et al., 2010). Moreover, water consumption increased with increasing Na intake (0.4 kg/d per g Na) (Meyer et al., 2004).

Dry and lactating cows increased water intake for dilution and excretion of excess nitrogen intake (Holter and Urban, 1992; Kume et al., 2010). Further, predictive models included ash and neutral detergent fiber as explanatory variables. To increase prediction quality, studies should not only refer to one experiment only but use two models. One model should include the feeding ration and the other should include animal-related factors and ration, to reduce root mean square prediction error, as multiple factors influence water intake (Torres et al., 2019).

Water provision and housing specific influences on water intake and drinking behavior

Ad libitum drinking water provision for dairy cows is crucial to support animal health and welfare regarding growth, pregnancy, and milk yield (Beede, 2005). Nevertheless, some interacting geographical, climatic or management conditions lead to water restrictions in watering amounts, frequencies or both (Singh et al., 2022). Inadequate cow : water trough ratios in study scenarios may per se limit outcomes, as a literature review conducted by Schilcher (2023) revealed a median of 3.9 cm trough length per cow in tank troughs (compared to the recommended 6 cm per cow in Table 1), respectively 0.6 bowl troughs per cow. Restriction of watering frequency from ad libitum to twice daily decreased milk yield by 16% in cross-breed cows (Thokal et al., 2004). When water access was restricted by 50%, milk yield dropped to 74% in British Frisian cows, and animals spent more time near the trough and behaved more aggressively. Cowan et al. (1978) found no effect on milk yield when cows only had water access 20 minutes before milking instead of 24 hours a day. Similarly, Hötzel et al. (2003) observed an effect of treatment (24 h vs. 30 min water availability per day) on water intake and the number of drinking episodes. Investigating the duration of water restriction on drinking behavior showed that impairments are stronger under warm than cold, respectively under grazing than under intensive conditions (Williams et al., 2017). Under the Mediterranean climate, water restriction increased respiratory rate, heart rate, rectal temperature, and the serum concentration of different blood parameters (glucose, triglycerides, cholesterol, urea, creatinine, total protein) (Benatallah et al., 2019).

Besides water restrictions, the influence of stress on water intake and related behavior were tested in two other stress inducing scenarios: crowding situations at the trough and under thermal stress.

Crowding at the trough led subordinate cows to shift their drinking times in summer (McDonald et al., 2020), and reduced daily drinking frequencies in winter (Cardot et al., 2008). Separating water bowls increased drinking frequency significantly (Andersson, 1987). Moreover, Andersson (1987) found some cows to be anxious to drink and either wait than drinking next to other individuals. Stress reactions and drinking patterns differ among individuals, making management strategies on a farm level inefficient. Nevertheless, individual drinking patterns appeared consistent over time among individuals, increasing its usability as an indicator for individual management systems (Melin et al., 2005). Schilcher (2023) reviewed the literature regarding the effect of housing conditions on dairy cow's water intake and drinking behavior. By comparably analyzing the reviewed different study conditions, and comparing cows kept in a free-stall barn with tethered cows, the total water intake (feeding and drinking water) was significantly lower in tethered cows (68.6 L/d) than in dairy cows held in free-stall barns (87.1 L/d). Cows in free-stall barns had a higher total water intake than cows on pasture (67.9 L/d) (Figure 6). Nevertheless, since August 1, 2013, according to section 2 (3) No. 2 of the Animal Welfare Act, it is prohibited to keep vertebrate animals permanently tethered to a device that does not allow them freedom of movement. The tethering of cows, which prevents them from moving freely, was therefore, except for very few exceptions in alpine regions, prohibited by this law.

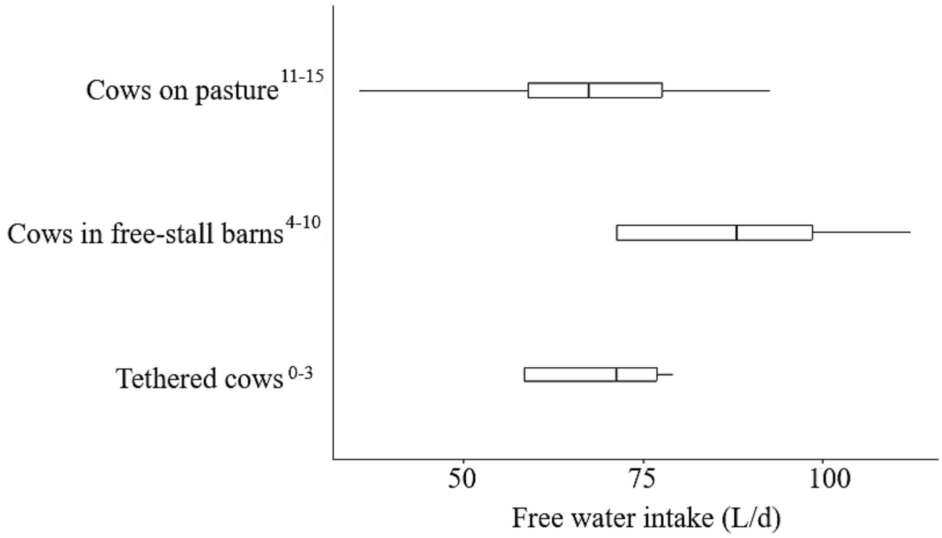

Figure 6: Water demand of dairy cows as a function of housing management (cows pasture, free-stall barn, tethered), extracted from data by Schilcher (2023). References: 0-3: Castle and Thomas (1975), Andersson (1985); 4-10: Castle and Thomas (1975), Cardot et al. (2008), Meyer et al. (2004), Ammer et al. (2018), Beck and Steingass (2000), Lanham et al. (1986). 11-15: Castle and Watson (1973), Castle (1972), Spörndly and Wredle (2005), Pereyra et al. (2010).

More recent studies on the water intake and drinking behavior of dairy cows under currently standard free stall barn conditions are missing. In the context of preventing heat stress, Laínez and Hsia (2004) found that providing wet pads and forced ventilation cooling systems doubled water intake compared to cows held in an open house without wet pads, reflecting a higher animal comfort and well-being. By providing both, wet pads and high-speed air movement by ventilation, the authors attempted to remove heat by conduction and convection (Laínez and Hsia, 2004) .

Investigating the effect of the trough location on water intake and drinking behavior, Coimbra et al. (2012) found that cows had a higher intake volume, drank more frequently, and drank longer when the water trough was located in a paddock instead of a corridor, indicating social stress with limited space. In the respective study scenario, 40 pasture-based paddocks à 900 m^2 each were connected by 4 m wide corridors. Providing shade did not affect drinking behavior. The watering system used affects the water volume consumed. Therefore, older studies under tie-stall conditions, showed that cows had a relatively low water intake per drinking episode (6.4 L) (Dado and Allen, 1994; Cardot et al., 2008). In Western Europe and Germany, most dairy farms provide a combination of open troughs and smaller valve troughs (Suevia Haiges GmbH, 2023). Nevertheless, no comparisons exist concerning the drinking patterns of cows offered water in troughs and bowls (Jensen and Vestergaard, 2021). According to Andersson et al. (1984), an increase in trough water flow rate from 2 L to 12 L reduced drinking frequency by up to 25% (40 vs. 30 drinking episodes per day). Nevertheless, these flow rates are relatively low for today's standard (up to 40 L/min) (Suevia Haiges GmbH, 2023). No evaluation of flow rates in the recent standard trough design has been conducted. Nevertheless, the surface area, height, volume, and material of troughs affected dairy cows' drinking behavior (Filho et al., 2004; Taxeira et al., 2006; Teixera et al., 2017). Investigations on the trough color's effect (green, grey, and red) on water intake and drinking behavior showed no differences in the volume consumed, the drinking time, or the number of sips in 18 lactating cows under a rotational grazing system (Teixera et al., 2017). Lukas et al. (2008) have concluded in their experiments that the calving of primiparous cows and the associated change of trough system leads to reduced water intake and potentially, to learning stress. Therefore, they demand that first calving cows are prepared for potentially altering trough systems before calving to avoid additional stress during and after calving. Assessing current recommendations concerning the water supply technique demands research on the effect of the design, location, stocking, and resulting competition on indoor housed dairy cows' drinking behavior and water intake (Jensen and Vestergaard, 2021).

Water characteristics-specific influences on water intake and drinking behavior

Regarding specific characteristics of the water offered, the effects of water temperature have been studied under several conditions, while water quality has rarely been considered. Water temperatures between 7 °C and 16 °C reduced body temperature and respiratory rate in Holstein-Fresian cows (26 – 30 kg milk/d), but dairy cows prefered water temperatures between 20 °C and 28 °C (Lanham et al., 1986; Milam et al., 1986; Wilks et al., 1990; Pereyra et al., 2010). Transient cooling effects started 40 min after drinking (Lanham et al., 1986). When consuming 10 °C compared to 28 °C water, DM intake and milk yield increased in 24 lactating Holstein cows held in individual tie-stalls (maximum ambient temperature 39°C) (Milam et al., 1986). Wilks et al. (1990) evaluated the effect of 10.0°C and 27.0°C drinking water in lactating Holstein. Cows consuming the colder water had a higher DM intake, milk yield, and water intake per kg DM, but overall water intake was higher when warm water was offered. Pereyra et al. (2010) could not find any effect of drinking water temperature (18 °C and 31 °C) on water intake or preference. However, in their experiments, cows had only access to two water troughs (à 18 and 31°C) twice per day for 10 minutes, and troughs were placed in a distance of 30 cm. Thus, the high demand to satisfy the severe thirst in a short time may have outweighed the individual's preferences. In conclusion, in cold ambient temperatures, dairy cows preferred heated drinking water; under hot weather conditions, they preferred cold water to counteract heat stress (Jensen and Vestergaard, 2021).

Impaired drinking water quality is expected to negatively impact dairy cows' water acceptability and the animals' digestive and physiological functions. Decisive factors for water quality are "odor and taste, physical and chemical properties, presence of toxic compounds, concentration of macro and micro minerals, and microbial contamination" (Beede, 2005). Regarding chemical properties, studies revealed that water intake decreased the higher the content of total dissolved solids (TDS) (Challis et al., 1987), sulfur (Beede, 2005), and sulfates (Weeth and Capps, 1972). Moreover, health and performance problems were linked to high amounts of TDS (Beede, 2005), nitrates (Kahler et al., 1974), and iron (Beede, 2005) in drinking water. Heifers preferred water treated with a reverse osmosis system or a municipal city water treatment over "hard classified" local untreated water in South Dakota (Senevirathne et al., 2021). Knowledge about the influence of water pH, hardness, or magnesium contents on dairy cow water preference, consumption, behavior, health, and welfare under practical conditions is rare (Beede, 2005)

Drinking represents a direct and major exposure to water-related microbial contaminants. Feces are the primary source of contamination (LeJeune and Gay, 2002). However, heavy soiling may also occur by feed residues, and therefore, water troughs need to be placed in adequate distance to feed bunks (KTBL, 2008). Primary indicators for the biological water quality are total bacteria, total coliforms (CC), fecal CC, fecal streptococcus per 100 mL drinking water, and biofilm formation (Beede, 2006; van Eenige et al., 2013). Further, scientists detected *Salmonella* spp, *Campylobacter* spp, Aeromonads, and blue-green algae in cattle water troughs (Galey et al., 1987; LeJeune and Gay, 2002; Waldner and Looper, 2007). Potential outbreaks of waterborne diseases can adversely affect animal and human health (Giri et al., 2020). The exposure of water troughs to solar radiation, especially under warm ambient temperatures, potentially increases water temperature and worsens its physicochemical and biological quality (Challis et al., 1987; Beede, 2006). Microbial contaminations highly rely on the drinking water source (Kamphues et al., 2007; Senevirathne et al., 2021). Water intake decreased when drinking water was contaminated with manure (19.8 L to 6.5 L; 0.0 mg and 0.05 mg fresh manure per g water), but flavoring drinking water showed no effect (Thomas et al., 2007). To the authors' knowledge, no controlled studies on the effect of impaired water due to neglected cleaning on water quality and related water intake have been published to this date, and no research has been conducted on the corresponding effects on specific drinking behavior.

1.4.2. Water consumption in response to environmental conditions

Stress reactions in hot climates occurred not only due to crowding at the trough but also substantially due to high air temperatures. A dairy cow's thermoneutral zone ranges from - 5 – 21 °C, depending on physiological status (Meyer et al., 2004). Air temperatures exceeding this range, combined with an increasing humidity and solar radiation, lead to heat stress in dairy cows. Heat stress is defined as a state in which the organism fails to maintain a constant body temperature by regulating the thermal energy balance (metabolic heat production exceeds environmental heat loss) (Becker et al., 2020). Dairy cows drink more to compensate for heat dissipation through evaporation. Holstein cows are particularly susceptible to heat stress due to a higher metabolic heat production induced by their higher milk yield. Moreover, compared to light colored coats, Holstein cows absorb more solar radiation due to their black coats (Becker et al., 2020). In beef cattle, Brown-Brandl et al. (2006) found darker colored breeds under hot weather conditions to have a higher respiration rate, panting scores, and surface temperatures, more extensive changes in behavior, and generally more stress than light coated breeds but a lower water intake. Meyer et al. (2004) predicted water intake to rise by 1.52 kg/d for each degree Celsius in ambient temperature (ambient temperatures range - 5.6 – 23.3 °C, mean: 8.6 °C). Cows milked in automatic milking systems (AMS) likewise showed positive and negative water consumption peaks in summer and winter (Krauß et al., 2016). Additionally, Cardot et al. (2008) observed under cold conditions 4.5 – 10.1 drinking episodes daily, while other studies under hot weather conditions indicate a range of 8 – 20 drinking episodes (average 13.6 ± 6.7 drinking episodes) (González Pereyra et al., 2010), respectively in average 14 ± 5.6 drinking episodes (Dado and Allen, 1994). In Summer, Laínez and Hsia (2004) found 142 Holstein cows drinking significantly more than in winter (61.9 L/d vs. 36.6 L/d), but drinking duration did not differ. Drinking frequency, drinking time, and the time searching for water increased with thermal stress in 100 Holstein cows held in a free-stall barn on a commercial farm. During study periods the ambient temperatures averaged 24.1 ºC and 32.8 ºC (Matarazzo et al., 2003). Investigating the impact of the temperature-humidity index) (THI) (daily ambient temperature: 19.2 °C) on different feeding rations and the water consumption, drinking episodes per day averaged feed dependent between 7.3 ± 0.6 and 8.8 ± 0.6 drinking episodes and increased with increasing THI (Ammer et al., 2018). Moreover, under heat stress conditions, several authors also indicate an increase in competitive interactions at the water troughs (Schütz et al., 2010; Polsky and Keyserlingk, 2017; McDonald et al., 2020). In total, fewer studies investigated the water intake of dairy cows in cold seasons (n = 12) than in warm seasons (n = 6) (Williams et al., 2017). Nevertheless, cows showed a higher DM intake under cold stress conditions to increase metabolic heat as a coping mechanism, potentially leading to a higher water intake (Singh et al., 2022). Investigations of dairy cows drinking behavior in moderate warm ambient temperatures under commercial farm conditions are missing.

Concerning the relative humidity, both positive (Lukas et al., 2008; Pereyra et al., 2010) and negative (Castle, 1972; Stockdale and King, 1983) relations were described. Rainfall possibly decreased (1) the ambient temperature, (2) the DM content of the ration, (3) increased relative humidity in winter and thereby reduced the water demand (Cardot et al., 2008). Castle (1972), Cardot et al. (2008) and Stockdale and King (1983) observed a positive correlation between rainfall and water intake (r = 0.6). Moreover, the higher the wind speed, the lower the water intake (Stockdale and King, 1983).

1.4.3. Water consumption in response to animal related influences

Due to their lower heat tolerance capacity, *Bos taurus* cattle drink more than *Bos indicus* cattle (Payne and Hutchison, 1963). The higher production of milk-related heat means that dairy breeds have a particular demand for water compared to beef and wild breeds. The demand depends besides the genetic constitution on their ability to adapt to heat in their environment (Golher 2020). Additionally, the efficiency of converting feed into energy is linked to their metabolic body size, which, in turn, affects their water demands (Ingram and Mount, 1975). Larger metabolic body sizes generally mean a higher water demand. Higher body weights increase nutrient demands, leading to a higher water intake, influenced by factors like metabolic rate, intake capacity, and water dynamics (Meyer et al., 2004). Among dairy cattle breeds, Holstein-Frisian cows consume the most water. This breed is the most productive in terms of milk excretion and represents the largest breed of dairy cows. Due to wide ranging study conditions, no cross-breed evaluation has been conducted. Therefore, Figure 7 compares feed, drinking, and total water intake of different breeds in the context of DM content of the ration, body weight, and milk yield. Data was extracted from a literature review conducted by Schilcher (2023). Body weight and milk yield of respective breeds are relatively low for today's dairy cows, as most studies regarding dairy cow water consumption took place before 2010. Numerically, free water intake and total water intake are highest for Holstein, Holstein-cross breed cows, and (Holstein)-Swedish red cows. Nevertheless, the multitude of mentioned factors per se influence the literature based indicated water intake amounts. Thus, drawing scientifically solid conclusions about the cross-breed differences is hardly practicable.

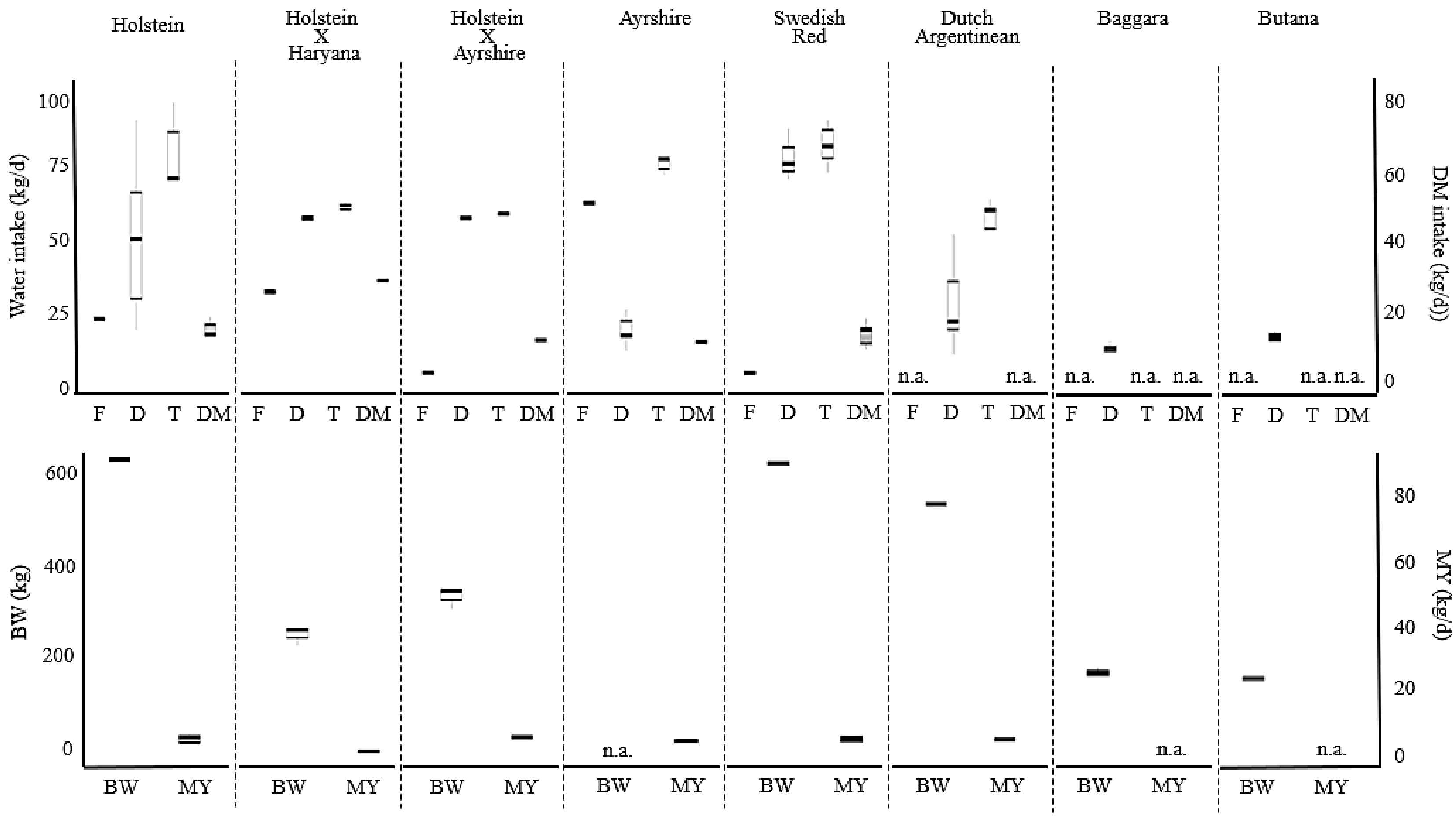

Figure 7: Water intake (feed (F), - drinking (D) and total (T) water) in relation to DM intake, body weight (BW) and milk yield (MY) of dairy cows depending on their breed. Data extracted from studies conducted in 1972 until 2018 (n = 18). The feed water content was calculated using the following equations: DM (kg)/ DM content (%) = fresh matter (kg); Fresh matter (kg) x water content (%) = water in feed (kg), n.a. = data not available.

Water consumption as a function of the physiological state

Calves

The first stage of calf development is characterized by transitioning from a monogastric organism to a ruminant organism. The abomasum is the functional stomach at birth, while the reticulum, rumen, and omasum are undeveloped (Heinrichs and Lesmeister, 2005). Microbial population growth requires ruminal water. Ninety seven percent of the water consumed through milk replacers via a nipple goes to the abomasum, but water consumed voluntarily ends up primarily in the rumen (Toullec and Guilloteau, 1989; Yohe et al., 2019). Calves primary demand for water for the excretion of uremic substance, namely detoxification processes, and for feed, respectively DM intake. Thereby the demand for water depends on the feeding regime (eg. milk or milk replacer fed calves), as increasing nutrient concentration of milk replacer, especially of protein and DM, increased the water intake (Jenny et al., 1978; Bartlett et al., 2006; Cowles et al., 2006).
Recent studies indicate that milk or milk replacer fails to adequately fulfill a calf's total water demand in terms of performance and health. Drinking water access from birth tendentially increased preweaning milk consumption per day and, as a result, body weight and heart girth. Postweaning calf disclosed higher body weight, hip height, body length, feed efficiency, as well as the digestibility of neutral and acid detergent fiber (Wickramasinghe et al., 2019). The latter indicates that providing water from birth improved the rumen function. Therefore, providing free water from birth on is recommended (Kertz et al., 1984; Wickramasinghe et al., 2019).

Heifers and dry cows

Heifer rearing in dairy cow management focuses on optimally supporting growth, mammary gland development, fertility, and the animal's milk production potential. Its success depends on nutritional strategies from birth, across puberty, and during pregnancy, but seldomly includes water supply. Post-pubertal nutritional management determined growth rate and body weight at calving, factors, that affect first lactation milk yield (Lohakare et al., 2012). Compared to adult cows, growing heifers are more susceptible to potentially impaired water quality in terms of weight gain and health (Beede, 2005; Grout et al., 2006). Therefore, providing adequate high-quality water supports optimal growth (Beede, 2005; Senevirathne et al., 2021). The larger the animal, the more water occupies the digestive tract (15 – 35% of the animals' total weight).

Therefore, body size and body weight determine water intake. Thus, with progressive growth, water demand rises (Odwongo et al., 1985; Golher et al., 2021). The reason for this is primarily an increased DM intake during growth (Winchester and Morris, 1956). Nevertheless, recent studies only predicted water intake for growing beef cattle (Meador, 2017). Generally, juveniles have a faster water turnover, leading to a lower tolerance for water restrictions than mature animals (Jensen and Vestergaard, 2021)

With gestation, the water demand for maintenance rises due to tissue growth and embryonic fluid up to 28 L/d. Thus, water demand increases with progressing pregnancy and an increase in the fetus growth rate (Golher et al., 2021). Despite pregnancy, dry cows' water demand is less than that of lactating cows, as respiratory and cutaneous losses are lower besides the lack of water loss due to milk excretion. In both physiological states, 78% of the total water intake (drinking and feeding water) was contributed by free water intake. Holter and Urban (1992) predicted water intake of dry cows as a function of DM intake, crude protein intake, and body weight ($R^2 = 0.64$, $P < .001$, $n = 60$). Nevertheless, few studies addressed the water supply of dry and growing cows (Williams et al., 2017; Silva et al., 2018).

Lactating cows

The state of lactation and the production capacity influence dairy cows water demand (Brandstetter et al., 2019; Golher et al., 2021). Water consumption of 24 multiparous Holstein dairy cows in transition increased in a study by Osborne et al. (2002) from 57 to 73 L/d from the week before to the week after calving. Similarly, Huzzey et al. (2005) found that the frequency of drinking episodes and the total drinking time increased from the pre- to the post-calving period. The transition period marques the end of pregnancy and represents the most vulnerable period of a dairy cow's life, as it comes with changes in nutrition, the start of lactation, including changes in physiology and social behavior due to the reintegration into the herd. Thus, water restrictions increase cows' susceptibility to metabolic and infectious diseases (Huzzey et al., 2005). Therefore, stress reducing feeding management is crucial, including ad libitum access to fresh water under minimization of competition at water troughs (Grant and Albright, 1995).

Cows in early lactation need to adapt to a different feeding ration and simultaneously adapt to the initiation of lactation (Gross, 2023). Both affect dairy cow's water metabolism and dynamics (Murphy et al., 1983). Brandstetter et al. (2019) observed cows in early lactation to drink shorter (8 min/d) than cows before (20 min/d) or around calving (20 min/d). In contrast, Laínez and

Hsia (2004) could not identify a significant difference in drinking time among high and low-yielding Holstein cows (15.3 min/d and 14.3 min/d, respectively); but, dry cows drank significantly shorter (8.2 min/d). Recording the drinking durations requires for unlimited flow rates of the water troughs, however, most studies doesn't indicate the respective technical specifications. Cardot et al. (2008) investigated the drinking behavior of 41 Holstein-Frisian cows in mid-lactation. They observed a positive correlation between the number of drinking episodes with the lactation number ($r = 0.41$), and milk yield ($r = 0.37$), even though drinking episodes and days in milk (DIM) were only lowly correlated ($r = 0.09$; $P < 0.01$), potentially due to great variations on a daily and individual basis. Similarly, Meyer et al. (2004) found a positive correlation between lactation number and water intake.

Differences in water intake depending on parity are hardly investigated (Lukas et al., 2008). However, Dado and Allen (1994) found that lower producing, primiparous cows ate smaller meals, during a longer time, ruminated shorter, and drank significantly less (63.2 L/d) than multiparous, high producing cows (89.5 L/d) ($P < 0.05$). The reason for this may lie in the higher milk yield and associated milk-related water losses of the multiparous cows, but also in the higher body weight and size, and thus, the gastrointestinal tract volume (Meyer et al., 2004). (Murphy et al., 1983; Meyer et al., 2004; Cardot et al., 2008). Meyer et al. (2004) demonstrated by regression analysis that a dairy cow's water demand rises by 1.3 kg for each milk produced. Kume et al. (2010) observed a drinking water intake of 2.0 – 2.7 kg for each kg of milk produced for cows with a milk yield of 33 – 35 kg/d, respectively, a drinking water intake of 2.6 – 3.0 for daily milk yield of less than 26 kg/day.

1.5. Research aims and outline of the thesis

Reviewing the literature highlighted the importance of providing sufficient water of adequate quality to optimize dairy cows' health, welfare, and performance at all production stages. The biological water quality is rarely the focus of investigations, although it represents a potentially limiting water intake factor. It can be assumed that the trough design plays a key role in drinking water quality, and therefore, considering interaction effects is crucial. Previously, water supply and quality had only been assessed using resource and management-related indicators. Nevertheless, under experimental study conditions, the behavior of dairy cows changed when the water provided was contaminated, showing the potential use of behavioral indicators.

However, specific animal-related indicators have not yet been evaluated under practical farming conditions.

This thesis aimed to investigate the effect of neglected trough cleaning on the water quality and drinking behavior at two different trough designs under cold conditions (low risk scenario) on a commercial farm. Thus, dairy cows' drinking and related behavior was characterized (Figure 8). A reflection of the impaired water quality due to neglected cleaning in the dairy cows' specific drinking behavior was hypothesized (**H1**) (Burkhardt et al., 2022).

Further, in this thesis the same scenario under moderate warm conditions was tested (higher risk scenario). Considering the interaction of the trough design and cleaning interval, this study aimed to investigate the effect of climatic conditions (cold versus moderate warm) on water quality and drinking behavior (Figure 8). As elaborated in the previous chapters, microbial growth in water troughs increases with rising temperatures. Therefore, a higher impact (soiling or contamination) of neglected cleaning under warm ambient temperatures on the biological water quality, and thus, dairy cows drinking behavior was expected (**H2**) (Burkhardt et al., 2024).

In order to accurately assess and correctly interpret behavioral changes, factors influencing the analyzed behaviors need to be known and adjusted for (Rushen et al., 2012). Recent studies indicate that social hierarchy significantly influenced dairy cows' behavior at the trough, and thus, should be considered in behavioral analysis, alongside specific body and performance traits, on a herd and individual animal level (Keyserlingk et al., 2009). Therefore, this thesis investigated the effect of body and performance traits, as well as social hierarchy, on individual cows' water intake and drinking behavior under experimental conditions (Figure 8). A variation of the drinking behavior among individuals was hypothesized, depending on specific body and performance traits, and that competitive success determines access to a trough near the milking parlor. High-yielding cows involving more frequently in competition, due to their higher water demand, was assumed. Further, cows with high competitive success spending more time at the trough, and drinking more was expected (**H3**) (Burkhardt et al., 2025).

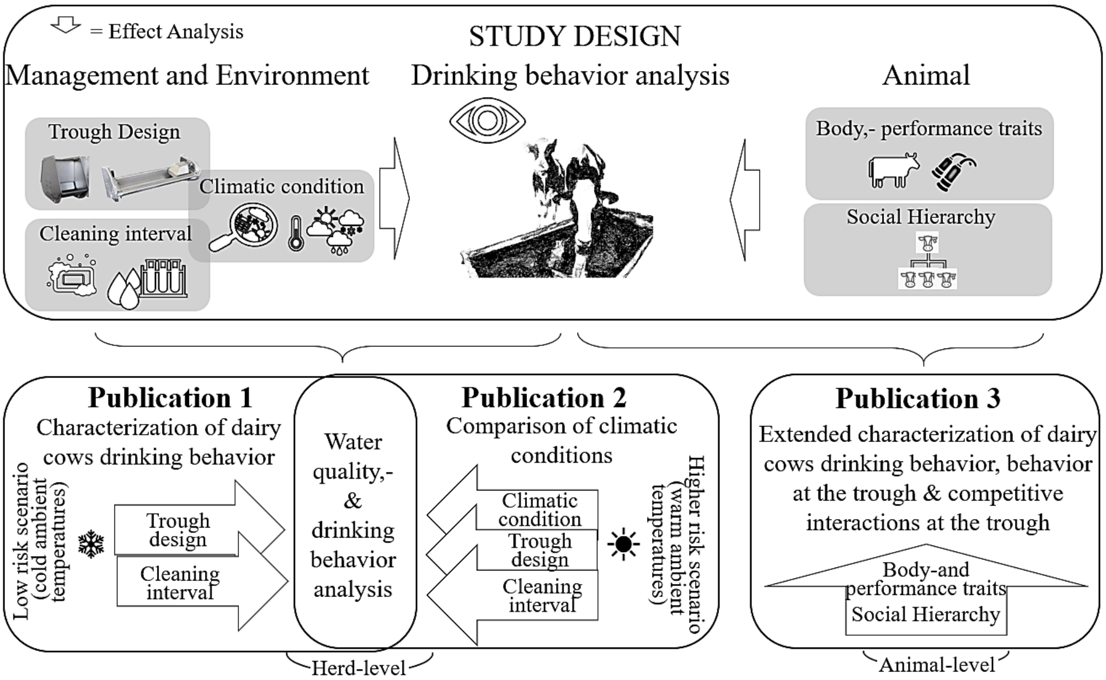

Figure 8: Study design of the thesis, including key aspects and a conceptual overview of the conducted effect analyses to assess dairy cow´s water supply using drinking behavior variables. **Publication 1** focuses on the characterization of dairy cows drinking behavior, further investigating the interaction effect of trough design cleaning interval on drinking behavior and water quality under a low risk scenario. **Publication 2** focuses on the comparison of trough design and cleaning interval effects in two climatic conditions on drinking behavior and water quality. Therefore, the same scenario in a higher risk scenario was conducted and compared with data of the low-risk scenario in **Publication 1**. **Publication 3** investigate the effect of body- and performance traits on the individual drinking behavior, considering social hierarchy.

1.6. References

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2. Material and Methods

2.1. Study design

The general objective of this thesis was to develop an animal-related assessment of dairy cows' water supply management under practical conditions based on drinking behavior indicators. A special emphasis should be given to the evaluation of climatic conditions, the trough design, and the cleaning interval. Therefore, a video-based behavioral analysis was performed under practical-farm conditions, using 13 drinking behavior variables. Effects of the trough cleaning interval were tested using a Latin square design, allowing the access of cleaned and not cleaned troughs of each design during the trials in two climatic conditions (cold and moderate warm ambient temperatures). Further, the drinking water quality was analyzed at the start and end of the study trials, and monitored on a daily basis using rapid tests. Two studies have arisen from this project, a first focusing on the general characterization of drinking behavior (Burkhardt et al., 2022) and a second focusing on the comparison of two climatic conditions on drinking behavior and water quality (Burkhardt et al., 2024), both under the consideration of the drinking design and the cleaning interval **(Publication 1 & 2)**.

The characterization of drinking behavior, on which the first two studies are based, was expanded in a third study **(Publication 3)** to include a further 20 variables relating to behavior in the drinking trough environment, with a special emphasis on competitive interactions, resulting in a total of 33 behavioral variables. Thereby, under experimental conditions, effects of body and performance traits, respectively the social hierarchy on (1) the access of the trough and (2) the drinking behavior after milking on an individual animal level were investigated (Burkhardt et al., 2025) (Figure 8).

2.2. Experimental procedures

Video recordings were conducted in two scenarios. Each scenario started after pre-trial periods, guaranteeing proper experimental running. In a first scenario in 2019/2020 **(Publication 1&2)**, dairy cows drinking behavior held in a conventional free-stall barn was recorded for two hours after feeding at all troughs in a total of 60 d (two tank troughs, length: 2.00 m; width, 0.43 m; depth, 0.15 m, 70 L, and two double valve troughs, length, 0.73 m; width, 0.32 m; depth, 0.10 m; variable volume 5-15 L). Following a 2 x 2 Latin square design, cows could choose between a daily cleaned or uncleaned trough of each design in four consecutive 15 d trials, two trials under cold ambient temperatures (2 x 15 d in December/February 2019/2020, n = 135 cows) and two trials under moderate warm ambient temperatures (2 x 15 d in September 2020, n = 144 cows) (Burkhardt et al., 2022, 2024). Further, at the start and end of each trial, the biological and physico-chemical water quality was analyzed, and the biological water quality was monitored on a daily basis using adenosine triphosphate (ATP) rapid tests and photographs.

In a second scenario **(Publication 3)**, the drinking-related and agonistic behaviors of n = 42 dairy cows after milking were recorded during 22 milkings at a milking parlor facing valve trough, considering body and performance traits, respectively the social hierarchy. Social hierarchy was determined by an “index of displacements”(Burkhardt et al., 2025).

2.3. Behavioral analysis

The video recordings were analyzed using the Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba, 2016) following an ethogram developed in the pre-trial period of the first study (Burkhardt et al., 2022, 2024) and completed in the third study (Burkhardt et al., 2024). Core element of the behavioral characterization builds the definition of a drinking episode, starting with the cows’ head crossing the edge of the through, signaling simultaneously the beginning of a drinking episode and the tasting period. The tasting period ended either with the cow taking more than five continuous sips, having less than three seconds between two sips or stepping away from the trough. If a cow left the trough by stepping away after tasting, the drinking episode was scored as “tasting only”. The whole drinking episode was characterized by periods of water intake, in which the cows was taking sips, and drinking breaks. Regarding non consumption behaviors, the first two studies considered agonistic behaviors, interruptions due to agonistic behaviors, and swallowing difficulties in the analysis (Burkhardt et al., 2022). The third study extended the analysis of non consumptive behaviors by a more detailed description of agonistic interactions between cows (reacting, ignoring, staring, poking, pushing aside, fighting, leaving), of social behaviors (accepting and giving care), of activities after milking (drinking, brushing, eating concentrate, eating a partial mixed ration (PMR), total episodes in the observation zone), as well as the measurement of the water volume consumed, resulting in a total of n = 33 behavioral variables (Burkhardt et al., 2025).

2.4. Water quality analyses

As a primary element of **Publication 1 & 2**, the effect of water cleanliness on dairy cows drinking behavior was investigated. Therefore, on a daily basis, the water quality of each trough was visually assessed using a three-stage evaluation scheme, as well as quantitatively assessed using ATP rapid tests, pH, and temperature meters.

At the start and end of each trial, water samples of each trough were obtained from a water depth of 2–5 cm, imitating cows' drinking depth. Hereinafter, the microbiological water quality (*Escherichia coli* (*E-coli*) count, total CC, aerobic total viable count (TVC) at 20°C and 36°C) was determined by pour plate. Physicochemical analysis was performed following 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) (Burkhardt et al., 2022, 2024).

2.5. Environmental measures

Ambient temperature and humidity were measured during all trials in 10-minute intervals using weather loggers that were placed above the troughs (Burkhardt et al., 2022, 2024; Burkhardt et al., 2025).

2.6. Data analyses

The obtained data of **Publication 1 & 2** were analyzed using the FREQ and MEANS procedures in SAS version 9.4 (SAS Institute Inc., Cary, NC) for categorical variables (shown as distributions) and continuous variables (shown as means ± standard error), respectively. If necessary, outcome variables were log **(Publication 1)** or cubic-root transformed **(Publication 2)** to achieve normal distributions. Linear mixed effect models with "trough design" (tank troughs vs. valve troughs), "trough cleaning status" (cleaned vs. uncleaned) in **Publication 1**, and adding climatic condition (cold vs. moderate warm ambient temperature) in **Publication 2** as fixed factors calculated differences between continuous variables. For categorial variables, the MASS package (version 7.3 – 53) in R version 4.1.1 based on Rawat (2017) was used to apply a binary logistics regression modeling the effects of "trough design" and "trough cleaning status" in **Publication 1** and comparably illustrated in **Publication 2**. Correlative relationships were calculated by Spearman's rank correlations in SAS (PROC CORR Spearman) (Burkhardt et al., 2022, 2024).

In **Publication 3**, the averaged sum of n = 33 behavioral variables (n = 42 cows) were descriptively analyzed using R version 4.1.1. Mean ± standard deviation, median, minima, maxima, range, percent range, and frequencies were calculated using the "calculate_stats" function. A t-test was performed to investigate behavioral differences depending on the milk yield (< 30 L, > 30 L). Correlative relationships were calculated by Kendall correlations in R using the "ggcorrplot" package (Burkhardt et al., 2025).

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.

For detailed descriptions of the experimental procedures, conditions, and statistics please refer to the respective publications. A short overview is provided in Table 3.

Table 3: Summary of material and methods applied in the current thesis (Publication 1,2 & 3), including the experimental procedure and conditions.

	Publication 1	**Publication 2**	**Publication 3**
Video-recording*	Two hours after feeding, daily	Two hours after feeding, daily	During milking, twice daily
Trough cleaning interval	2 x 2 Latin square design	2 x 2 x 2 Latin square design	-
Facility (number of dairy cows)	Conventional farm (n = 135)	Conventional farm (n = 135/144)	Experimental farm (n = 42)
Drinking behavior analysis	n = 13 behavioral variables	n = 13 behavioral variables	n = 33 behavioral variables
Laboratory water analysis	Physico-chemical, biological	Physico-chemical, biological	-
On-farm water quality monitoring	Water ATP value Water pH value Water temperature	Water ATP value Water pH value Water temperature	Electrical conductivity Water pH value Water temperature
Environmental measures	Ambient temperature Relative humidity Light irradiation	Ambient temperature Relative humidity Light irradiation	Ambient temperature Relative humidity Light irradiation
Target variables	Drinking behavior, water quality	Drinking behavior, water quality	Drinking and agonistic behavior
Explanatory variables	Trough design, cleaning interval	Climatic condition, trough design, cleaning interval	Body-, and performance traits, social hierarchy
Statistical Analysis	Mixed effect models Post-hoc tests Correlation analysis	Mixed effect models Post-hoc tests Correlation analysis	Descriptive analysis T-test Correlation Analysis

*Water consumption of dairy cows is associated with milking and feeding times (Andersson, 1987; Laínez and Hsia, 2004; Cardot et al., 2008).

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

Burkhardt, F. K.; Hayer, J. J.; Heinemann, C.; Steinhoff-Wagner, J., 2022. Drinking behavior of dairy cows under commercial farm conditions differs depending on water trough design and cleanliness. Appl. Anim. Behav. Sci. 256:105752. https://doi.org/10.1016/j.applanim.2022.105752.

Authors contributions:

FKB, JJH and JS-W conceived the idea and design of the experiments. **FKB** conducted the on-farm experiments, as well as collected and analyzed the data. JJH and CH conducted the laboratory analysis of the microbiological water quality. **FKB** and JJH led the writing and JSW, respectively CH led the editing of the manuscript. All authors approved the manuscript after critically contribution. The development of the drinking behavior assessment protocol was part of the unpublished master thesis of **F.K.B.,** submitted to the Faculty of Agriculture, University of Bonn on 15 September 2020.

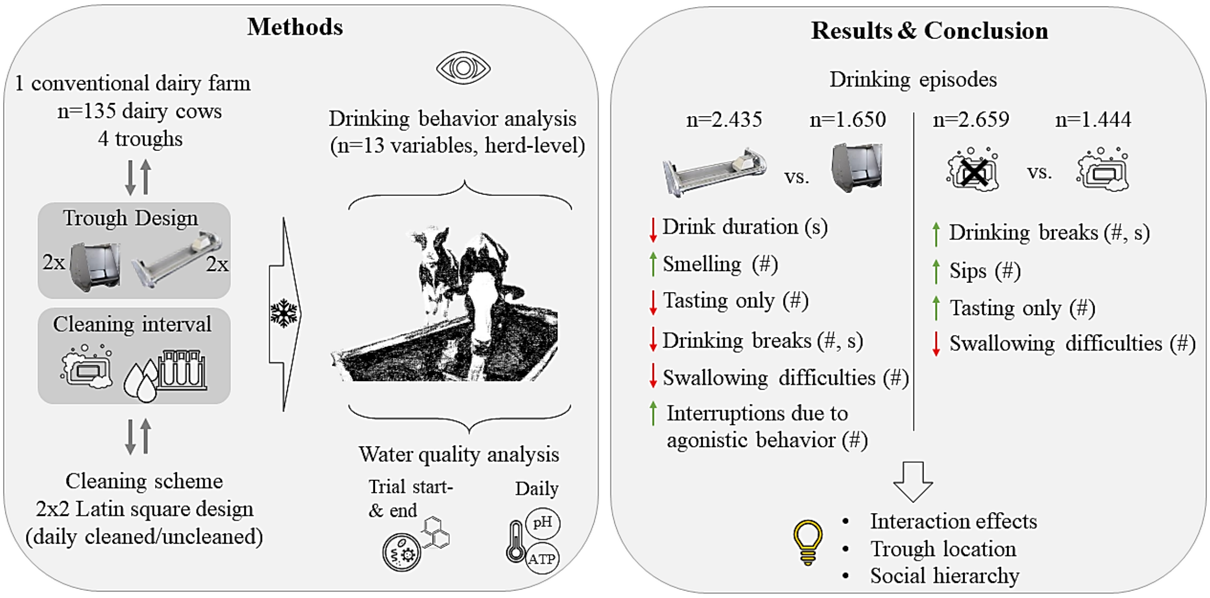

Figure 9: Graphical abstract of **Publication 1** illustrating the study design under presentation of the methods used, results and main conclusions. Core element is the characterization of dairy cows drinking behavior and the water analysis on a herd level, considering the influence of the trough design and cleaning interval.

Summary

The "absence of prolonged thirst" is considered an iceberg indicator to theoretically reliably predict the overall welfare of dairy cows. Analyzing the drinking behavior might provide useful insights into improving water supply (e.g. choice of trough design or cleaning interval). Thus, **Publication 1** aimed to characterize dairy cows' drinking behavior under practical farm conditions using 13 behavioral variables. Thereby, investigating the hypothesis that the drinking behavior is altered by trough design and cleaning interval.

At a commercial farm (n = 135 dairy cows), following a 2 x 2 Latin square design, cows were allowed to freely choose between a daily cleaned and an uncleaned tank trough (70 L), respectively, a daily cleaned and an uncleaned double valve trough (variable volume, 5 – 15 L) during two consecutive15 d trials. Drinking behavior was video recorded daily for two hours after feeding. At the start and end of each trial, biological and physicochemical water quality was analyzed, and on a daily basis the trough water temperature, pH and ATP value was monitored. ATP represents soiling and bacteria, and is therefore an useful hygiene tool do assess the trough water quality.

The biological water quality influenced the drinking behavior, as the water ATP value correlated with the " total number of drinking episodes"($r = 0.3$, $P < 0.001$), the "total duration of drinking" ($r = -0.2$, $P < 0.05$), the duration of "water intake" ($r = -0.2$, $P < 0.05$), the number of "sips per drinking episode" ($r = -0.2$, $P = 0.05$), and "swallowing difficulties"($r = -0.4$, $P < 0.0001$). Trough water ATP values fluctuated more at uncleaned troughs, than cleaned troughs. The initial and final water quality measurements did not differ statistically. The interaction of trough design and cleaning interval strongly affected dairy cows' drinking behavior. Eight of the 13 behavioral variables were altered by trough design and six by the cleaning interval (Figure 9). Cows visited tank troughs more frequently, respectively uncleaned troughs. However, feeding regime, trough location, and social hierarchy were assumed decisive, as the feeding truck was mixing near one of the tank troughs, likely provoking a temporary accumulation of cows in the tank troughs environment during the observation period. Moreover, when cleaning the referred trough daily, displacements increased, potentially to the next trough, an uncleaned tank trough. These factors are assumed to account for a higher number of drinking episodes at tank troughs than valve troughs, respectively, at uncleaned than daily cleaned troughs (Burkhardt et al., 2022).

4. Effect of Climatic Condition, Type of Trough and Water Cleanliness on Drinking Behavior in Dairy Cows

Burkhardt, F. K., J. J. Hayer, C. Heinemann, and J. Steinhoff-Wagner. 2024. Effect of Climatic Condition, Type of Trough and Water Cleanliness on Drinking Behavior in Dairy Cows. Animals. 14(2): 257. https://doi.org/10.3390/ani14020257

FKB, JJH and JS-W conceived the idea and design of the experiments. **FKB** conducted the on-farm experiments, and collected and analyzed the data. JJH and **FKB** conducted the laboratory analysis of the microbiological water quality. **FKB** led the writing and JJH, JSW, respectively CH led the editing of the manuscript. All authors approved the manuscript.

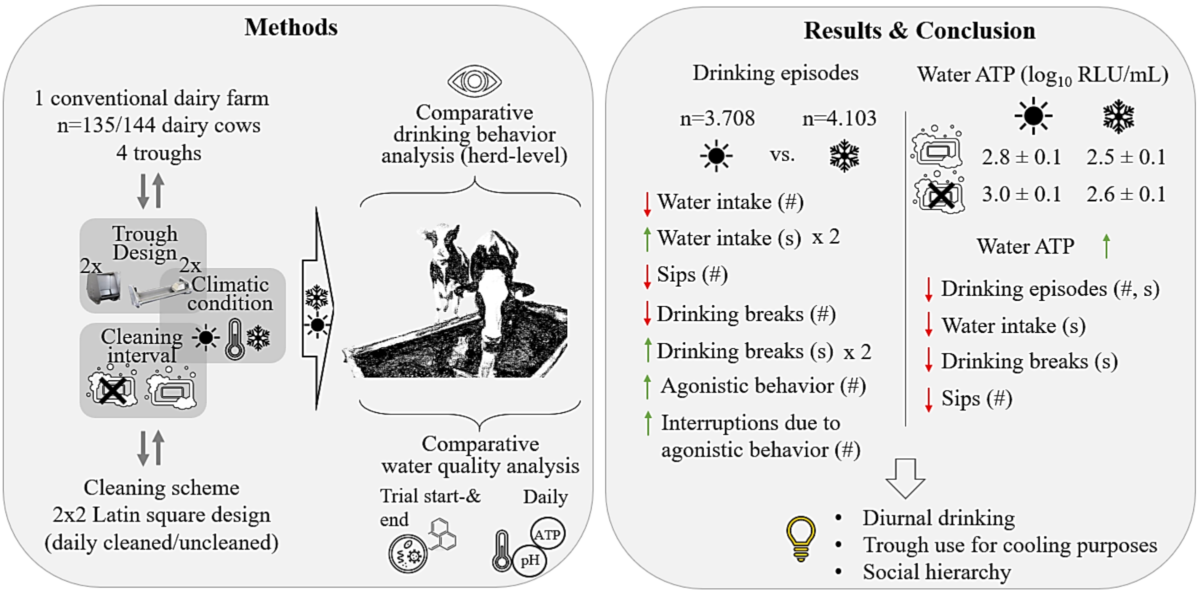

Figure 10: Graphical abstract of **Publication 2**, illustrating the study design under presentation of the methods used, results and main conclusions. Core element is the comparative behavior and water analysis on a herd level in two climatic conditions (cold and moderate warm ambient temperatures), considering the influence of the trough design and cleaning interval.

Summary

Increasing temperatures lead to declining freshwater availability while the risk of waterborne diseases rises. Simultaneously, livestock water requirements are expected to triple. Therefore, on-farm water management strategies are needed, to recognize and react to early management environmental or inter-animal-induced signs of stress, thereby supporting the animals' adaption mechanisms and maintaining animal health, welfare, and performance.

Thus, **Publication 2** aimed to comparatively analyze dairy cows' drinking behavior (n = 8081 drinking episodes) and trough water quality in two climatic conditions (cold and moderate warm ambient temperatures) under practical farm conditions, considering trough type and cleaning interval. Therefore, the data set from **Publication 1** was completed with new data of the same scenario under moderate warm ambient temperatures. Thereby, the hypothesis should be tested that the drinking behavior is altered by the interaction of climatic conditions, trough designs and cleanliness.

At a commercial farm with a herd size of n = 135 (cold ambient temperatures), respectively 144 dairy cows (moderate warm ambient temperatures), following a 2 x 2 Latin square design, were allowed to freely choose between a daily cleaned and an uncleaned tank trough (70 L), a daily cleaned and an uncleaned double valve trough (variable volume, 5 – 15 L) during four consecutive15 d trials (two trials under cold ambient temperatures, two under moderate warm ambient temperatures). Drinking behavior was video-recorded daily for two hours after feeding. At the start and end of each trial, the biological and physicochemical water quality was analyzed. On a daily basis, the trough water temperature, pH, and ATP value were monitored.

Warm ambient temperatures amplified the soiling in troughs of neglected trough cleaning. At uncleaned troughs, trough water ATP values were highest, and CC and TVC exceeded reference values under moderate warm ambient temperatures. Further, neglected cleaning during warm ambient temperatures led to shorter drinking episodes, longer but fewer water intake periods, longer drinking breaks, and fewer sips compared to cleaned troughs, respectively this scenario at cold ambient temperatures. Further, risks for agonistic behaviors at tank troughs were higher at warm than cold ambient temperatures. Nevertheless, against the assumption, fewer drinking episodes were registered under warm ambient temperatures. In this context, the diurnal feeding rhythm of cows is assumed to be decisive in the frequency of the water troughs used, as the study investigated dairy cows' behavior during the same time of the day for two hours after the morning feeding under cold and moderate warm ambient temperatures. However, cows shifted

drinking times in dependence of seasonal changes in ambient temperatures, as described by several authors.

Under practical conditions, considering the effects of the trough design, cleanliness, location and potentially influencing management practices, the number of drinking episodes, the water intake periods and drinking breaks in number and duration, the number of sips, and the number of agonistic behaviors might be valuable indicators optimizing dairy cow water supply and hygiene management (Burkhardt et al., 2024).

5. Association between individual animal traits, competitive success and drinking behavior in dairy cows after milking

Burkhardt, F. K., R. Wahlen, J. J. Hayer and J. Steinhoff-Wagner, 2025. Association between individual animal traits, competitive success and drinking behavior in dairy cows after milking. Animals 15(4): 534.

FKB and JS-W conceived the idea and design of the experiments. RW conducted the on-farm experiments, as well as collected the data. **FKB** supported in the data collection and analyzed the data. **FKB** led the writing and JJH, JSW, respectively RW led the editing of the manuscript. All authors approved the manuscript after critically contribution.

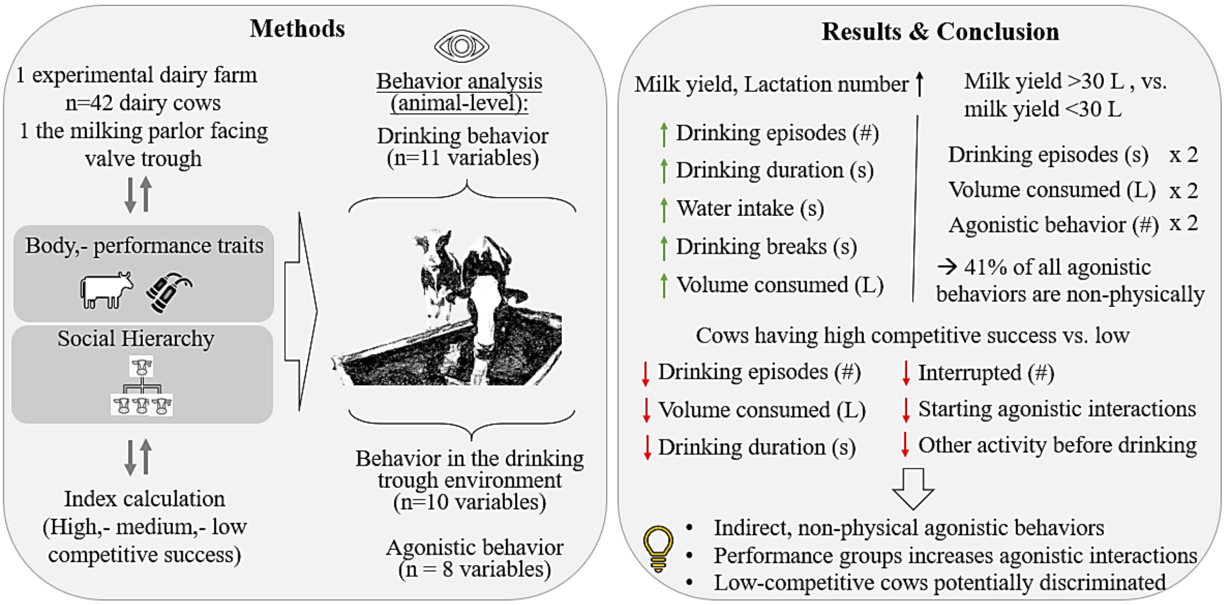

Figure 11: Graphical Abstract of **Publication 3**, illustrating the study design under presentation of the methods used, results and main conclusions. Core element is the extended behavior analysis on an individual animal level, considering the influence of body and performance traits, respectively the social hierarchy.

Summary

Dairy cows' demand for water is particularly evident after milk secretion, as milk consists of an average of 87% water. Therefore, troughs next to the milking parlor are expected to be mostly frequented for water intake and thus, provoke particularly frequently competitive behavior. Thus, **Publication 3** aimed to characterize dairy cows' individual behavior at water troughs next to the milking parlor, including drinking and agonistic behavior and activities after milking. Especially, body and performance traits and social hierarchy should be considered. On an experimental farm with 42 lactating dairy cows, the behavior after milking (n = 33 behavioral variables) in the drinking trough environment of each individual cow was video recorded twice daily during 22 milkings. Animals were weighed and measured regarding body size before the trial. Performance traits were extracted from the herd register of the experimental farm. Competitive success was determined for each individual using a literature-based index calculation.

Body and performance traits affect the individual drinking behavior: the higher the milk yield and lactation number, the more drinking episodes and water consumed, and the higher the total duration of drinking, of water intake, and drinking breaks. High-yielding cows (> 30 L) drank approximately twice as much, as long, and were approximately twice as often involved in agonistic interactions compared to low-yielding cows (< 30 L). Consequently, more troughs are needed in high performance groups to minimize competition. Competitive interactions comprise 41% non-physical contacts, leading in 7.6% of interactions to interruptions. Cows with high competitive success most frequently started agonistic interactions, were less interrupted while drinking, and consequently, drank in average 30 s shorter. Furthermore, they drank numerically less in fewer drinking episodes at the trough next to the milking parlor than cows with low competitive success. Nevertheless, cows with low competitive success drank more frequently after pursuing another activity first.

To summarize, dairy cows' drinking behavior differed between individuals, reflecting the herds heterogeneity in terms of body and performance traits. The higher the individual milk production, the more frequently visited cows the trough, the longer drank cows, respectively displayed the water intake periods and drinking breaks, and the more water was consumed. Agonistic interactions were not only displayed in direct physical contact. Of all agonistic interactions, 41.0% were indirect ("staring" or "ignoring"), causing 7% of all drinking interruptions. Predominantly, cows with high competitive success started agonistic interactions.

Cows with low competitive success were interrupted more frequently (low success, mean: 2.0 ± 1.2 times vs. high success, mean: 1.4 ± 1.1 times) and, therefore, needed several attempts to fulfill their water demand. This study highlighted the need to take physiological needs and individual differences into account in recommendations for water supply (Burkhardt et al., 2025).

6. General discussion and conclusion

6.1. General discussion

Given dairy cows' exceptionally high demand for water due to their milk production-related water loss, and potential limitations in their capability to access water directly can lead to tremendous welfare impairments. The German Welfare Act lays down the general purpose of legal regulations for animals as to „protect life and well-being of animals out of human responsibility for them as fellow creatures“ (TierSchG, 1972). In order to fully assess and ensure animal welfare in dairy cows, monitoring physical, health, and animal behavior – and thus their affective state – is necessary in addition to good housing and management. Therefore, on-farm animal welfare assessments using animal-related indicators are explicitly demanded (EFSA, 2012). Despite the essential role of water in the maintenance, health, and welfare of dairy cows, no specified legal regulation exists concerning dairy cows' water supply management based on animal-related indicators. The recording of behavioral parameters offers several advantages over health and physiological parameters.

Most importantly, behavioral observations are non-invasive and minimize stress or disturbances (Whitham and Miller, 2016). Further, these observations enable direct assessments of the animals' well-being (EFSA, 2012), both positive and negative (Lagerkvist et al., 2011; Whittaker and Marsh, 2019). They are potentially automatically monitorable, reducing subjectivity and increasing feasibility (Whittaker and Marsh, 2019). Hence, the overall objective of this thesis was to find a range of behavioral variables in dairy cows at water troughs to adequately assess water supply management using animal-related indicators. Thereby, relevant management, environment, and animal-related influencing factors, as well as the applicability under practical farm conditions should be considered. The overall aim was to potentially deriving management measures providing a direct operational added value for farmers. To do so, **Publications 1 and 2** investigated the effect of the climatic condition, the trough design, and the trough cleanliness on the biological water quality on 13 drinking behavior parameters (Burkhardt et al., 2022, 2024). **Publication 3** examined the impact of body and performance traits and social hierarchy on 33 behavioral parameters at water troughs, with special emphasis on agonistic interactions (Burkhardt et al., 2025). Hence, the thesis provides a total of n = 33 drinking-related behavioral parameters to assess dairy cows' water supply. The following chapter discusses existing resource, management, and animal-based recommendations for dairy cows' water supply, combining published literature and results of derived publications. Thereinafter, dairy cows’ behavior at the trough, representing potential animal-related indicators, and influencing factors are discussed. The final section provides a conclusion and outlook.

Recommendations for dairy cow water supply

On a national level, some primary resource-based recommendations exist regarding water supply constructions. The KTBL (2020), QM-Milch (2022), and Welfare Quality® (2009) require at least six cm of water surface per cow. Regarding bowl troughs, one bowl for 10 – 15 animals is recommended. Bowl troughs are therefore rated almost equally in their adequacy compared to tank troughs comprising one-meter length (100 cm / 6 cm = 17 animals). Moreover, following these recommendations, bowl troughs are even favored over tank troughs comprising less than 100 cm trough length. The water depth is not considered, although it became apparent from **Publication 1 & 2** that dairy cows favor tank troughs with a higher depth (Burkhardt et al., 2022, 2024). Tank troughs not only enable a more natural water intake with less swallowing difficulties, but cows additionally seemed to use troughs for cooling. The trough use for cooling purposes was reflected in the length of displayed periods of water intake. Water intake periods were characterized as cows having direct water contact, which was approximately doubling in the trial periods under warmer ambient temperatures (Burkhardt et al., 2024). According to available recommendations, however, farmers would most likely decide in favor of bowl troughs by economic reasons, as these costs approximately 82 Euros compared to 935 Euros for a tank trough of one-meter length (Suevia Haiges GmbH, 2023). Current recommendations do not consider the potential economic losses caused by a reduced milk yield or enhanced metabolic diseases due to insufficient water intake resulting from inadequate trough designs.

Moreover, compared to the feed space allocation comprising 36 – 61 cm per animal (Erickson and Kalscheur, 2020) under consideration of the elaborated significance of water for lactating dairy cows, the ratios appear unequal. The 1:1 animal-to-feeding space ratio reduces competition (Hetti Arachchige et al., 2014). However, as indicated in **Publication 1 and 2**, tank troughs are prone to agonistic behaviors while predominantly used for water consumption (Burkhardt et al., 2022, 2024).

Moreover, groups of high-yielding cows are particularly susceptible to agonistic behaviors. High-yielding cows (producing > 30 L milk/d) drank twice as much, as long, and were twice as much involved in agonistic interactions **(Publication 3)** (Burkhardt et al., 2025). Hence, an insufficient number or length of water troughs can jeopardize animal welfare, especially in those groups. Thereby, following Jensen and Vestergaard (2021), not only the number but also the distance of troughs must be adequate, as increasing the number of water troughs alone does not automatically increase drinking frequencies in troughs placed next to each other, as investigated by Andersson (1987). In the study scenario of **Publication 1 & 2**, a few dominant cows seemed to block tank troughs, limiting the access for low-ranked cows, as indicated in **Publication 3** (Burkhardt et al., 2022, 2024; Burkhardt et al., 2025). Thus, visits to areas other than the water troughs after milking, as displayed predominately by low-ranked cows, are used as an indicator of the water supply in **Publication 3** (Burkhardt et al., 2025). Due to the high water loss by milk excretion, regular milking times most likely lead to synchronized thirst peaks for all animals, severely restricting animal welfare if drinking access is limited by space or social behavior. Therefore, water provision during milking may be a suitable solution to allow individual animals to drink without social interruptions. Studies demonstrated that water restriction has a highly negative effect on dairy cows' milk yield (Hötzel et al., 2003; Thokal et al., 2004), health (Benatallah et al., 2019), and behavior (Williams et al., 2017). Enabling animals’ access to water during milking, and thereby guaranteeing an uninterrupted water intake at least twice a day per individual may lead to animal health, welfare, and performance benefits. Concentrate feeding in AMS, salt-lick-stones in conventional milking systems, and a lack of water troughs in the waiting area may per se increase dairy cows' water demand. Still, considering the water loss due to milk excretion, combined with those factors most likely increases the water demand before, during, and after milking. Considering the aspects mentioned above, current recommendations of water supply seemed not target orientated, highlighting the need for studies explicitly addressing the determination of requirements regarding the water provision on-farm.

Regarding the trough construction, recommendations are based on water troughs' functioning, dimensions, and materials. Reflecting dairy cows' natural behavior while drinking, water depths of at least 60 mm are recommended to allow the lips to fully submerge and prevent air from flowing through the mouth while drinking (Schönholzer, 1958). Due to the variable volume of valve troughs, water depth may fall below 60 mm. Further, following Jensen and Vestergaard

(2021), muzzle-operated troughs rest the water intake speed, as represented by valve troughs or equivalent alternatives (e.g. bowl troughs). Those trough types are frequently used in dairy barns located in Western Germany (Suevia Haiges GmbH, 2023), as investigated by Hayer et al. (2022a). **Publication 1** described that the interruption of vacuum forming during drinking when pressing the valve might be causing the observed high number of swallowing difficulties at valve troughs compared to tank troughs in **Publication 1 & 2**, thus making tank troughs preferable. As expected, cows drank more frequently from the tank than from valve troughs. Findings that cows prefer higher volume troughs over smaller volumes are in line with Filho et al. (2004), even though compared water volumes are relatively large for Western European standard water troughs (Burkhardt et al., 2022, 2024). However, water levels are recommended to be at least 80 cm, and stainless steel for trough material focuses on minimizing contaminations, not on the nature of the animal in drinking. Understanding animal welfare in specific situations demands investigations of the animal's behavior in natural environments. These approaches provide insights into mitigating environmental stressors and support ethical guidelines, contributing to animal health and welfare.

Management-based recommendations for water supply are limited to daily trough cleaning for minimizing bacterial contamination in water troughs (Welfare Quality®, 2009). The "good feeding practice" section of the Feed Hygiene Regulation (EC) No. 183/2005 also demands constructing a bacteria reducing drinking environment. However, so far, no study has investigated the impact of neglected trough cleaning on water quality or dairy cows' behavior. Investigating the status quo of dairy water troughs' hygiene status, Hayer et al. (2022b) rated the inside of 43.8% of cattle troughs as "heavily soiled." Comparing their results regarding CC and *E-coli* counts with the results of LeJeune et al. (2001a), they stated comparability of the hygiene status in US and German farms. Moreover, Hayer et al. (2022a) indicated shorter trough cleaning intervals to be associated with higher odds for *E-coli* presence in water troughs. This is in line with findings of Smith et al. (2008), demonstrating higher *E-coli* counts in troughs emptied the week before compared to later visits.

Valve troughs form a type of bacteria-reducing drinking trough as they have a self-cleaning effect due to the low drinking volume and frequent rinsing. Accordingly, as shown in **Publication 1**, lower bacterial burdens were observed in valve troughs compared to tank troughs (Burkhardt et al., 2022). In line with this finding, Hayer et al. (2022b) found that the risk for increasing coliform counts increased with enhanced trough volume. Following the

literature, contamination with enterobacteria in water troughs is attributable to the water troughs' proximity to the feed bunk, the troughs' protection from sunlight, the minimization of protozoa, and high ambient temperatures (LeJeune et al., 2001a; Hayer et al., 2022a).
Contrary to these findings, as described in **Publications 1 & 2**, no *E-coli* were detected under warm ambient temperatures, but in both cleaned and uncleaned troughs under cold ambient temperatures (Burkhardt et al., 2024). However, these findings are attributable to the punctual analysis of the microbial water quality solely at the start and end of the trials. Daily rapid testing of the trough water ATP values showed significantly higher values at warm ambient temperatures, respectively uncleaned troughs, than at cold ambient temperatures **(Publication 2)**. The author recommends frequent water analysis using rapid tests based on these findings, as cows are susceptible to feed or water quality impairments even if reference values were not exceeded, as demonstrated by **Publication 1** (Burkhardt et al., 2022). These findings emphasize the need for preventive measures. Additionally, neglecting cleaning had a major impact on the animals' behavior, as agonistic behaviors doubled at cleaned tank troughs. This led to the inverse effect of a higher number of drinking episodes at uncleaned troughs, as described in **Publication 1 & 2** (Burkhardt et al., 2022, 2024). The role of agonistic behaviors has already received attention from several authors and, thus, been included as an indicator for evaluating the water supply by KTBL (2020) and the Welfare Quality® (2009).

In average a maximum of five agonistic interactions per cow and hour should be recorded, calculated as the proportion of "the frequency of head butts, displacements, chasing, fighting, and chasing up" out of total social behaviors using an index calculation (Welfare Quality®, 2009). Afterward, the index is score-transformed using index-spline functions and put into relation. However, the on-farm applicability seems complicated. The reason for monitoring requirements of agonistic interactions is the potential animal welfare impairment due to agonistic behaviors characterized by physical injuries, stress, and behavioral changes. Monitoring agonistic interactions can improve herd management by (1) detecting the need for interventions in the group composition of management practices (Krahn et al., 2023) and (2) in resource allocations (Thompson and Green, 2023), as well as (3) the respective effectiveness of interventions (Thompson and Green, 2023). In the literature, authors described agonistic behaviors in the form of direct, physical interactions as replacements (Foris et al., 2019), pushing, butting, pressing, and sniffing (Guzhva et al., 2016; Krahn et al., 2023). As described in **Publication 3,** those behaviors alone do not reflect sufficiently social competition and, thus, exclude relevant, potentially resource limiting behaviors such as threatening, avoiding, or a general mutual awareness between individuals, that lead to behavioral changes (Burkhardt et al., 2024). Following Krahn et al. (2023), the transition from direct, physical interactions to threatening and avoiding (non-physical behaviors) is a phenomenon of established relationships. As part of this thesis, in **Publication 3,** types of agonistics interactions in detail were identified to draw an overall picture of behavioral patterns that potentially led to a limited accessibility of water troughs. Behavioral analyses provide insight into management-related deficits but need to be considered in interaction with each other, an, thus, demand for a broader spectrum of behavioral patterns. Besides physical and non-physical agonistic behaviors, the studies summarized in this thesis identified the number and duration of drinking episodes, water intake periods and drinking breaks, and the number of sips, as reliable indicators providing insights into dairy cows' welfare during water consumption. However, as behavioral analysis proved to be complex and demand for a certain know-how, various indicator assessments limited their indicator pool to a few indicators, that are easy to assess (Flint et al., 2016). An relatively easy approach directly applicable for the farmer may represent a direct observation of the lowest and highest ranking cows for 30 min after feeding. During this time span, cows displayed the most drinking episodes during the two hours recording period starting with morning feeding (Burkhardt et al., 2022), and those indicator animals are usually well-known in most of the dairy farms or easily identified by the farmer. The suitability of the water trough location could be checked by observing if specific troughs are repeatedly used or avoided by the highest or lowest ranking cows. Moreover, the involvement in agonistic interactions may provide insights in the accessibility of a specific trough for all animals. Hereby, observational data potentially displays the necessity of adjusting the number, location, distance or cleanliness of troughs.

Current approaches to assess water supply using animal-related indicators

Characterizing dairy cows' drinking behavior, which is the core element of **Publication 1** regarding drinking-related behavior and **Publication 3** regarding agonistic interactions, formed the basis for extracting reliable behavioral patterns and the subsequent effect analyses. Most studies addressed dairy cows' water supply by investigating their water intake under experimental conditions. However, as Sadrzadeh and Kamyabi (2021) described, measuring the water volume consumed by individuals is impossible under practical-farm conditions with standard tank troughs. Tank troughs allow several animals simultaneously to drink, thus, only measuring the water volume consumed on a herd level might feasible. Offering water during milking may enable individual water consumption measurements. However, in this context, the expression “water intake” seems misleading, as measuring the water consumed using flow meters, automatic waterers, or calibrated scales includes spilled water losses and, thus, must be considered inaccurate. Counting and comparatively analyzing the number of sips per animal may represent an adequate alternative. Firstly Hötzel et al. (2003) described dairy cows taking sips trough “a movement of the animal’s throat swallowing water, while its mouth was submerged” and later adapted in the current studies (Burkhardt et al., 2022, 2024). A positive correlation between the number of sips and the water volume consumed, described in **Publication 3,** supports this finding (Burkhardt et al., 2025). Hötzel et al. (2003) used a counter for tallying sips, while the current thesis recorded sips by video. Liquid intake was also investigated in rodents to better understand thirst using video recordings, specialist lickometers, and sipper devices (Godynyuk et al., 2019). Another more recent method investigated the actual ingested water amount by using bolus telemetry, as discussed in the context of precision livestock farming. Studies indicated that monitoring ruminal temperature and pH helped determining changes in the ruminal fluid (AlZahal et al., 2009), which seemed to mirror water intake. Moreover, a recent device (smaXtec animal care technology®) combines the measurement of drinking cycles and water intake via bolus telemetry with climate sensor data. However, compared to manual devices described as a tool being “less labour intensive, time consuming and cannot result in injuries” (Ammer et al., 2016), health risks due to misapplications or technical defects may exist with every direct animal-based application other than video recordings. So far, Burkhardt et al. (2022) was the first study investigating more detailed behavioral patterns during drinking, such as the type and duration of tasting, the number and duration of water intake periods, drinking breaks, and swallowing difficulties (Burkhardt et al., 2022). It might be advantageous if studies refocus from "technically possible" to "applicable practices at the farm level" and, thus, directly add value for farmers.

However, recent studies have focused primarily on automated evaluable behaviors. The automatic evaluation of behavioral parameters is a novel approach in the field of animal welfare assessment. A fundamental issue regarding such an approach is the technical implementation without knowledge of the basic relationships. Solutions are, therefore, not very robust against influencing factors if these are unknown. Moreover, it faces various additional difficulties, including the complexity of behaviors, technical limitations, accuracy, precision, subjectivity, and variability (Rushen et al., 2012). However, combining different sensor types, such as image analysis tools with accelerometers or GPS data, potentially increases the accuracy of behavior recognition. For example, combining leg mounted and collar mounted accelerometers in cows has improved the differentiation of similar behaviors (Tran et al., 2022). Moreover, providing context for the behaviors observed by incorporating influencing factors, such as environmental data, is crucial for accurately assessing animal welfare (Nielsen, 2022). Validating these systems against traditional methods may also improve the accuracy. Algorithms can be refined, thus improving reliability (Rushen et al., 2012). Traditional methods were direct observation (Genther and Beede, 2013), video recording (Andersson, 1987), electronic water bins (Melin et al., 2005; Ammer et al., 2018; McDonald et al., 2020), radio frequency identification collars (Cardot et al., 2008), or instantaneous scan sampling technics (Coimbra et al., 2012). However, those methods led to variations in the frequency and duration of recorded drinking episodes, attributable to different definitions of the start and end of a respective episode. Authors defined the drink initiation as the time a cow accesses the trough (Andersson, 1987), the cow is in contact with the water surface (Coimbra et al., 2012; Genther and Beede, 2013; Williams et al., 2017) or the identification system used recognizes the cow (Melin et al., 2005; Cardot et al., 2008). As the current thesis aimed to draw an overall picture of dairy cows' behavior at the trough, video recording was considered the appropriate method. However, manually analyzing video recordings is a highly time-consuming and methodological challenging approach. Two hours of video recording, demanded for 2-3 hours of analyzing, summing in at least 480 h of video analysis, considering n = 60 d of trials for **Publication 1& 2**. In **Publications 1 & 2,** the same trained researcher analyzed all recordings, thus no inter-observer shifts of analysis results existed; however, inter-observer reliability was tested and particularly approved in **Publication 3.** Time-lapse cameras were used to meet challenges regarding cost effectiveness, transferability, and storage. Memory cards can be used for data transfer due to the small amount of data, which facilitates data transfer and handling.

Regarding the practicability, a location specific spot sampling analysis by behavioral analysis may provide direct insights of the animals' welfare applicable on-farm, but requires basal information about when to schedule the spot and how to avoid influencing factors. The scope of behavioral observations may be reduced to informative and easy to measure parameters, such as the frequency of the highest or lowest ranking cows visit a specific trough after milking, the duration these cows stay at the trough, the number of sips taken by these cows, or the involvement of selected, easily accessible agonistic interactions.

Dairy cow water consumption in response to various influencing factors

Dairy cows' drinking behavior is complex and needs to be evaluated in the context of specific management, environmental, and animal-related factors, and their interaction. To do so, **Publication 1** aimed to investigate the influence of the drinking trough design and the cleaning interval on dairy cows' drinking behavior under practical conditions. Changes in dairy cows' drinking frequency depending on the dimension and design of water troughs were expected. Cows frequently visited high volume tank troughs (70 L) rather than valve troughs (5 – 15 L). In line with these findings, Taxeira et al. (2006) observed dairy cows to drink more (12.9 vs. 4.2 L/3 min), spend more time drinking (34.6 vs. 10.7 s/3 min), and take more sips (22.5 vs. 6.5 sips/3 min) from a trough with larger surface area. In another experiment investigating the effect of trough height on drinking behavior, cows showed tendencies to drink more (10.9 vs. 7.4 L/3 min) and longer (28.9 vs. 19.6 s/3 min), and to take significantly more sips from the higher than lower trough (19.9 vs. 12.4 sip/3 min). Comparing relatively large troughs, multiparous lactating Holstein cows drank less (0.7 vs. 9.3 L/3 min) in a shorter time (2.4 vs 27.3 L/3min), and took fewer sips (1.6 vs. 17.6 L/3 min) out of a 189 L trough (68 × 126 × 30 cm) than out of a 568 L trough (95 × 139 × 60 cm) (Filho et al., 2004; Teixera et al., 2017). Moreover, the placement of the feeding truck during feed mixing in the direct vicinity of one of the tank troughs potentially led to the highest number of drinking episodes after feeding, regardless of the study scenario. The influence of specific daily adjustable management practices on dairy cows behavior in general was demonstrated for the feeding time, frequency and delivery (DeVries and Keyserlink, 2005; Cardot et al., 2008), the transfer into the calving area (Matamala et al., 2021), the time cows spent on pasture (Wagner et al., 2017), and the regrouping and grouping by performance (Keyserlingk et al., 2008). Moreover, as described in Figure 5, dairy cows showed drinking-related behavioral responses to the feed composition (Cardot et al., 2008), the degree of manure contamination of the drinking water (Schütz et al., 2019), and the stocking density (Andersson, 1987). Further, as described in **Publication 1 & 2,** dairy cows altered their behavior depending on the trough cleanliness. The results highly indicate dairy cows' behavioral responses on the cleaning interval, supporting elaborated recommendations for daily trough cleaning, especially under warm conditions.

Additionally, in **Publication 1 & 2**, the placement of the tank troughs near the milking parlor can be assumed to have a major influence on the drinking frequency at these troughs. The depending on the cleaning interval: when cleaning the most visited tank trough daily, agonistic interactions doubled, potentially leading to displacements to the nearest trough- an uncleaned tank trough. Consequently, contrary to expectations, more drinking episodes were registered at uncleaned troughs than cleaned troughs, regardless of the climatic condition (cold or moderate warm ambient temperatures). Neglected trough cleaning under cold ambient temperatures resulted in greater fluctuations in the ATP values but resulted in comparable values in daily cleaned and uncleaned troughs (Burkhardt et al., 2022). As hypothesized, moderate warm ambient temperatures led to greater soiling, significantly higher ATP values, and reference values exceeding CC and TVC counts at 20 °C in uncleaned troughs (Burkhardt et al., 2024). As ATP values showed no linear course but fluctuated in all trials, it would be advisable to obtain a more holistic picture of bacterial growth due to neglected trough cleaning and, thus, to gain insights into the development and displacement of bacterial cultures among each other. Bacteria form complex microbial communities through direct physical contact, metabolic exchange, biofilm formation, and genetic material exchange (Kolenbrander et al., 2006; Baudy et al., 2021; Hayer et al., 2022a), and, inter alia, can further stimulate *microalgae* aggregation (Zhao et al., 2020). Thus, bacterial interactions play a crucial role in the bacterial burden and diversity of water environments. Few studies have addressed the bacterial burden in dairy cows' water troughs, even though it is expected that high bacterial burdens may cause welfare and health issues (LeJeune et al., 2001b; Hayer et al., 2022b). However, studies found gram-negative bacteria, CC, and *streptococci* in bedding materials, as well as the microbial composition of dairy cows' rumen linked to mastitis (Hogan et al., 1989; Zhong et al., 2018). Water quality fluctuations may change the rumen pH value and thus lead to changes in dairy cows' health and behavior (Sanftleben, 2014).

Further, some authors investigated dairy cows' preference for clean vs. manure-contaminated water, indicating a clear preference for clean water (Willms et al., 2002; Schütz et al., 2019). In line with these findings, at warm ambient temperatures, cows spent more time at cleaned troughs, displayed more extended periods of water intake, and, thus, stayed longer in contact with the water. To summarize, assessing water cleanliness using behavioral parameters is possible, but the interaction effects must be considered. Therefore, as discussed in **Publication 2,** it is insufficient to account for drinking frequencies, but detailed behavioral changes depending on the trough location should be considered (Burkhardt et al., 2024). As demonstrated in **Publication 1 & 2** the analysis of specific, potentially appearing secondary behavioral patterns may be significant, for example illustrated by changes in the duration of water intake periods which is dependent on the ambient temperature or swallowing difficulties at specific trough designs.

Warm ambient temperatures not only increase the bacterial burden in water troughs (LeJeune et al., 2001a; van Eenige et al., 2013; Hayer et al., 2022b) but also lead to a higher water demand in dairy cows (Nardone et al., 2010; Rojas-Downing et al., 2017). Several authors investigated water intake and drinking behavior under heat stress conditions and indicated increased water consumption-related behaviors with rising temperatures (Matarazzo et al., 2003; Laínez and Hsia, 2004; Meyer et al., 2004). Dairy cows' behavior under a moderate warm climate, as it prevails most of the year in Western Europe, has not yet been the subject of research. However, as dairy cows thermoneutral zone ranges at - 5 – 21 °C, moderately warm temperatures in late summer leading to behavioral changes in dairy cows drinking behavior was assumed. In general, a higher number of drinking episodes in moderate warm than cold ambient temperatures were hypothesized. Against the assumption, more drinking episodes were observed under cold than warm ambient temperatures. The reason for this could be dairy cows' diurnal feeding and drinking rhythm, that are controlled endocrinologically and regulated by internal timekeeping mechanisms. These circadian clocks allow mammals to adapt to environmental changes (Casey and Plaut, 2022).

Consequently, as described in **Publication 2**, cows shifted their drinking times to cooler times, e.g., early morning, late afternoon, and night (Ray and Roubicek, 1971; Cardot et al., 2008). As the present thesis focused on a detailed but, therefore, highly time-consuming analysis of drinking, and related behavior using 13 (**Publication 1 & 2**) and 33 (**Publication 3**) behavioral parameters, a limitation of the recording period to a specific period was needed. Therefore, daily shifts in drinking behavior could not be displayed. However, a general adaptive alliteration of the drinking behavior in the cold and moderate warm ambient temperatures was observed: under moderate warm ambient temperatures, cows doubled the time spent in contact with the water, e.g., the duration of water intake periods, without visibly drinking more, respectively taking more sips. Thus, they changed their behavior from short, altering periods of water intake and drinking breaks under cold ambient temperatures to less but longer respective periods under warm ambient temperatures (Burkhardt et al., 2024). Changes in behavioral patterns in moderate warm ambient temperatures could demonstrate early signs of adaption mechanisms due to climate dependent discomfort with rising temperatures. This is in line with observations of McDonald et al. (2020), indicating, on the one hand, that particularly low-ranked cows shifted drinking times at the trough in times of lower competition, and on the other hand, the use of troughs by cows for cooling purposes the higher the ambient temperatures.

Moreover, they observed some high-ranked cows blocking water troughs for cooling. This is in line with observations described in **Publication 2** that cows stayed longer in direct contact with the water, represented by extended water intake periods with relatively consistent total drinking times under warm ambient temperatures (Burkhardt et al., 2022). McDonald et al. (2020) summarized that shifting behavior may be a helpful indicator of when to provide cooling. Yadav et al. (2021) found specific physiological parameters to alter and first signs of discomfort at ambient temperatures of 25 °C. More research in recognizing earlier signs regarding discomfort due to rising ambient temperatures and, thus, implementing earlier heat stress preventing measures for dairy cows could help minimize discomfort on a herd level, thereby counteract discrimination of low-ranked cows and improving animal welfare.

A holistic approach to an animal-related welfare assessment demands the consideration of welfare on a herd and an individual-animal level. Monitoring differences in individual behavior in dependence on social hierarchy helps to identify potential resource or management-related risks for the discrimination of individual animals. Therefore, **Publication 3** is based on a study conducted under experimental conditions. This enabled the inclusion of individuals' specific body and lactation characteristics in the behavioral analysis, which was later considered in the context of the social hierarchy (Burkhardt et al., 2025).

Particularly in the context of social behavior in association with high ambient temperatures, it is essential to consider animal welfare at the individual animal level, as dairy cows are known to compete for resources as water troughs, and thus, water intake can be limited for animals with low competitive success (McDonald et al., 2020). For a more detailed examination of this phenomenon, **Publication 3** addressed the animal individual behavior at the water trough under warm ambient temperatures at a milking parlor facing trough after milking. It was assumed, that drinking-related behavior and social, respectively competitive interactions would be particularly prominent at this trough. The reason for this assumption was the far-reaching recommendation of placing water troughs of adequate size close to the milking parlor, as dairy cows have to compensate for water loss through milk secretion after milking and, apart from feeding times, have the highest water intake after milking (Beede and Collier, 1986; Andersson, 1987; Cardot et al., 2008).

As expected, the experimental herds' heterogeneity regarding body and performance traits was reflected in the individual drinking behavior, which varied considerably (Burkhardt et al., 2025).

One reason for this might be the variation in milk-related traits of the experimental herd. Cows displayed a range of 304 d in days in milk, a range of 6 lactations in lactation numbers, and a range of 32.4 L in milk yield. In a 50:50 ratio, experimental cows gave above and below 30 L daily milk. Due to the high water content of milk, dairy cows' water demand depends, as described in the previous chapters, on their milk performance, physiological state, and age. Following Vijayakumar et al. (2017), lactation number correlated positively with the milk yield, and dairy cows reached the maximum milk yield in their third lactation. Accordingly, cows in the third lactation would have the highest water demand. **Publication 3** describes a positive correlation between milk yield and lactation number, the number and duration of drinking episodes, water intake periods and drinking breaks, and the water volume consumed (Burkhardt et al., 2025).

Krahn et al. (2023) reviewed the concept of social dominance and different methods to categorize individuals within the herd by agonistic interactions. They distinguish between three levels of dominance relationships: (1) between a pair of individuals, (2) between individuals within a group, and (3) the individual within the dominance structure. A widely applied method, also used in **Publication 3,** is calculating the competitive success of individuals by the proportional success in competitive interactions (Burkhardt et al., 2025). Even though called the "dominance index," it rather represents an "agonistic success index," as individuals' identity in each interaction is not considered. Therefore, it rather is used to illustrate agonistic success when distinguishing between individuals is impossible (Krahn et al., 2023). As the study scenario of **Publication 3** intended to illustrate dairy cows' social behavior at the water trough after milking and exhibit a limited time and spatial design, this index was considered appropriate to display competitive success at the drinking trough. Considering body and performance traits in the context of competitive success, as described in **Publications 3,** no significant correlations of body traits, but only milk production-related traits were found (Burkhardt et al., 2025). This is in contrast with earlier studies, indicating a positive correlation between body weight and body condition score with the social hierarchy of dairy cows (Andersson et al., 1984; Hohenbrink and Meinecke-Tillmann, 2012; Burkhardt et al., 2025). However, the experimental Brown Swiss herd used in **Publication 3** varied less in body traits (8.8 – 39.8 %) than in milk production-related traits (53.1 – 85.7 %). As discussed earlier, concerning correlation analyses between body traits and water intake, breed-specific differences are likely influential, as different dairy breeds vary in body weight (Velayudhan et al., 2022), energy partitioning (Bines and Hart, 1978), and in their genetic constitution. Genetic markers, such as copy number variations (CNVs) associated with traits related to body size, fertility, and milk yield, showed differentiation among various cattle breeds (Xu et al., 2016). Given the potential crucial influence of dairy cows' breeds in different behavioral patterns, cross-breed studies addressing animal behavior in the context of specific influencing traits are needed. Comparing the drinking behavior characterization of **Publication 1** (n = 4103 drinking episodes, mean ± SD of all drinking episodes) with the results of **Publication 3** (n = 467 drinking episodes, mean ± SD per cow) indicate, that Holstein-Friesian drink longer (123 ± 90 s vs 78.4 ± 47.5 s), but take less sips than Brown Swiss cows (20.0 ± 17 vs. 52.4 ± 31.9 sips). Studies investigating breed-specific behavioral differences are rare, and no studies have addressed differences in water consumption depending on the cattle. However, breed-specific differences exist regarding physiological traits (Hagen et al., 2005), body traits (Dippel et al., 2009), milk performance traits (Bobić et al., 2014), dietary invention (Noel et al., 2019), and social behavior (Keyserlingk et al., 2008). Given the breed-specific change of the experimental animals from Holstein-Frisian cows to a herd of Brown Swiss cattle, it is highly probable that this has an influence per se on drinking behavior and, in particular, on competitive interactions of the individuals among each other (Keyserlingk et al., 2008).

In the context of competitive success, cows with high competitive success predominately using the trough next to the milking parlor was expected. Even though this hypothesis was not confirmed, cows with low competitive success rather pursued another activity before drinking (Burkhardt et al., 2025). Interpreting this phenomenon as cows “do wait” for free access seems speculative. However, a certain shift in drinking times to avoid competition under hot weather conditions was described by McDonald et al. (2020). Nevertheless, cows lose up to 34% of their total water intake during milk excretion (Murphy, 1992; Beede, 2005), so they feel most thirsty after milking. Thus, it is very unlikely that cows are intrinsically motivated to engage in another activity other than drinking after milking. Therefore, further investigation is required into the underlying causes, which may be related to social behavior.

Moreover, contrary to expectations, cows with high competitive success drank less and, on average, 30 s shorter than cows with low competitive success. Notably, cows with high competitive success got fewer interruptions from drinking by agonistic interactions but started those more frequently. When considering the ratio of drinking duration and water volume consumed (all cows: 0.11 L/s, high success: 0.12 L/s, medium success 0.11 L/s, low success 0.09 L/s), as discussed in **Publication 3**, it is noticeable that low-yielding cows are potentially discriminated and need more attempts to fulfill their demand (Burkhardt et al., 2025). Findings from **Publication 1** that a few high-ranked cows potentially displace lower-ranked cows from cleaned to uncleaned troughs support this assumption. Additionally, **Publication 3** indicates that cows with high competitive success started twice as often agonistic interactions, leading to interruptions for 72% of the herd during drinking, of which 83% left the observation zone at least once. Based on these observations, conclusions about overall animal welfare cannot be drawn by simply measuring the amount or duration of water intake at specific water troughs; instead, an interaction of various factors and behaviors needs to be considered to assess animal welfare at the farm level. However, current studies primarily focus on behavioral patterns that are automatically monitorable in the current state of technology; however, interaction effects are rarely included, highlighting the deficiencies of these technologies. Therefore, developing technologies combining behavioral analysis with specific interaction effects is highly desirable. A first approach presents the smart sensing system Tang et al. (2021) developed. Combining radio-frequency identification readers, motion detectors, water level and flow meters, as well as temperature sensors, it enables the recording of the animals' water intake, drinking frequency, the duration per visit and day of each animal, the water temperature, possibly extended to water pH, turbidity and mineral levels in future.

6.2. General conclusion

In conclusion, adequate, high-quality water provision is an essential foundation for dairy cows' welfare, health, and performance. In the current thesis, the feasibility of using behavioral parameters for an animal-based assessment of dairy cows' water supply management was tested and rated as valuable. Still, it demands the consideration of interacting behavioral patterns and influencing factors, as drinking behavior changes depending on several factors such as trough design, water quality, or availability. The number and duration of drinking episodes, water intake periods and drinking breaks, the number of sips, and agonistic interaction, including non-physical interactions, were identified as reliable indicators providing insights into dairy cows' welfare during water consumption. In contrast to assessments of the water intake, behavioral assessments have been proven to be highly sensitive to changes in the trough design and cleanliness and, therefore, have a high potential for implementation in welfare assessments. Nevertheless, especially in research settings, interactions with each other, with specific management practices, and the trough design and placement need to be considered. However, the on-farm behavioral observation demands a certain know-how in behavioral analysis, time, and the technical resources.

Solutions directly applicable to gain initial insight into the welfare of the animals at the drinking trough could be to analyze the behavior of the lowest and highest-ranking cows in the first 30 min after feeding, as, in general, management deficiencies in the water supply primarily endanger the well-being of lower-ranking animals. Those are, therefore, particularly worthy of protection.

Mainly, high-yielding cows interact agonistically at the trough, and therefore, grouping in performance groups highly demands adequate trough accessibility, ensured by water in sufficient quantity and distance of troughs. Limited accessibility of troughs due to agonistic interactions is not exclusively apparent by direct, physical interactions but as a result of a highly complex hierarchical structure, which is characterized inter alia by avoidance, ignoring, and staring at others. First signs of welfare impairments may be feasible to recognize by installing flow meters for the detection of changes in the water consumption on a herd level. Future studies should investigate technological approaches that combine monitoring a wide range of behavioral parameters and simultaneously measuring specific influencing factors.

6.3. References

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8. Appendix