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Master Thesis

(In)consistency of body language in dairy cattle

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Ronja LANDVOGT, BSc

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Supervisor:

Univ.Prof. Dr.med.vet. Christoph Winckler
Division of Livestock Sciences
Department of Sustainable Agricultural Systems

Co-supervisors

Dr. Sara Hintze

Division of Livestock Sciences
Department of Sustainable Agricultural Systems
University of Natural Resources and Life Sciences Vienna

Prof. Linda Keeling

Department of Animal Environment and Health
Swedish University of Agricultural Sciences

Affidavit

I hereby declare that I have authored this master thesis independently, and that I have not used any assistance other than that which is permitted. The work contained herein is my own except where explicitly stated otherwise. All ideas taken in wording or in basic content from unpublished sources or from published literature are duly identified and cited, and the precise references included.

I further declare that this master thesis has not been submitted, in whole or in part, in the same or a similar form, to any other educational institution as part of the requirements for an academic degree.

I hereby confirm that I am familiar with the standards of Scientific Integrity and with the guidelines of Good Scientific Practice, and that this work fully complies with these standards and guidelines.

Vienna, 22.08.2022 Ronja LANDVOGT *(manu propria)*

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Abstract

Good welfare of farmed animals is of increasing importance in society. In this context, animal behaviour and body language are promising indicators of animal welfare, including (positive) affective states. As a prerequisite for reliable assessments, this master thesis focuses on the occurrence and consistency of different measures of body language of dairy cattle, which may in future be used in on-farm animal welfare assessment protocols. We investigated consistency on individual animal as well as on herd level with regard to time periods between observations (short - from one day to the next, medium - 2-3 days later, long - 11-17 days later) and age category (cow, heifer, calf) while also taking basic body position (lying, standing) into account. An ethogram describing the body language of dairy cattle was developed and inter-observer reliability tested and proven. Over a period of 6 weeks, 4 observers performed live observation rounds of 15 observation cycles per animal and day in 24 cows, 13 heifers and 12 calves of the Holstein (HF) and Swedish Red and White (SRB) breed. In total 5,491 and 2,126 one-min observation cycles were recorded for lying and standing animals, respectively. Occurrences and consistencies of several postures and behaviours differed across basic body positions and age categories. However, the length of the time periods between observations did not systemically affect consistency. This study may serve as a reference for further investigations of body language in dairy cattle. For future on-farm animal welfare assessment purposes, sub-sampling of age categories and animals in different body positions is recommended. Due to unchanged (in)consistency of many postures over different time periods it is however irrelevant when observations take place. To obtain reliable data, multiple observation days should be considered to fully reflect the body language of dairy cattle.

Kurzfassung

In unserer Gesellschaft werden Forderungen nach höheren Tierwohlstandards immer häufiger. Verhaltensforschung kann hierbei wertvolle Aussagen liefern, auch über den mentalen Zustand von Tieren. Als Voraussetzung für zuverlässige Beurteilungen thematisiert diese Masterarbeit die Häufigkeit des Auftretens und die Konsistenz der Körpersprache von Milchkühen, die in Zukunft in Protokollen zur Beurteilung von Tierwohl in landwirtschaftlichen Betrieben verwendet werden könnten. Die Konsistenz wurde sowohl auf Einzeltier- als auch auf Herdenebene, in Bezug auf verschiedene Zeiträume zwischen den Beobachtungen (kurz – von einem Tag zum nächsten, mittel - 2-3 Tage später, lang - 11-17 Tage später) und Alterskategorien (Kuh, Färse, Kalb) untersucht, wobei auch die Grundkörperhaltung (liegend, stehend) berücksichtigt wurde. Dazu wurde ein Ethogramm entwickelt und die Übereinstimmung zwischen Beobachterinnen bestätigt. Über einen Zeitraum von 6 Wochen führten 4 Beobachterinnen Live-Beobachtungsrunden mit 15 Beobachtungszyklen pro Tier und Tag bei 24 Kühen, 13 Färsen und 12 Kälbern der Rassen Holstein und Swedish Red and White durch. Insgesamt wurden 5.491 für liegende und 2.126 einminütige Beobachtungszyklen für stehende Tiere aufgezeichnet. Das Auftreten und die Konsistenz von Körperhaltungen und Verhaltensweisen unterschieden sich je nach Grundkörperhaltung und Alterskategorie. Die Länge der Zeiträume zwischen den Beobachtungen hatte jedoch keinen systematischen Einfluss auf die Konsistenz. Für künftige Tierwohlbewertungen in landwirtschaftlichen Betrieben wird eine gesonderte Beobachtung von Alterskategorien und Tieren in verschiedenen Körperpositionen empfohlen. Aufgrund der unveränderten (In-)Konsistenz vieler Körperpositionen über verschiedene Zeiträume hinweg ist es jedoch unerheblich, wann die Beobachtungen stattfinden. Um die Körpersprache von Milchkühen vollständig zu erfassen, sollten mehrere Beobachtungstage in Betracht gezogen werden.

1. Introduction

Good welfare of farmed, non-human animals is of increasing importance in society. Consumers and NGOs criticise current animal husbandry practices and demand improved standards. The German Ethics Council published a position paper about animal welfare (Deutscher Ethikrat 2020) and political debates are held internationally. Animal welfare has been included in the "Farm to Fork" strategy 2020 as part of the European Green Deal (European Commission 2020).

However, no universal definition of the term animal welfare exists. For this work, the concept of defining animal welfare as a synergy of basic health and functioning, natural living as well as mental health comprising affective states was applied (Fraser 2008). Affective states are an overarching term including moods, feelings and emotions as well as states assigned to neither feelings nor emotions, like hunger or thirst (Fraser 2008; Kremer et al. 2020; Brambell 1965). Positive animal welfare adds to this in that it includes the presence of positive affective states besides the absence of negative affective states (Boissy et al. 2007; Keeling et al. 2021).

Science offers several approaches to assess animal welfare. One focus lies on environmental aspects, e.g. housing and husbandry procedure, so called indirect "resource-based" measures, the other on animal related aspects, including physiological, pathological or ethological indicators, so called "animal-related" measures. This master thesis focuses on ethological indicators, mainly body language, as an animal-related measure for on-farm animal welfare assessment of dairy cattle.

Several researchers have shown that body language as part of behavioural indicators might be promising to assess both positive and negative animal welfare in general (Mendl et al. 2010) and in cattle specifically (Hintze et al. 2020; Battini et al. 2019; Lange et al. 2020; Oliveira and Keeling 2018; Proctor and Carder 2014; Mendl et al. 2010, 2010; Keeling et al. 2021). Various postures of multiple body parts, e.g. ear, neck and tail posture, were observed in situations assumed to be of positive or negative valence for the animal. By this, researchers aim to validate behavioural indicators of positive and negative affective states. Scientific approaches to use behaviour in cattle to assess positive animal welfare are presented below.

- Ears of dairy cows were investigated as suitable measure for positive animal welfare (Proctor and Carder 2014). The authors concluded that a certain ear posture (low ears) could be a useful indicator of a positive and low arousal emotional state since it occurred significantly more often during a presumably positive and low arousal situation (being scratched by a human). A similar result was seen for visible eye white in dairy cattle, where less visible eye white is associated with positive animal welfare (Proctor and Carder 2015) and more visible eye white with states of high arousal (Lambert and Carder 2017).
- De Oliviera and Keeling (2018) analysed neck, ear and tail postures of individual dairy cows in routine activities and linked variations in body language to valence and arousal dimensions of emotion. The different postures were not interpreted regarding their validity as indicator of positive animal welfare.
- Whether valence and arousal in cattle can be measured by eye white and ear postures was the research aim of Battini et. al. (2019). They associated *ears down* or *backwards* and *half-closed eyes* with more relaxed animals.
- Also for sheep, ear and tail postures were suggested as valuable indicators for positive animal welfare, when more studies on validation exist (Reefmann et al. 2009).

In addition to positive affective states, the following approaches have been taken to study negative affective states in non-human animals using behavioural indicators:

- Scientists focused on evaluating pain in dairy cattle via different behavioural indicators, such as complex behaviour (e.g. response to approach) or body posture (e.g. head position and facial expressions). Only a few indicators showed significant difference between the group in

pain and the group treated with analgesics i.e. “attention towards the surroundings”, “head position”, “ears position”, “facial expressions”, “response to approach” and “back position”. Accordingly, these behaviours can be useful to measure pain in dairy cattle (Gleerup et al. 2015).

- Several species have been studied regarding their facial expressions when in pain. By analysing detailed facial expressions when animals are in pain, scoring systems for pain were developed. These grimace scales have been described for rodents initially and are now generated for further domestic and wild animal species (Mogil et al. 2020; Häger et al. 2017).
- In addition to body language, (in)activity might be a valuable, though not yet validated, indicator for positive and negative effective states in animal welfare research (Hintze et al. 2020; Fureix and Meagher 2015).
- Additionally, *tongue rolling* was stated as possibly valuable indicator of negative animal welfare (Schneider et al. 2019). Furthermore, *nose pressing* behaviour might link to negative states of animal welfare as it is possibly related to stress relief (Gutmann et al. 2013).

To assess animal welfare and the effect of interventions aimed to improve animal welfare, valid, reliable and feasible indicators are necessary. In addition to the crucial work of validating behaviour and different parts of body language as indicators of affective states, studies on the consistency and feasibility are very important. Especially when considering that long-term mood, in contrast to short-term emotional states, is relevant in on-farm welfare assessments, consistency of body language over time under non-changing and familiar conditions is of interest.

The existing literature on consistency in behaviour of cattle mainly focuses on assessing the personality or temperament of animals, which is defined as consistent individual behaviour over time and across contests (Carter et al. 2013; Stamps and Groothuis 2010). Personality is often assessed in experimental setups such as novel object test, novel human test and novel arena test which interrupt the animal’s familiar routine and create an artificial situation. Additionally, the focus of those studies often lies on complex behaviours such as fear responses (Foris et al. 2018; Marçal-Pedroza et al. 2020; Neave et al. 2020). Low to moderate consistency could be found for *activity/exploration* and *boldness* over a period of six months (Foris et al. 2018). *Temperament* in dairy cattle was assessed by measuring several behavioural indicators (entrance time, crush score, flight speed, flight distance) which showed consistency over a period of 135 days (Marçal-Pedroza et al. 2020). Various definitions of *activity* have been proven as consistent behaviour in experiments over a period of three months (Schrader 2002), six months (Foris et al. 2018) or even two lactation periods (Müller and Schrader 2005).

Beyond the complex concept of personality, consistency of body language has not been described well in the literature. Therefore, we focus on the consistency of body language in dairy cattle. Consistency in this case is defined as the same pattern of occurrence of body language being repeatedly observed over different time periods. Since many postures and behaviours that are studied are likely to reflect short-term affective states, the repeated observation over time is one way of possibly reflecting long-term positive animal welfare in on-farm assessments until more suitable mid- and long-term indicators are available (Keeling et al. 2021).

This study is part of a three-year research project carried out at the Swedish University of Agricultural Sciences from December 2019 to November 2022. The overall goal is to create an on-farm assessment protocol that allows detecting especially positive affective states of animal welfare instead of only focusing on negative affective states. However, in this part of the project we focus on consistency of body language without interpretation of the valence of the situation.

We furthermore observe the animal’s behaviour in familiar surroundings and under routine management practices. If the findings of this study were to be applied in animal welfare assessments on commercial farms on a regular basis, a focus on routine procedures and familiar environments promises to be less time and cost intensive. Additionally, we investigate situations where the body language is “undisturbed” by short-term events namely feeding, drinking, queuing to be milked, being

milked, brushing or walking. Hence long-term mood will be more visible and less blurred by short-term emotions (Nettle and Bateson 2012; Kremer et al. 2020; Webb et al. 2019).

If animal welfare shall be assessed based on changes in patterns of behavioural indicators, it is important to know, whether animals show consistent body language over time, e.g. from one day to the next or two weeks later, in different contexts, e.g. when lying or standing, and during their development, e.g. when they are a calf or a milking cow. The aim of this study was thus to describe and compare consistency in dairy cattle on these three levels: Age category (cow, heifer, calf), basic body position (lying, standing) and time periods (short, i.e. from one day to the next, medium, i.e. 2-3 days later, long, i.e. 11-17 days later).

1.1. Research questions

1. How reliably can body language in dairy cattle be observed during live observations with multiple observers?
2. What is the overall occurrence of different body postures in different age categories of dairy cattle, i.e. cows, heifers, calves and in different basic body position, i.e. lying and standing?
3. Assessment of consistency:
    3.1. How consistent is body language within individuals of different age categories of dairy cattle (cow, heifer, calf) in different basic body positions (lying, standing) and over different time periods (short, i.e. from one day to the next, medium, i.e. 2-3 days later, long, i.e. 11-17 days later)?
    3.2. How consistent is body language in dairy cattle on herd level in different basic body positions (lying, standing) across a period of seven weeks?

2. Animals, material and methods

2.1. Housing and husbandry

The Swedish Livestock Research Centre keeps around 550 Swedish Red and White (Swedish: "*Svensk röd och vit boskap"*; SRB) and Holstein-Friesian (HF) cattle. Dairy cows account for 280 animals, of which 250 are lactating. Additionally, approximately 270 heifers and calves are kept. Key production figures of the farm are given below (Table 1).

Table 1 **Key figures for milk production at Lövsta dairy facilities in 2016.** More recent figures were not published. (Faculty of Veterinary Medicine and Animal Sciences - The Swedish Livestock Research Centre 2017)

	All	**SRB**	**HF**
Average number of cows	265	160	105
Milk yield (kg ECM)	10,282	9,780	10,789
Fat (%)	4.2	4.3	4.1
Protein (%)	3.4	3.5	3.4
Dry period (days)	67	71	72
Number of calvings	280	169	111
Calving interval (months)	13.0	12.8	13.4
Number of inseminations	2.2	2.3	2.1
Calving to first insemination (days)	75	72	78
Recruitment percentage	44	n.a.	n.a
Somatic cell count (1000 cells/ml)	175	n.a.	n.a.
First calving age (months)	26.2	n.a.	n.a.

Lactating dairy cows are housed in four groups of approx. 60 - 64 animals each (Figure 2). The animals are milked by DeLaval VMS™ milking robots with one robot per group. The cubicles are equipped with mattresses and wood shavings, the latter being delivered by a rail-based bedding robot above the cubicles (Figure 1). One brush is available per group. Heifers, calves and dry cows are not provided with brushes. Automatic wire-driven scrapers remove the manure in the pathways. Dairy cows are fed with grass silage seven times a day via a feeding system including belt distributors and an automatic feed cart following a rail path in the ceiling. Occasionally, a mix of corn and grass silage is fed (ca. 25% corn

silage). Total mixed ration (TMR) is not provided. However, dairy cows have access to concentrates via concentrate feeders and during milking in the milking robots.

Figure 1 **View of resting area in dairy cow group 4.**

During the first weeks of life, calves are housed individually or pairwise in igloos under a roof outside the main farm building. Colostrum is given for three days followed by whole milk from Lövsta's own cows. Free access to hay, silage, concentrates and water is available. All calves are dehorned and male calves are sold. The calves are weaned at eight weeks of age. After weaning they are moved to a designated calf area in the main farm building. In the calf area, calves are kept in group pens bedded with straw or wood shavings, which is distributed manually. They remain in this housing system up to an age of five to six months.

Subsequently, older calves are moved to the free-stall area for older calves and heifers (Figure 3). This area is equipped with cubicles, rubber mattresses and wood shaving bedding. In total 261 lying places are available for older calves and heifers, separated into seven groups corresponding to different age classes. The feeding consists of roughage. At an age of 14 to 15 months the animals are inseminated. A concentrate feeder is available only for inseminated and pregnant heifers.

Dry cows are housed in the same area as young animals and heifers (Figure 3). Again, the cubicles are equipped with rubber mattresses and wood shaving bedding. For calving, cows are moved to individual calving pens. The feed is similar to the one for young animals but supplemented with minerals. A concentrate station is available for individual feeding (Faculty of Veterinary Medicine and Animal Sciences - The Swedish Livestock Research Centre 2017).

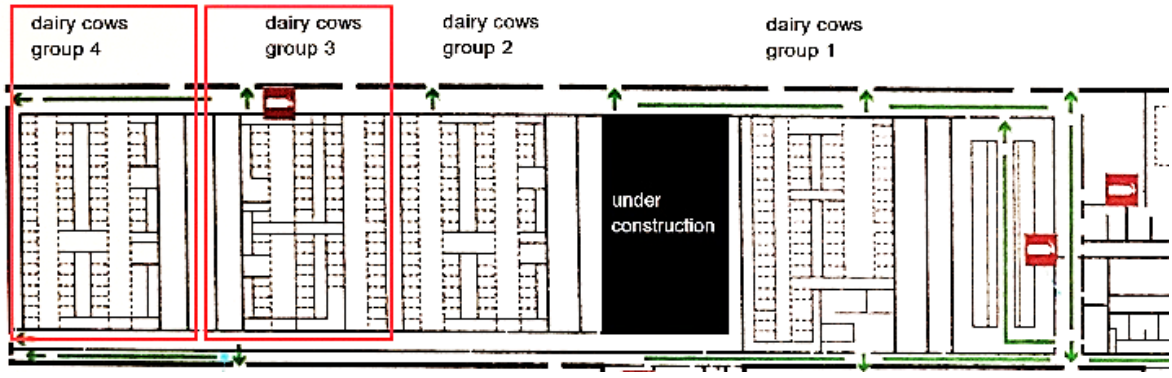

Figure 2 **Housing area for lactating cows.** Only cows from groups three and four were part of this study, indicated by the red framing.

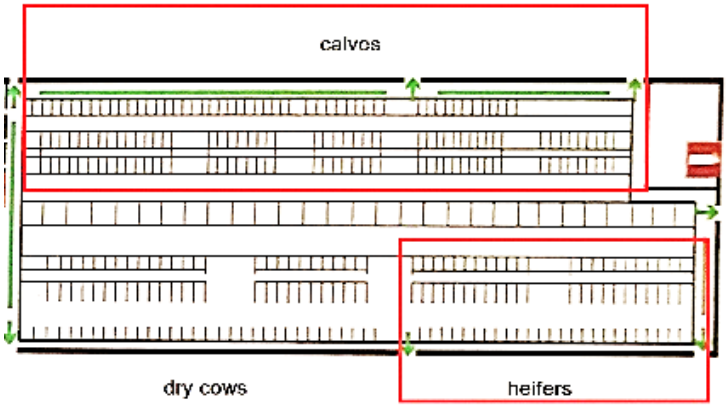

Figure 3 **Housing area for dry cows, heifers and calves older than 6 months.** Only heifers and calves were part of this study, indicated by the red framing.

During summer the animals have access to pasture which consists of 70 ha cultivated pasture and 100 ha natural pasture. Cows are released on cultivated pasture in the beginning of May, young animals older than six months graze on natural pasture from mid April to September/October. Lactating cows have access to pasture at least half a day and are supplemented with silage and concentrates while they stay in the barn.

Claws are trimmed and inspected weekly on a rotating schedule. Additional information about housing and feeding can be found in the booklet “Resources at the Swedish Livestock Research Centre” published by the Faculty of Veterinary Medicine and Animal Sciences 2017.

2.2. Animals

For the experiment, 49 Holstein (HF) and Swedish Red and White (SRB) female dairy cattle including 24 lactating dairy cows (lactation numbers 1 to 7), 13 pregnant heifers and 12 calves which were older than six months, were chosen from the pool of animals available at the research farm. A broad range of lactation numbers and an even distribution across breeds was aimed for, where possible. Except for one, all animals were generally healthy without obvious abnormalities. Only one dairy cow showed signs of *Bilateral convergent strabismus with exophthalmus (BCSE).* This genetically inherited eye defect can influence visual capabilities, depending on its severeness (Mömke and Distl 2007). She was included in the experiment as she was one of the few older animals available and her milk yield was normal during all her lifetime (milk yield, fertility etc.), according to the staff. Only dairy cows from group 4 and group 3 were chosen since group 1 and 2 were used for other experiments (Figure 2). Cows expected to calve within two months from the start of the experiment were excluded. During the period of observations one calf (Number 2241) was moved to another group on 05.11.2020 and therefore no longer served our inclusion criteria. A new calf (Number 2272) was added to the experiment instead, from 16.11.2020 onwards.

All selected animals, meaning cows, heifers and calves, were marked with an individually coloured collar around their neck. Each colour combination was assigned to a specific experimental animal. Hence, they could be identified as experimental animal as well as differentiated from each other from far away. This reduced disturbances and potential stress for the animals since there was no need to check the ear tags up close.

Standard deviation regarding ages per age category was higher in cows than in heifers or calves as they had various ages (Table 2).

Table 2 **Overview of breed, age and lactation number (for dairy cattle) of the experimental animals.**

Age category	Total number	HF	SRB	Age (months) (MEAN ±SD)	Lactation number (MEAN ±SD)
Cows	24	10	14	47.2 ±17.7	2.3 ±1.4
Heifers	12	7	5	21.3 ±1.3	
Calves	12*	6	6	10.8 ±3.2	

*One calf was replaced by another calf during the experiment due to changes in group composition.

2.3. Experimental design

Live observations were performed for six weeks between 26.10.2020 and 06.12.2020. Individual animals with low data availability were additionally observed from 06.12.2020 to 11.12.2020. Half of the animals per age category (i.e. twelve cows, six heifers, six calves) were observed in weeks one, three and five, whereas the other half was observed in weeks two, four and six (Table 3).

Observations were carried out by three researchers on four days per week during daytime between 07:30 AM and 08:00 PM. Each researcher was assigned to eight cows, four heifers and four calves. If the assigned observer was not able to observe an animal during the day, other observers took over the task if possible. One observer was replaced by a fourth back-up observer for 9 % of the observation cycles recorded in total during the experiment.

Table 3 **Observation schedule.**

Age category	**Number of animals**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**
Cows	12 (1-12)	x		x		x		As required
	12 (13-24)		x		x		x	As required
Heifers	6 (1-6)	x		x		x		As required
	6 (7-12)		x		x		x	As required
Calves	6 (1-6)	x		x		x		As required
	6 (7-12)		x		x		x	As required
X = observation weeks								

Each animal was observed once a day either while lying (on two days a week) or while standing (on the other two days). To avoid possible biases, different combinations of order of observations while lying (L) or standing (S), e.g. S-S-L-L or S-L-S-L in one week, were determined beforehand. If the animal could not be observed while standing during a day, she was observed lying instead. Cows were only observed in the resting area, not in the feeding area. Heifers and calves were observed in the whole pen.

2.4. Behavioural observations

Each observer positioned herself in at least five-meter distance from the focal animal, close enough to be able to see all relevant aspects of the body language but as far away as possible to not disturb the animal. No ladders or stools were used. Directly facing the animal and direct eye contact with the animal were avoided whenever possible.

Each observation round included 15 individual instantaneous observation cycles, with each observation cycle lasting for minimum one minute (Figure 4). One observation round could not exceed 30 minutes.

Each cycle started with 30 seconds of observing the animal's behaviour and assessing her (in)activity status (as in Hintze et al. 2020). During the following 2 seconds, the body language of the focal animal was observed based on the ethogram developed for this purpose, including abnormal oral behaviour (Table 4). The information was then recorded on a portable tablet (Microsoft surface Go 2; Windows 2010), using the software Mangold INTERACT light. After noting the information, the observer waited until a whole minute had passed since the start of the next observation cycle. This was repeated until all 15 observation cycles per round were recorded. After the last observation cycle, the next individual was spotted, and a new observation round started.

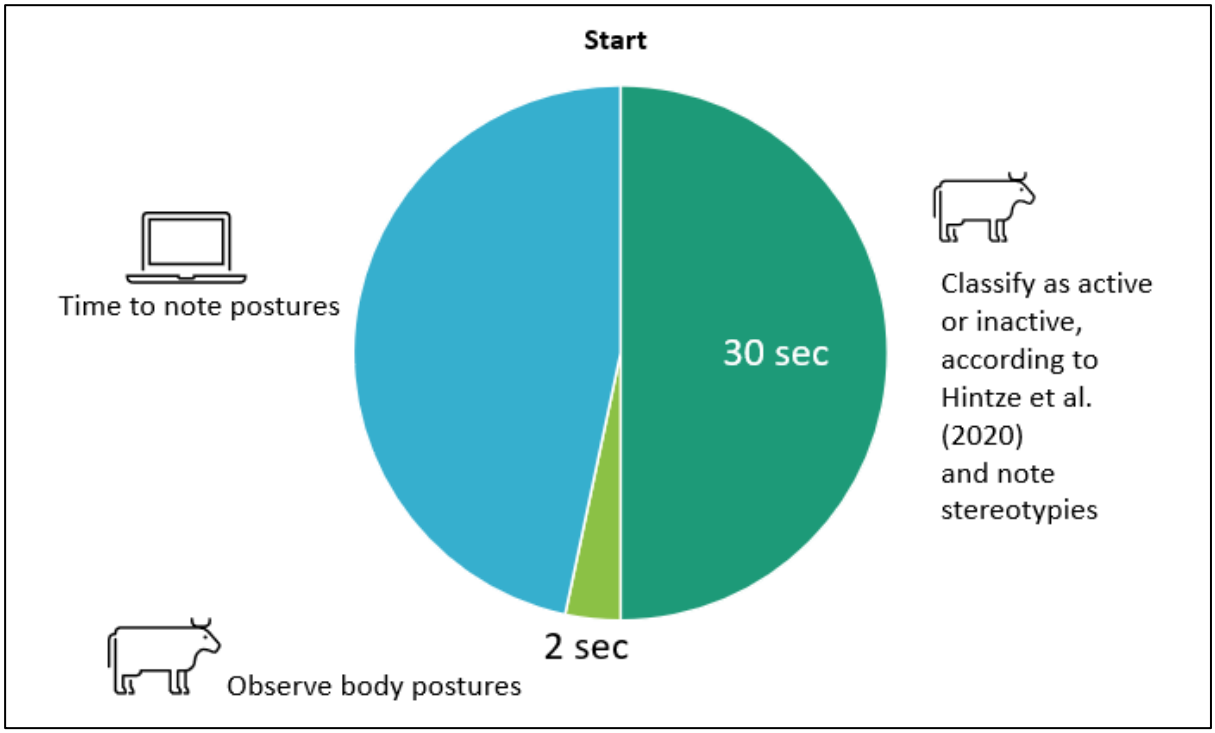

Figure 4 **Schematic description of one observation cycle.**

If necessary, an observer could start the next observation cycle later than one minute after the first observation cycle. Defined reasons for delaying the start of an observation cycle were:

- The animal is currently walking
- The animal is currently *active*, e.g.: animal is self-grooming, grooming or being groomed or mounted/mounting
- Observer needed to reposition because the animal moved or was out of sight due e.g. to conspecifics blocking the sight
- Observer needed to correct mistakes done while typing in information
- Observer needed to wait for the software to recover from "freezing"
- Observer needed to adjust to external circumstances in the farm building (e.g. feed wagon passing)
- Unusual rise in arousal level occurred, noticed through an overall increase of mooing and motion activity throughout the farm building or a sudden startling movement of the observed animal. This could be triggered by people working in the pen, animals being moved by farm workers or sudden and unusual loud noise.

If an observation round had to be stopped before the end of the 15th cycle (e.g. by an external event), a minimum of 8 observation cycles in a time period of 30 minutes had to be reached for the observation round to be included in the data analysis.

An observation round was discharged if the following incidences occurred during an observation round and exceeded 30 minutes, the maximum time frame for one observation round:

- The animal changed basic body positions i.e. stood up or laid down.
- The animal became *active* i.e. started brushing or feeding, walked into the milking area, walked into the feeding area, was being groomed or started grooming a conspecific.
- Veterinary students entered the pen of the observed animal.

2.4.1. Ethogram

An ethogram (Table 4) was developed aiming to describe the body language of dairy cattle, including cows, heifers and calves older than six months. In addition to body language, described by postures of individual body parts, relevant behaviours possibly providing additional information about animal welfare such as (in)activity (Hintze et al. 2020), basic body position (Tucker et al. 2021), rumination (Paudyal 2021) and abnormal oral behaviour (Gutmann et al. 2013) were described and observed in this study. The ethogram is based on recent literature (Oliveira and Keeling 2018; Hintze et al. 2020) but was refined and adverbeekapted to cover the body language of the animals from the different age categories. A version of the ethogram including pictures is available in the Appendix.

When finalised, the ethogram was translated into a coding scheme in the Software *Mangold INTERACT®*, and thus a fast and easy-to-use tool was created to perform live observations on farm.

Table 4 **Ethogram adapted from Hintze et al. (2020) and De Oliviera & Keeling (2018).**

Activity/body part	Posture	Definition
Activity	Inactive	According to Hintze et al (2020): • Maximum two steps forward or backward • Singe movement of one leg, including a kick after flies • Lying down or standing up without further movements • Stretching while standing or lying • Skin twitching • Urinating or defecating • Any tail movements • Head shaking (while standing or lying) • Snapping after flies by quickly throwing the head towards one side of the body • Ear movements • Eye blinks • Yawning • Coughing • Sneezing • Humming • Being licked without obvious reaction • Being nibbled without obvious reaction • Being mounted • Receiving a head butt without obvious reaction • Being displaced and being inactive thereafter Additionally counted as inactive: • defecating/urinating • stretching • repositioning when lying.
	Active	According to Hintze et al. (2020): • Scratching self with one foot more than twice • Scratching self on barn equipment or objects on pasture • Licking self more than twice • Sniffing an object or conspecific • Flehming • Mooing • Licking a conspecific • Nibbling on a conspecific • Mounting a conspecific • Head butting • Displacing a conspecific Additionally counted as active: • rubbing against an object or the floor more than twice • stepping more than twice (incl. stepping over manure-scraper) • scratching more than twice • licking more than twice • explorative sniffing for more than 5 seconds

		According to Hintze (2020): Note that some movements could be classified as both the animal still being inactive and the animal becoming active based on the number of times this movement was shown (up to two consecutive times: classified as still being inactive, more than two consecutive times: classified as becoming active).
Basic body position	Standing	Three or four claws on the ground, trunk does not touch the ground, no forwards or backwards movement, animal standing leveled or with front feet elevated (e.g. in cubicle).
	Lying	Trunk touches the ground.
Rumination	Ruminating yes	Any chewing motion performed more than three consecutive times.
	Ruminating no	No chewing motion visible.
Abnormal oral behaviour	No abnormal oral behaviour	No abnormal oral behaviour was observed (tongue rolling or nose pressing)
	Tongue rolling	The animal lies or stands and curls her tongue repeatedly to the left or right. The mouth is open and the tongue clearly visible.
	Nose pressing	The nose is pressed against a conspecific or obstacle for more than 20 seconds consistently.
Neck posture	Middle	The neckline is parallel to the ground, regardless of the animal's posture (e.g. standing elevated); an angle of 10° above or below parallel is within the range of "*neck middle*"; if the neckline is curved, an imaginary line is drawn from the back of the head to the part of the withers with the highest inclination; whenever the neck is not clearly "*above*" or "*below*" it is "*middle*".
	Above horizontal	Neck held above the imaginary horizontal line parallel to the ground (>10°).
	Below horizontal	Neck held below the imaginary horizontal line parallel to the ground (>10°); the muzzle is not lower than the carpal joints.
	Down	Muzzle is between the height of the carpal joint and the ground, can touch the ground or an object on the floor.
	Moving	More than one different neck postures are observed within the observation interval of 2 seconds.
Head orientation (standing)	The forehead determines the direction for "*head orientation*".	
	Facing straight	Forehead neither directed to the left nor to the right (less than 30° angle from the sagittal plane).
	Facing left	Forehead directed to the left at an angle of more than 30° from the sagittal plane.
	Facing right	Forehead directed to the right at an angle of more than 30° from the sagittal plane.
	Head moving	Forehead is in more than one of the postures above within the observation interval of 2 seconds.

Head orientation (lying)	Facing neutral	The forehead follows the natural curve of the spine and neck of a lying cow, or the forehead is facing straight ahead.
	Facing in	The forehead and neck are bend inwards, more than "*neutral*" but less than "*folded*".
	Folded	The forehead and neck are folded, the head is touching the body or ground.
	Facing out	The forehead is facing further out than "*neutral*".
	Head moving	The forehead is in more than one postures described above within the observation interval of 2 seconds.
Head contact	Head no contact	None of the below.
	Head contact floor	Nose, jaw, side of head or forehead touches the floor; if multiple contacts exist, "*contact floor*" is the predominant posture chosen.
	Head contact other	Nose, jaw, side of head or forehead touches the animal's own body, a conspecific or an obstacle in the barn.
Ear postures	If one ear was out of sight, the ear posture could not be determined.	
	Axial	Ears point straight out to the side, perpendicular to the head-rump axis.
	(Pushed) Forwards	Both ears are in front of the frontal plane; ears are directed forwards, with the tip of the ear at an angle of more than 30 degrees from the perpendicular axis ("scooped"); ears are pushed forwards, recognizable by e.g. visible tension in the backside of the ear, visible veins.
	Backwards	Both ears are behind the frontal plane and tip of the ears point up, higher than the base of the ear; not pinned.
	Ears pinned	Ears point backwards and up; ears are as tense as anatomically possible and parallel to each other.
	Asymmetric left	Right ear is axial or forwards and left ear points backwards (up or down).
	Asymmetric right	Left ear is axial or forwards and right ear points backwards (up or down).
	Drooping	Ears hang loosely downwards, falling axial to the head; base of ear pointing down, ears are completely relaxed.
	Moving	More than one different ear postures (as defined above) within 2 seconds of observation.
Eye closure	Open	Upper and lower eyelid are not in contact.
	Closed	Upper and lower eyelid are in contact, eyeballs invisible.
Tail postures and movements	Only movements initiated during the 2 second body posture observations were recorded. E.g. if the tail was wagged strongly just before the observation, but the ceasing of the swinging continued during the observation period of 2 seconds then this is recorded as "tail hanging".	
	Hanging	standing: tail hangs down without moving; minimal movements of tail tip may occur lying: tail hanging off the cubicle and touching the floor in the alley.

	Tail tucked between legs	standing: tail is tucked between the legs, touching the body and udder on the full length of the tail lying: tail is not hanging off the cubicle, tail does not need to touch the animal's body. Tail tucked between legs when lying is not necessarily associated with a negatively valenced state in this study.
	Tail wagging	Tail moves from side to side, initiated during 2 second observation interval; movement is not due to the animal's stepping behaviour.
Tail lifts	Lifted	Proximal tail lifted straight up, right or left; tail does not touch the body; gap visible between body and tail
	Not lifted	Tail is hanging down loosely or wagging.
Lying posture	Chest prone	The ventral part of the shoulder blade is not or not fully touching the ground.
	Flat on the side	The ventral part of the shoulder blade is fully touching the ground.
Lying side	Lying on the left	The animal is lying on the left side of the trunk.
	Lying on the right	The animal is lying on the right side of the trunk.
Front leg posture	Both front legs tucked in	Both front legs are bent at the carpal joint and placed under the body.
	One front leg stretched	One front leg is bent at the carpal joint and placed under the body, the other front leg is stretched; if the leg is not fully stretched, it is classified as tucked.
	Both front legs stretched	Both front legs are stretched.
Rear leg posture	Rear legs tucked	Angle between tibia and metatarsus of the rear legs is less than 90°.
	Rear leg stretched	At least one rear leg is stretched; Angle between tibia and metatarsus of the rear leg is 90° or more than 90°.

2.4.2. Adaptations of the experimental procedure

After pre-experimental pilot observations at the beginning of the experiment some procedures were adapted due to feasibility reasons:

- We started with a total of 36 cows but reduced the number of animals to 24. It was not possible to observe more than eight animals in the assigned basic body position per day and observer. Especially spotting focal animals in standing body position was time consuming.
- Initially it was planned to observe the animals for five seconds within one observation cycle before recording their body language. However, this time was reduced to two seconds to minimize potential changes in body postures during the observation interval.
- Due to illness of one of the three main observers a fourth observer had to substitute. The fourth substitute observer covered 687 (9%) out of 7617 observation cycles in total.
- To exclude a correlation of tail movements with increased occurrence of flies in the facilities a fly assessment was planned. However, the occurrence of flies decreased until the experiment started, possibly due to decreasing temperature outdoors as well as indoors. The fly assessment was therefore cancelled.

2.5. Inter-observer reliability testing

Before inter-observer reliability was tested, all four observers were trained using live and video observations. During the inter-observer reliability test, three observers plus the fourth substitute observer recorded the body language of one cow simultaneously and live. Only dairy cows, but neither heifers nor calves were observed for the inter-observer reliability testing since simultaneous observation of several observers was only possible in the cow area.

A total of 354 observation cycles per observer were recorded on 32 different animals across 16 days. One observer was replaced by the back-up observer for 62 of the 354 observations. The number of observations per observation round and cow ranged from 1 to 16 observation cycles during the inter-observer reliability testing.

2.6. Statistical methods

2.6.1. Inter-observer reliability

For each body part and posture, Fleiss' Kappa coefficients (Fleiss 1971) were calculated for the three main observers. Data for the fourth substitute observer were excluded from the inter-observer reliability calculation, since merging data collected by the substitute observer and her main observer would create an artificial observer which did not actually do the observations. Merging the data for test purposes predominantly resulted in higher inter-observer reliability than when only the three main observers were compared. Hence, we provided information about the minimum inter-observer reliability reached by only the three main observes. The range of pairwise Cohen's Kappa is given for all combinations of three observers. The number of observation cycles for pairwise comparison differed depending on the observers paired.

2.6.2. Data processing

Due to the exploratory nature of this study, descriptive statistical methods were used.

General data processing:

1) Observation rounds with fewer than 8 observation cycles per animal and day were excluded to ensure a representative number of observations cycles per animal on this day and to minimise the effect of outliers.
2) The same exclusion criterion applied to single postures within one observation round, i.e. if a specific body part was mostly out of sight, the data for this body part were excluded whereas another posture that was less out of sight may have been included within the same observation round per animal. Also at this level, a minimum of 8 sufficient observation cycles was the minimum requirement. For example, 15 observation cycles during one observation round may have always revealed information for the body part "ears", but only 3 observation cycles supplied data for "eyes" which were mainly out of sight. Therefore, the information for "eyes" was discharged since not a minimum of 8 sufficient observation cycles were reached. Nevertheless, the 15 observation cycles available for "ears" were included.
3) For each animal and day, an average percentage was calculated per body part and posture using pivot tables. This evened out the different numbers of observation cycles available per animal and day (8 to 15 observation cycles per day).

Data processing for percentages of occurrence per posture:

After step 1), 2) and 3) percentages of occurrences of each posture were calculated per basic body position, age category and animal based on all observation cycles available. No differentiation by date or time period was made. Following, the mean per age category was calculated form the means of each animal. This data is displayed in the stacked bar charts.

Data processing for absolute changes per postures:

After step 1), 2) and 3) the average percentages of occurrence were then compared for three different time lags (periods): “short”, i.e. one day after day x, “medium”, i.e. 2-3 days after day x, and “long”, i.e. 11-17 days after day x. Absolute changes (German: ”Beträge”) for each body part were calculated for all combinations between day x and further days within the given time period. For example, for observations on day 1, 2, 3 and 4, absolute changes on day 1 and 2, 2 and 3 as well as 3 and 4 were calculated for "short" and occurrences on day 1 and 3, 1 and 4 as well as 2 and 4 for “medium” time period, as “medium” comprises all days 2-3 days after day x. In the results section, the number of day-pairs giving information about changes of occurrences between days is stated below each boxplot. The absolute changes of postures for each time period were then averaged per animal and time period. This dataset formed the basis for the boxplots.

Data processing for percentages of occurrence on herd level:

After step 1), 2) and 3) the mean percentages of occurrences on herd level per basic body position and day were calculated for each posture. Hence, age categories were merged. Standard deviation and coefficient of variation were calculated across all available days.

2.7. Ethical statement

The project was approved regarding animal, human and environmental wellbeing by the Swedish Board of Agriculture (*Jordbruks verket – De regionala djurförsöksetiska nämnderna*) in Uppsala, Sweden. Application record number 5.2.18-11064/16 and record number 31-6443/11 for Uppsala Animal Trial Center (*Försöksdjursanläggningen*). Applicant and project leader was Prof. Linda Keeling.

3. Results

3.1. Inter-observer reliability

Inter-observer reliability between the three main observers measured via Fleiss' Kappa Coefficient (Fleiss 1971) was at least moderate (> 0,4) (Landis and Koch 1977) in 53 of the 55 postures observed (Table 5). *Eyes closed* and *ears pinned* had Fleiss' Kappa values lower than "moderate" (0.41-0.60). For *eyes closed* Fleiss' Kappa was low, since one observer never observed *eyes closed,* but pairwise comparison still showed low Cohen's Kappa (Cohen 1960) values for *eyes closed*. *Ears pinned* was observed with "fair" (0.21-0.4) to "moderate" (0.41-0.60) reliability for different pairs of observers.

Table 5 **Inter-observer reliability.** The inter-observer reliability is described by Fleiss' Kappa Coefficients (Fleiss 1971) for the three main observers. Additionally, the range of Cohen's Kappa coefficients (Cohen 1960) for all pairwise comparisons between the three main observers is stated. Coefficients below 0.4 are marked red, indicating a reliability less than "moderate" (0.41-0.60), "substantial" (0.61-0.80) or "almost perfect" (0.81-1.00) (Landis and Koch 1977). For binary postures, both coefficients were stated e.g. for *lying on the left* and for *lying on the right*, since the third option being "*out of sight*", might have led to differing results.

	Posture	**Fleiss' Kappa Coefficient**	**Range of pairwise Cohen's Kappa Coefficient**
Number of observation cycles compared		292	292-354
Number of observers		3	3
(In)activity	Active/Inactive	0.69	0.63 - 0.73
Basic body position	Lying	1.00	1.00
	Standing	0.99	0.99
Rumination	Ruminating no	0.93	0.92 – 0.94
	Ruminating yes	0.95	0.94 – 0.95
Abnormal oral behaviour	Tongue rolling	0.60	0.50 – 1.00
	Nose pressing	1.00	1.00
	No abnormal oral behaviour	0.82	0.75 - 1.00
Neck posture	Neck above horizontal	0.50	0.49 - 0.51
	Neck below horizontal	0.64	0.62 - 0.66
	Neck down	0.85	0.79 - 0.92
	Neck middle	0.63	0.58 - 0.66
	Neck moving	0.66	0.44 - 0.91
Head orientation (standing)	Facing straight	0.93	0.91 - 0.95
	Facing left	0.82	0.75 - 0.86
	Facing right	0.80	0.72 - 1.00
	Head moving (standing)	0.65	0.59 - 0.75

Head orientation (lying)	Facing neutral	0.96	0.94 - 0.97
	Facing in	0.66	0.58 - 0.71
	Folded	0.94	0.91 - 1.00
	Facing out	1.00	1.00
	Head moving (lying)	0.60	0.50 - 0.67
Head contact	Head contact floor	0.89	0.84 - 0.92
	Head contact other	0.49	0.49 - 0.51
	Head no contact	0.80	0.77 - 0.81
Ear postures	Asymmetric left	0.63	0.53 - 0.84
	Asymmetric right	0.59	0.56 - 0.62
	Axial	0.69	0.62 - 0.77
	Backwards	0.80	0.73 - 0.86
	Drooping	0.80	0.67 - 1.00
	Moving	0.58	0.52 - 0.67
	Pinned	0.39	0.23 - 0.59
	Forwards	0.83	0.81 - 0.85
Eye closure	Open	0.70	0.59 - 0.78
	Closed	0.09*	0.20*
Tail postures and movement			
	Tail hanging (standing)	0.92	0.89 - 0.97
	Tail hanging (lying)	1.00	1.00
	Tail tucked between legs (standing)	0.78	0.66 – 0.86
	Tail tucked between legs (lying)	0.95	0.94 – 0.98
	Tail wagging	0.79	0.75 - 0.86
Tail lifts	Tail lifted	0.73	0.66 - 0.77
	Tail not lifted	0.79	0.77 - 0.80
Lying posture	Chest prone	1.00	1.00
	Flat on the side	1.00**	n.a. **
Lying side	Lying on the left	0.94	0.92 - 0.98
	Lying on the right	0.96	0.95 - 0.98
Front leg posture	Front legs tucked	0.96	0.94 - 0.98
	One front leg stretched	0.90	0.88 - 0.92
	Front legs stretched	0.93	0.89 - 1.00
Rear leg posture	Rear leg stretched	0.81	0.78 - 0.84
	Rear legs tucked	0.81	0.81 - 0.82
	*one observer never observed *eyes closed*		
	***flat on side* was never seen		

3.2. Occurrences and consistency of body language on animal level

In the following chapter the results regarding (in)activity, body postures and complex behaviour such as rumination or abnormal oral behaviour are presented. Each subchapter describes a specific body part (e.g. ears), (in)activity or behaviour in detail. The descriptions consist of two parts.

Firstly, the percentage of occurrence of each posture of all recorded observation cycles per body part, age category and basic body position is displayed in a stacked bar chart. All occurrences of postures per body part are based on the numbers shown in Table 6. Note the different number of observation cycles available for all age categories and lying vs. standing animals.

Secondly, each posture per body part is described by illustrating absolute changes *(German: "Beträge")* of the occurrences per basic body position (lying, standing), age category (cow, heifer, calf) and time period (short i.e. from one day to the next, medium i.e. 2-3 days later, long i.e. 11-17 days later) using box-and-whisker plots. These graphs describe how likely it is to see the same percentages of occurrences of postures for an animal on one day as on another day. Additionally, the variability of change across individual animals is displayed, which cannot be seen in the stacked bar graphs. The lower the absolute changes, i.e. the lower the median, and the lower the variability in change, i.e. the smaller the boxes, the higher the consistency of occurrence of postures across individuals within one basic body position, age category and time period.

However, boxplots are based on different sample sizes in terms of number of animals and number of observation cycles and must be interpreted with caution when the sample size is low. Therefore, the number of animals and the number of observation cycles is given below each boxplot. The number of observed animals and the number of observation cycles for lying animals was often higher than for standing animals. Especially low are sample sizes for standing calves. If less than five observation cycles were available, no boxplot is displayed.

The scale of the y-axis in percentage can differ between body parts. This makes the comparison within body parts easier but has to be taken into account when comparing different body parts with each other.

Table 6 **Information underlying the calculation of percentages of occurrence for all postures.** Total number of animals and mean (± standard deviation, SD) of observation cycles per animal are given per age category and basic body position.

Basic body position	Age category	Number of animals observed	Observation cycles per animal (MEAN ±SD)	Total number of observation cycles
Lying	Cow	24	118.7 ±23.9	2849
Lying	Heifer	12	103.5 ±28.4	1242
Lying	Calf	13	107.7 ±30.4	1400
Standing	Cow	24	49.8 ±19.7	1194
Standing	Heifer	11	57.0 ±18.0	627
Standing	Calf	11	27.7 ±16.8	305

3.2.1. (In)activity

Lying animals were recorded as *inactive* during most of the observations (97.8 ±1.1, MEAN ±SD). When standing, on average 51.2 ±13.9 % of all observations were described as *active* across all age categories. However, the younger the age category, the more *active* the animals were, when standing (mean cows =38.5%; mean heifers=48.9%; mean calves= 66.1%) (Figure 5).

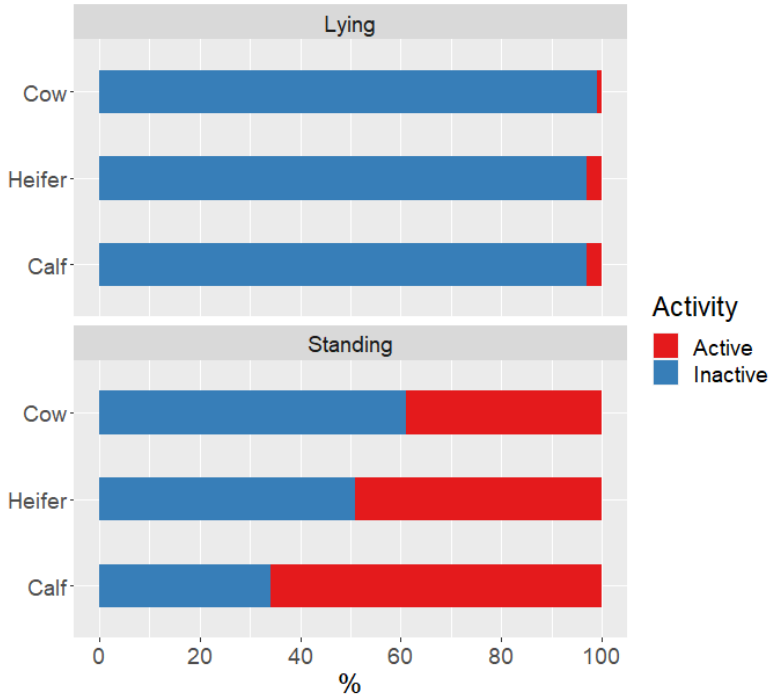

Figure 5 **(In)activity.** The mean occurrence (%) of (in)activity is given per basic body position (lying, standing) and age category (cow, heifer, calf).

Since activity and inactivity are mutually exclusive postures, i.e. if an animal is not *active*, she is *inactive* and vice versa, Figure 6 displays the absolute changes of both. Separate boxplots would look identical because they describe the same absolute change of (in)activity from one day to another. This equally applied to the mutually exclusive body parts eye closure (chapter 3.2.7.1), tail lifts (chapter 3.2.8), rumination (chapter 3.2.2), lying sides (chapter 3.2.9.1), lying posture (chapter 3.2.9.2) and rear leg posture (chapter 3.2.9.4).

Animals showed different patterns of consistency regarding their (in)activity depending on basic body position. In lying position, cows were slightly more consistently (in)active than heifers and calves (Figure 6). Lying animals showed similar and comparably smaller changes of (in)activity, regardless if the time period in between the days being compared was short, medium or long. Standing animals showed a greater change in occurrences of (in)activity than lying animals, across all age categories and time periods (but no information given for calves short- and mid-term). This means that standing animals could be largely *inactive* on one day, but mainly *active* on another day, throughout all age categories and independent from the time period between observations. Graphically this is displayed in a larger height of the boxes.

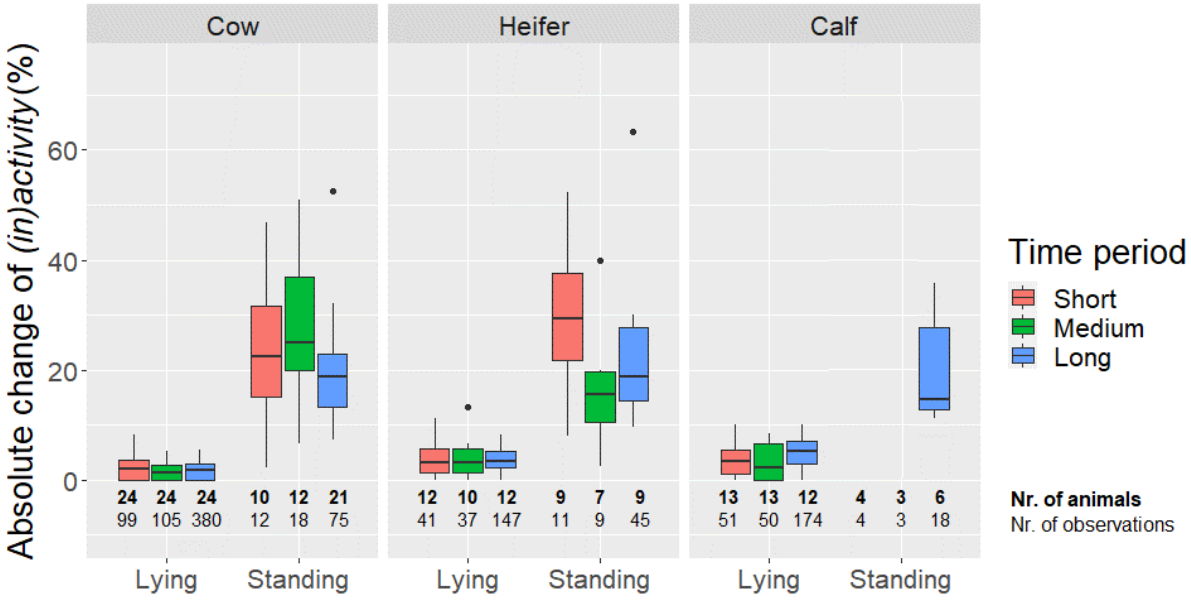

Figure 6 **Absolute changes of (in)activity.** Changes of (in)activity are given as absolute values describing the change in occurrences of (in)activity for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

3.2.2. Rumination

Lying cows were observed *ruminating* in less than half of the observations (mean cows=45.9%). Lying heifers and calves on the other hand ruminated in more than half of the observations (mean heifers=67.9%; mean calves=67.5%). When standing, cows ruminated in more than half of the observations, more often than during lying position. For heifers and calves the opposite occurred; they showed less rumination standing than lying. Standing calves ruminated in only 30.6% of standing observations (Figure 7).

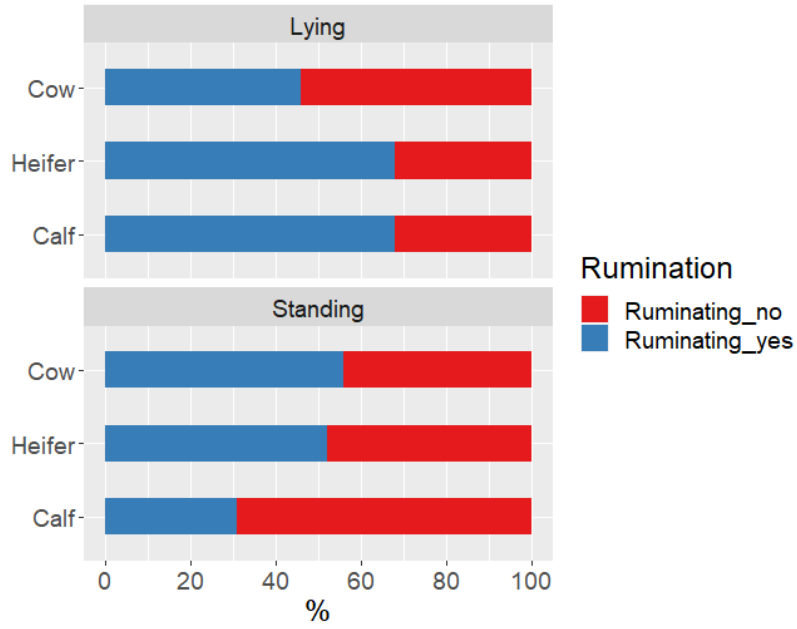

Figure 7 **Rumination.** The occurrence (%) of rumination is given per basic body position (lying, standing) and age category (cow, heifer, calf).

For all age categories *rumination* was variable. For *rumination* in cows and heifers, the variability of change decreased the longer the time period was or the more observations were available. For calves this trend was not visible (Figure 7).

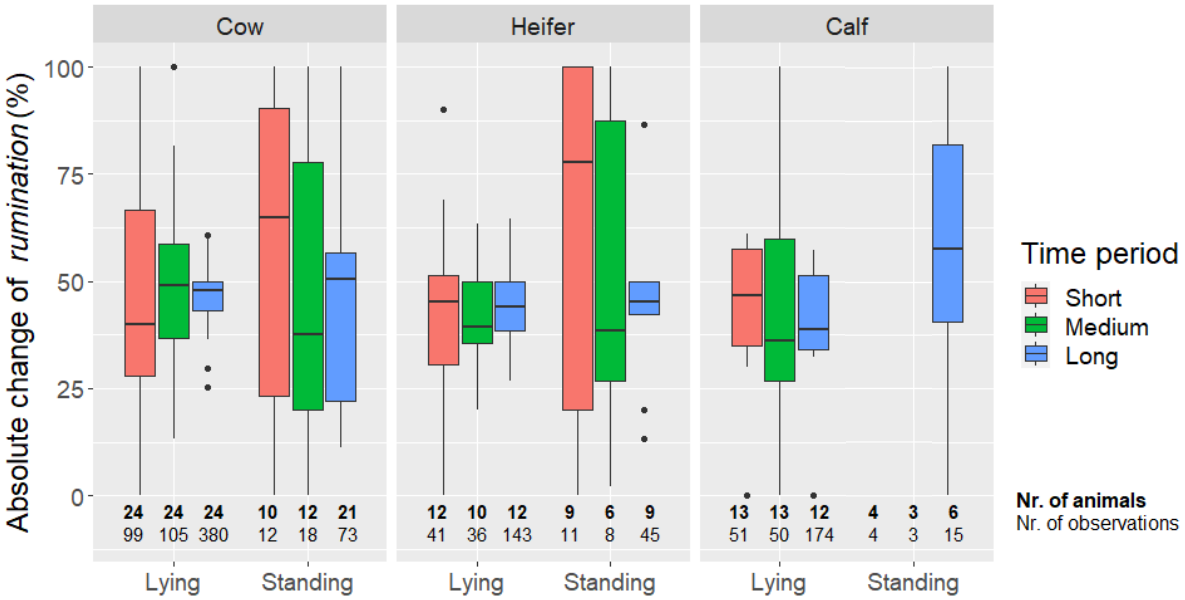

Figure 8 **Absolute changes of *rumination*.** Changes of *rumination* are given as absolute values describing the change in occurrence of *rumination* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

3.2.3. Abnormal oral behaviour

Abnormal oral behaviour (*tongue rolling* or *nose pressing*) occurred in 1.6% of all observations across age categories and basic body positions. *Nose pressing* was seen in 1.1% of observations, of which 0.8% were seen in standing heifers. *Tongue rolling* occurred in 0.5% of which 0.4% were seen in standing heifers (Figure 9).

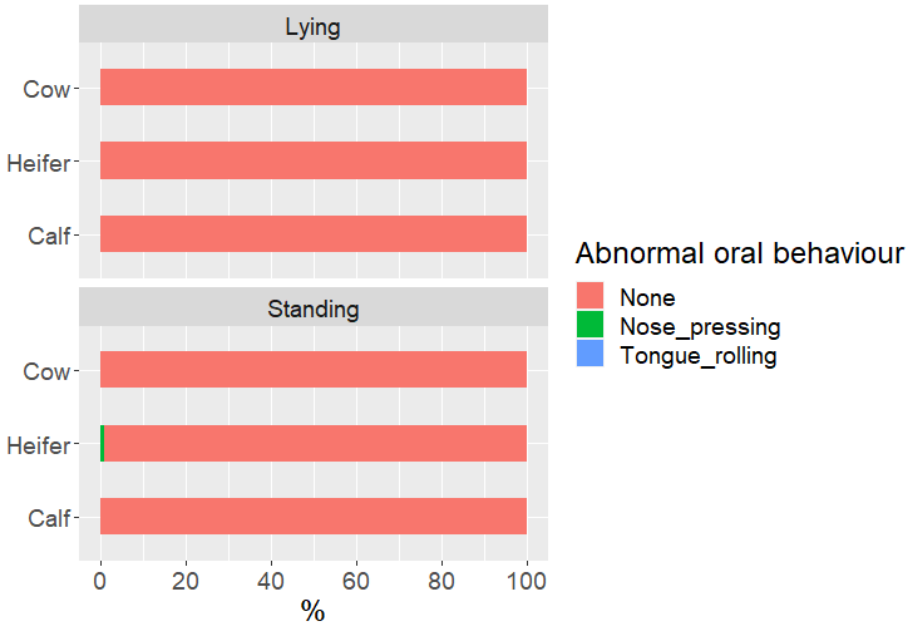

Figure 9 **Abnormal oral behaviour.** The occurrence (%) of abnormal oral behaviour is given per basic body position (lying, standing) and age category (cow, heifer, calf).

Changes in abnormal or non-abnormal oral behaviour were rare and most visible in standing heifers (Figure 10 - Figure 12).

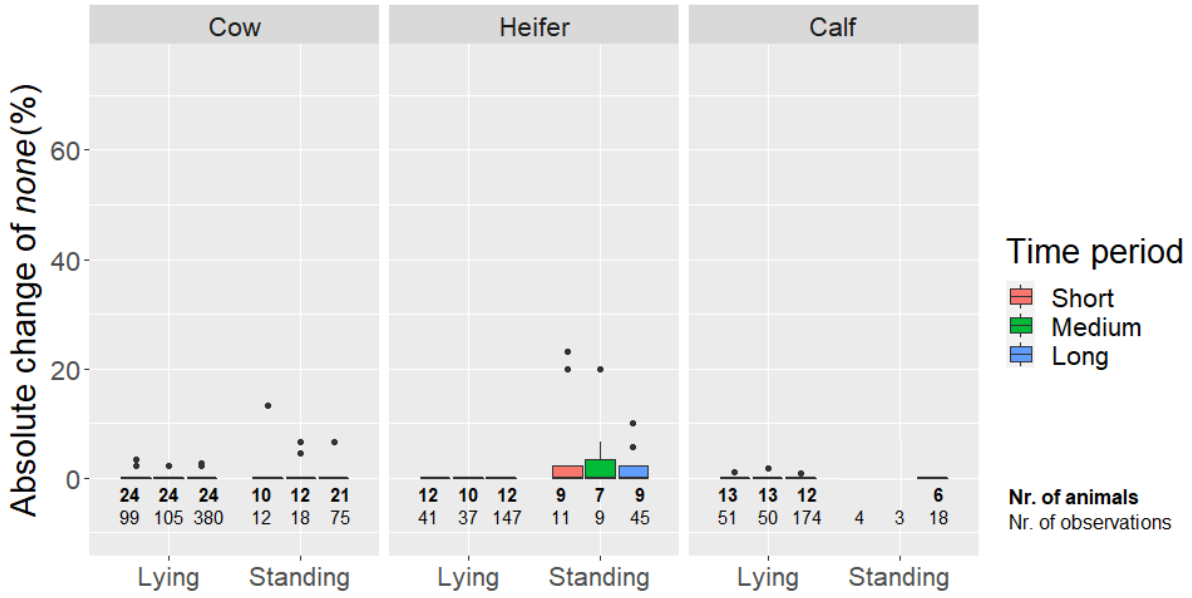

Figure 10 **Absolute changes of *no abnormal oral behaviour ("none").*** Changes of *no abnormal oral behaviour* are given as absolute values describing the change in occurrence of *no abnormal oral behaviour* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

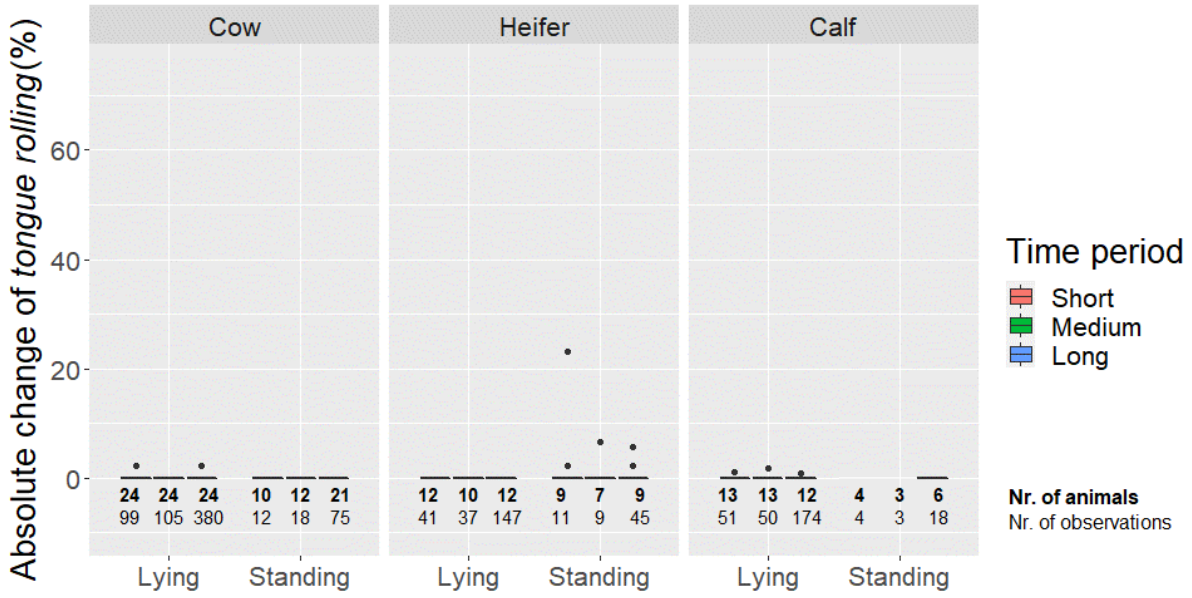

Figure 11 **Absolute changes of *tongue rolling*.** Changes of *tongue rolling* are given as absolute values describing the change in occurrence of *tongue rolling* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

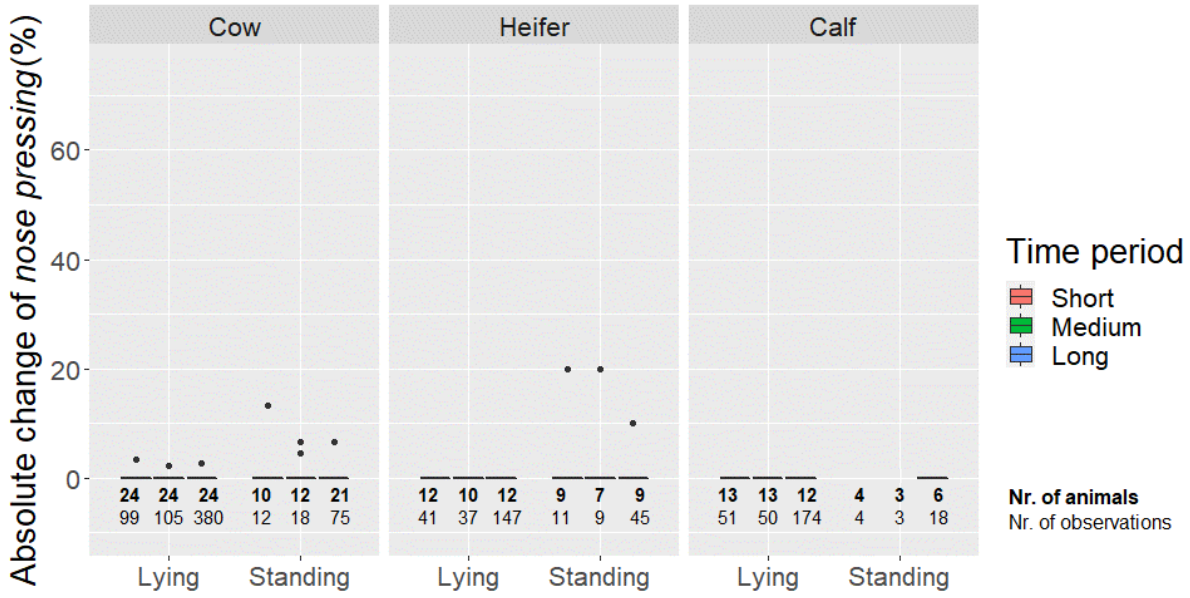

Figure 12 **Absolute changes of *nose pressing*.** Changes of *nose pressing* are given as absolute values describing the change in occurrence of *nose pressing* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

3.2.4. Neck postures

The younger the animal, the higher was the occurrence of the neck posture *above horizontal.* This was the case in lying and standing animals. In both basic body positions, cows held their *neck down* more often than heifers and calves. When lying, *neck middle* and *neck above horizontal* were the most frequently observed neck postures across age categories. Cows showed *neck middle* in more than half of the observations, whereas calves had their neck *above horizontal* in more than half of all observations.

For standing animals, *neck middle* and *neck below horizontal* were the most dominant postures, at least for cows and heifers. When not having their neck in the *middle*, standing calves showed *neck above horizontal* more often than *neck below horizontal*. Furthermore, calves were seen with a *moving neck* more often than the other age categories. Additionally, cows showed the greatest occurrence of *neck down* across age categories when standing, touching or almost touching the ground with their muzzle (Figure 13).

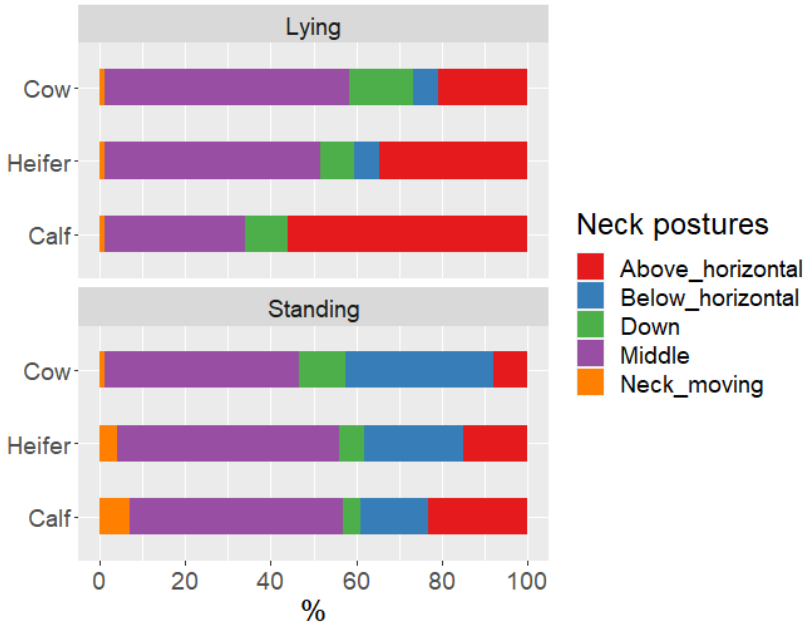

Figure 13 **Neck postures.** The occurrence (%) of neck postures is given per basic body position (lying, standing) and age category (cow, heifer, calf).

Neck above horizontal showed a greater change in lying animals than in standing animals across all time periods and age categories. The opposite was visible for *neck below horizontal*. Here the absolute changes were larger for standing animals. For *neck down*, lying animals showed slightly larger changes of occurrences than standing animals. Changes in *neck moving* posture were low since this posture only occurred rarely. Highest changes were seen in standing calves (only long time period available) (Figure 14 - Figure 18).

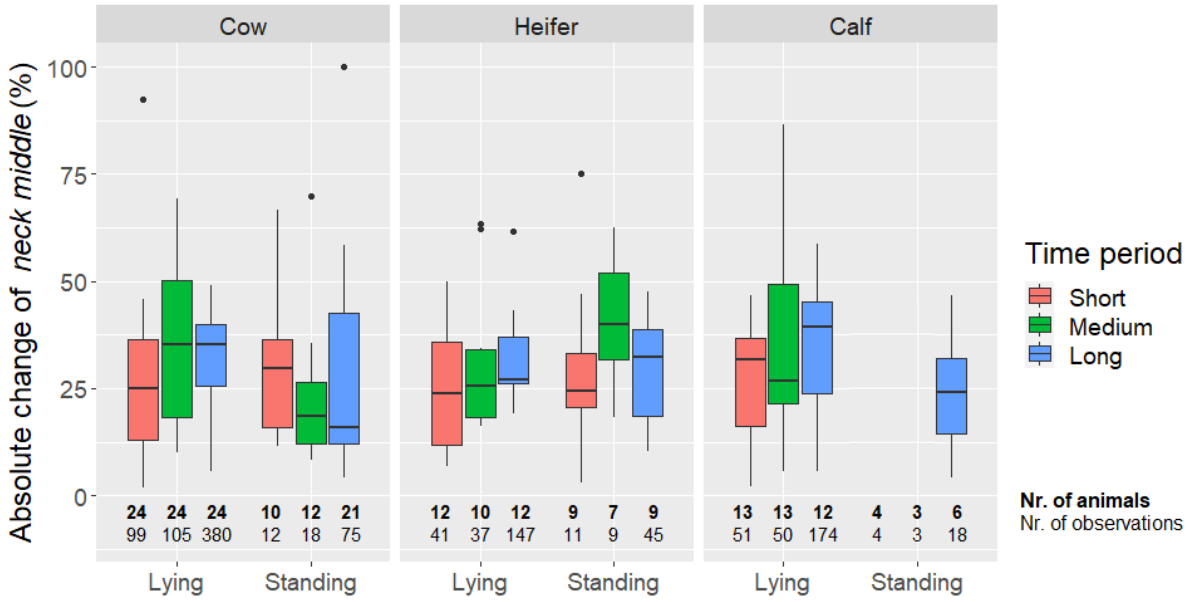

Figure 14 **Absolute changes of *neck middle*.** Changes of *neck middle* are given as absolute values describing the change in occurrences of *neck middle* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

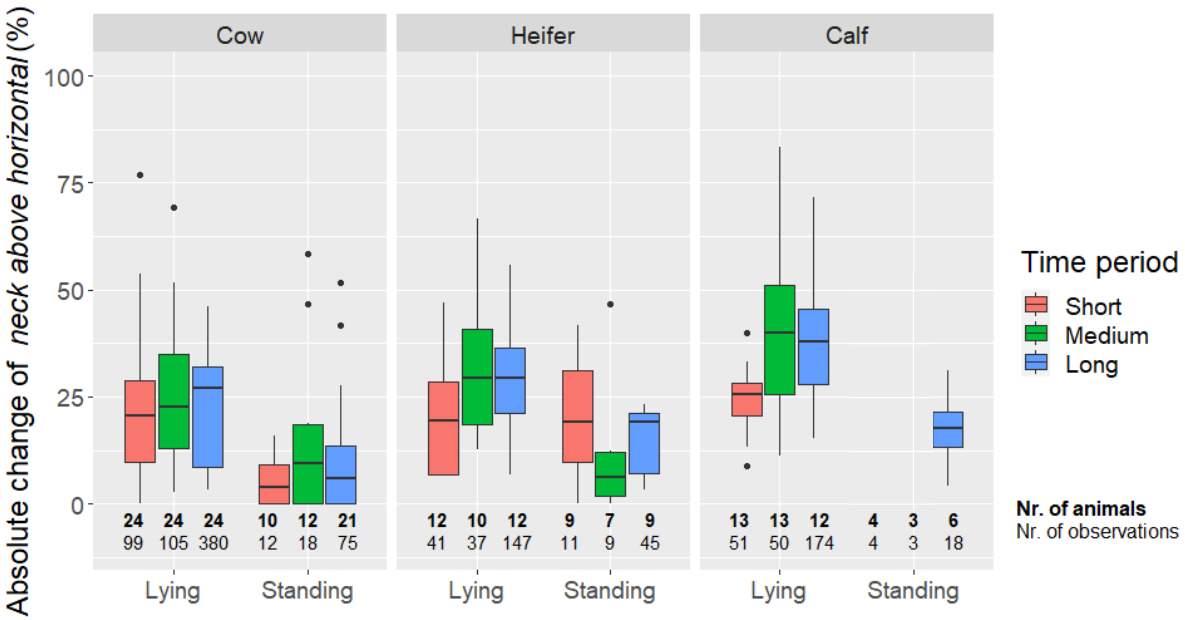

Figure 15 **Absolute changes of *neck above horizontal.*** Changes of *neck above horizontal* are given as absolute values describing the change in occurrences of *neck above horizontal* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

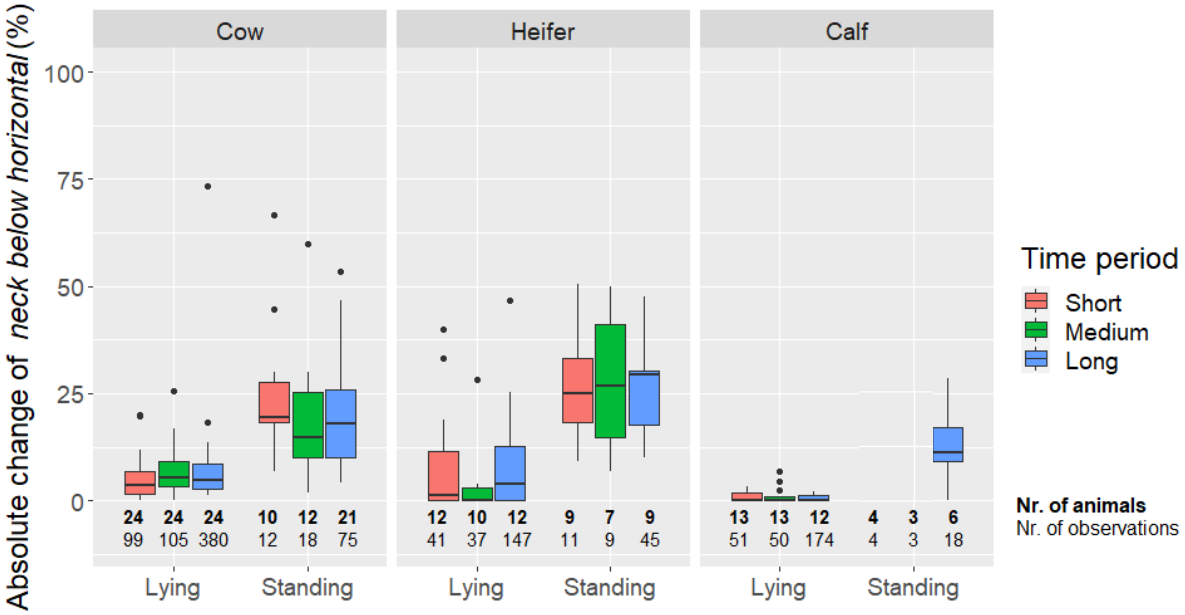

Figure 16 **Absolute changes of *neck below horizontal.*** Changes of *neck below horizontal* are given as absolute values describing the change in occurrences of *neck below horizontal* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

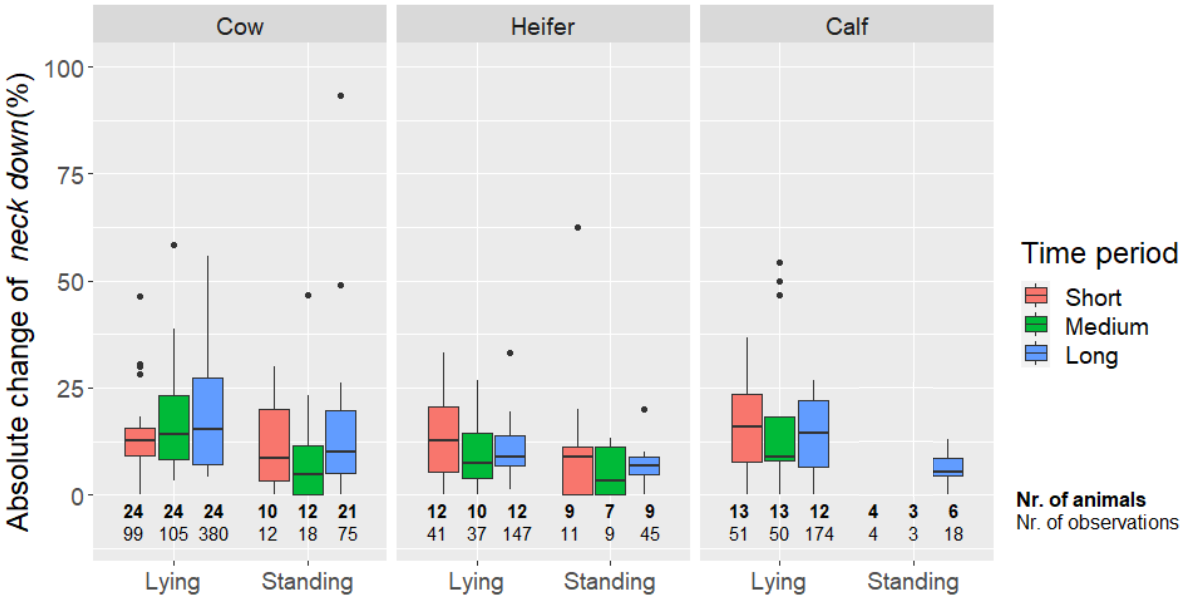

Figure 17 **Absolute changes of *neck down.*** Changes of *neck down* are given as absolute values describing the change in occurrences of *neck down* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

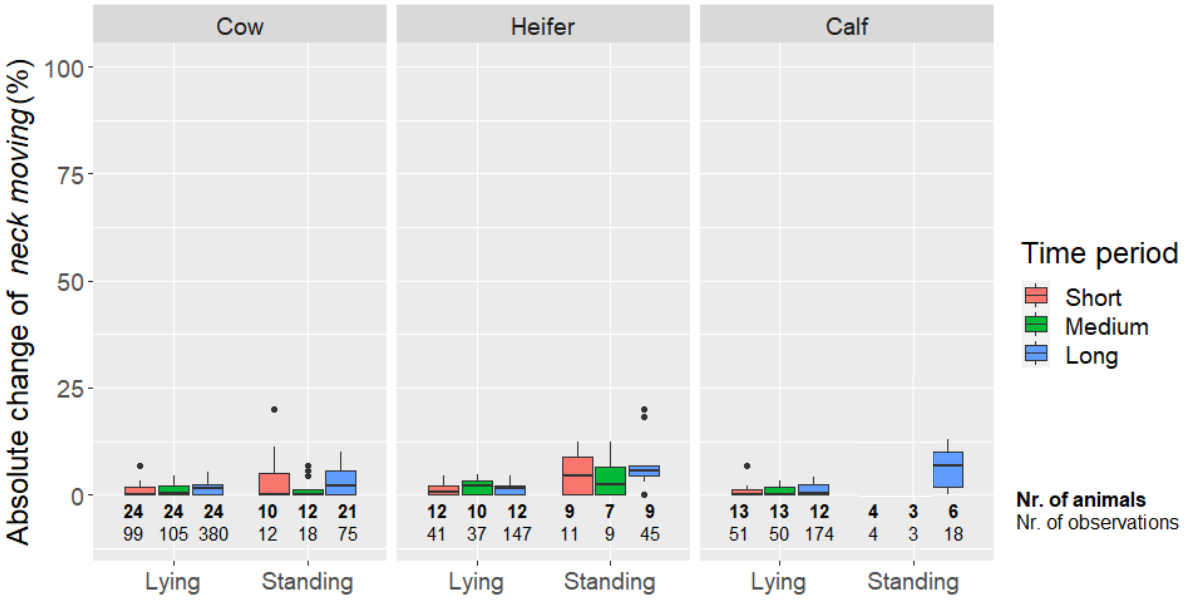

Figure 18 **Absolute changes of *neck moving.*** Changes of *neck moving* are given as absolute values describing the change in occurrences of *neck moving* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

3.2.5. Head orientation

Lying animals were mainly *facing neutral*, regardless of age category. The two other common head orientations were *folded* or *facing in*. Cows showed a *folded* posture more often than heifers and calves (mean cows = 11.1%; mean heifers= 5.1% mean calves=8.1%). Calves on the other hand showed more of the *facing in* head orientation than cows and heifers (mean cows=6.7%; mean heifers=5.3%; mean calves=11.2%). When lying, animals very seldomly showed a *moving head* orientation (mean=1.1%).

When standing, *facing straight* was the most dominating head orientation. *Facing left* and *facing right* showed almost equal occurrences across age categories. Standing animals *moved* their head more often than lying animals. The younger the age category, the higher was the occurrence of a *moving head*, when standing (mean cows=5.1%, mean heifer=9.3%, mean calves=14.8%) (Figure 19).

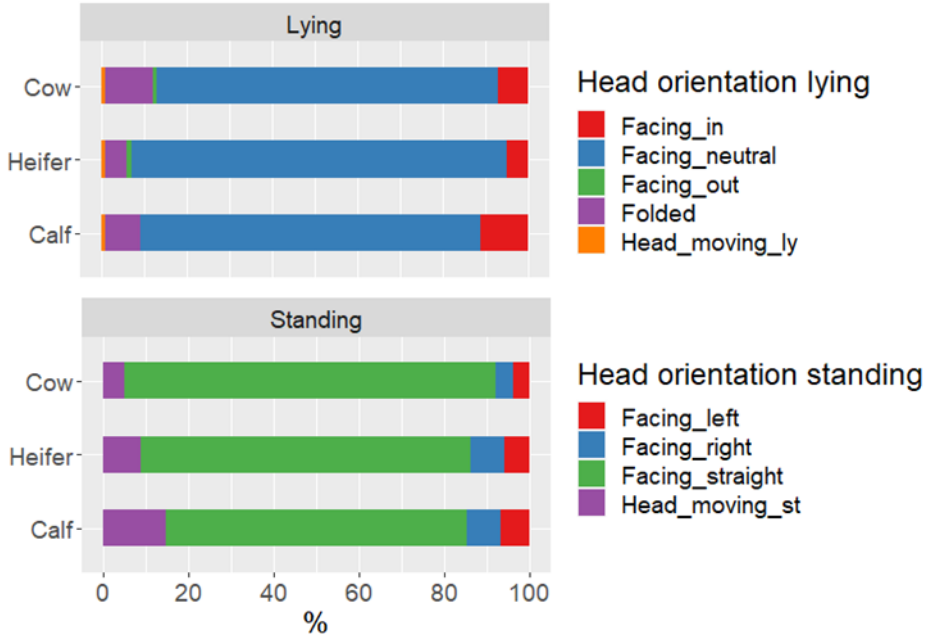

Figure 19 **Head orientations.** The mean occurrence (%) for head orientations is given per basic body position (lying, standing) and age category (cow, heifer, calf). Note that the ethogram for head orientation differed for lying and standing animals.

Regarding *facing neutral* (Figure 20), the dominating head orientiation in lying animals, no clear pattern for absolute changes could be seen across time periods for different age categories. However, calves showed a greater absolut change of *facing neutral* than cows and heifers, which is displayed by a larger box height. The same is true for *facing in* head orientation. Additionally heifers showed the least change during *facing neutral* and *folded* head orientation. *Facing out* and *head moving* occurred only during few observations, therefore absolut changes were small (Figure 20 -Figure 24).

For standing animals, cows showed less absolut change of *facing straight* than heifers and calves. For calves, this was only assesed for long periods of time. During *facing right* and *facing left* as well as *head moving* no clear pattern could be seen across age categories or time periods (Figure 25 - Figure 28).

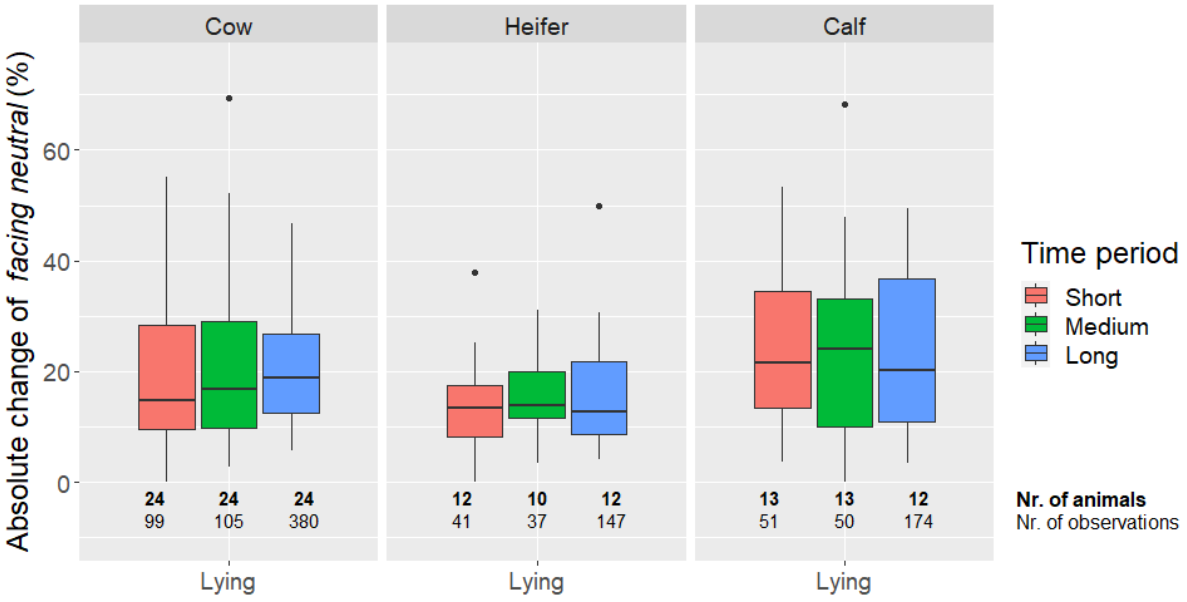

Figure 20 **Absolute changes of *facing neural, lying*.** Changes of *facing neutral* are given as absolute values describing the change in occurrences of *facing neutral* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

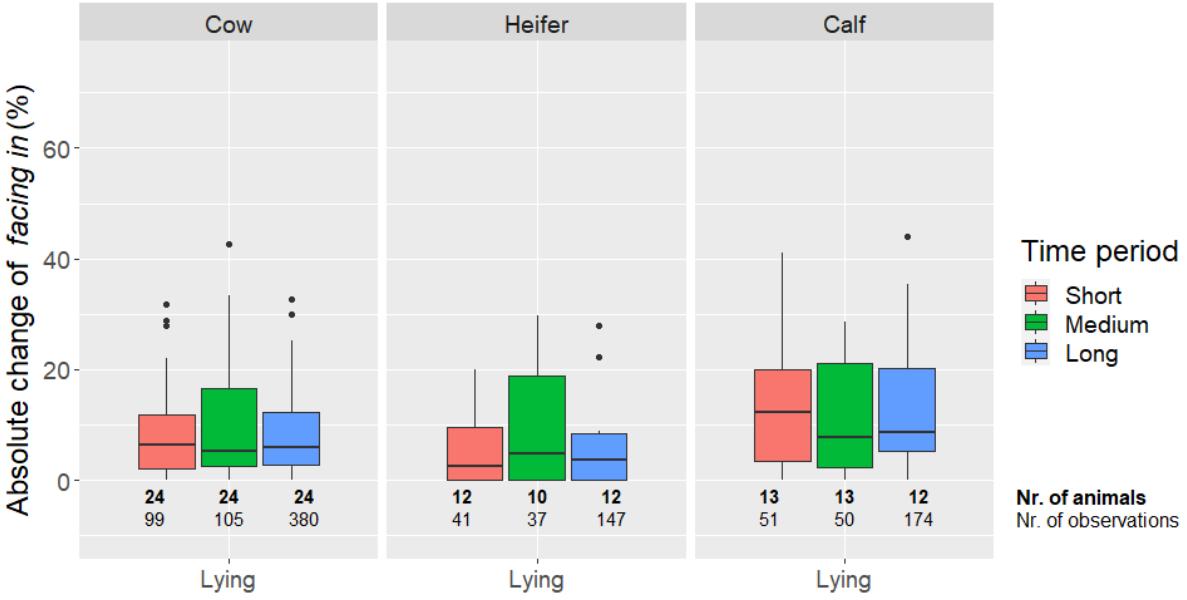

Figure 21 **Absolute changes of *facing in, lying*.** Changes of *facing in* are given as absolute values describing the change in occurrences of *facing in* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

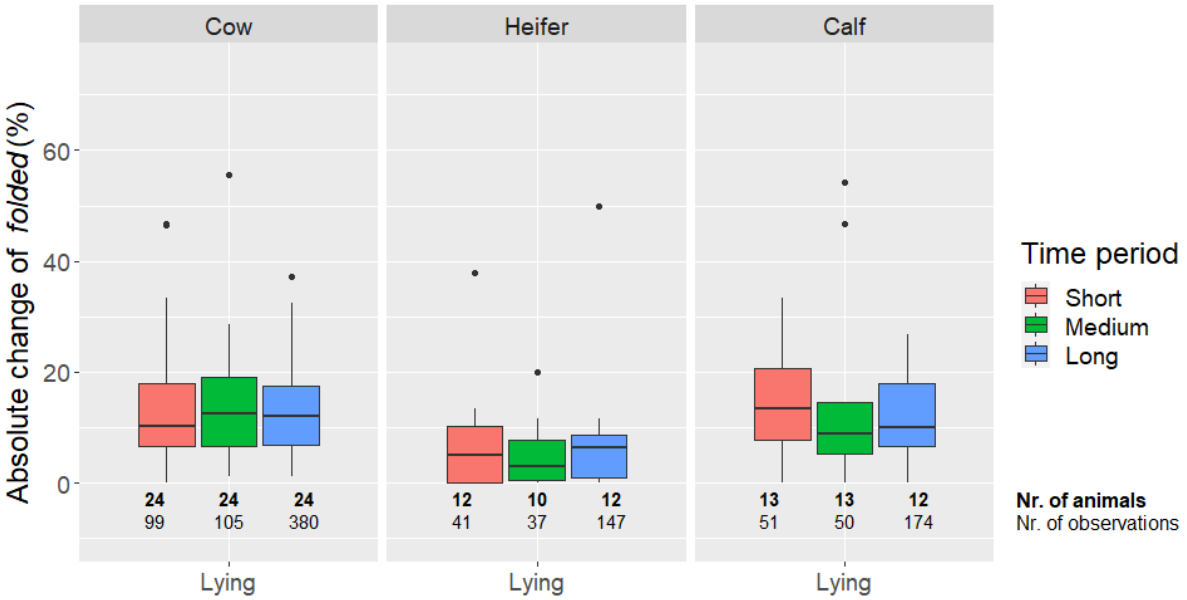

Figure 22 **Absolute changes of *folded, lying*.** Changes of *folded* are given as absolute values describing the change in occurrences of *folded* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

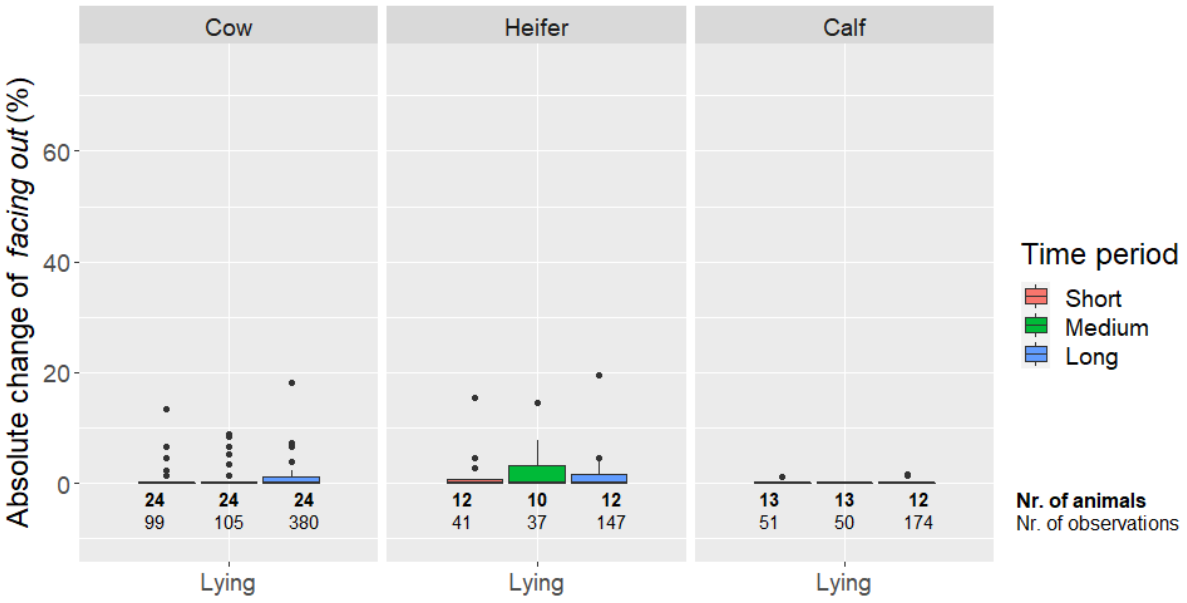

Figure 23 **Absolute changes of *facing out, lying*.** Changes of *facing out* are given as absolute values describing the change in occurrences of *facing out* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

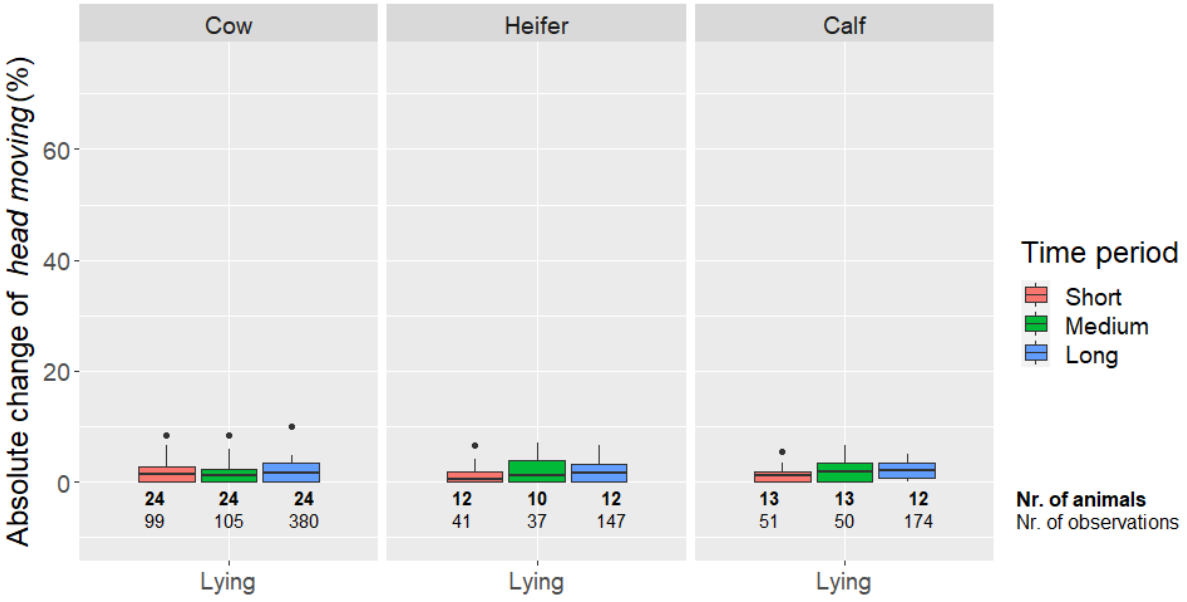

Figure 24 **Absolute changes of *head moving, lying*.** Changes of *head moving* are given as absolute values describing the change in occurrences of *head moving* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

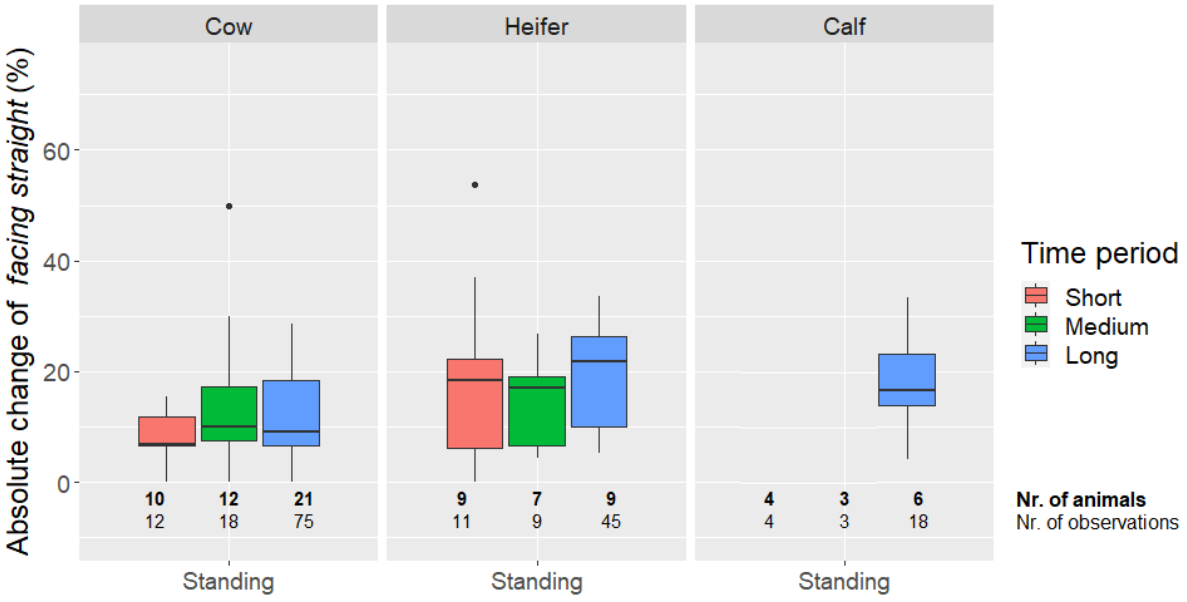

Figure 25 **Absolute changes of *facing straight, standing*.** Changes of *facing straight* are given as absolute values describing the change in occurrences of *facing straight* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

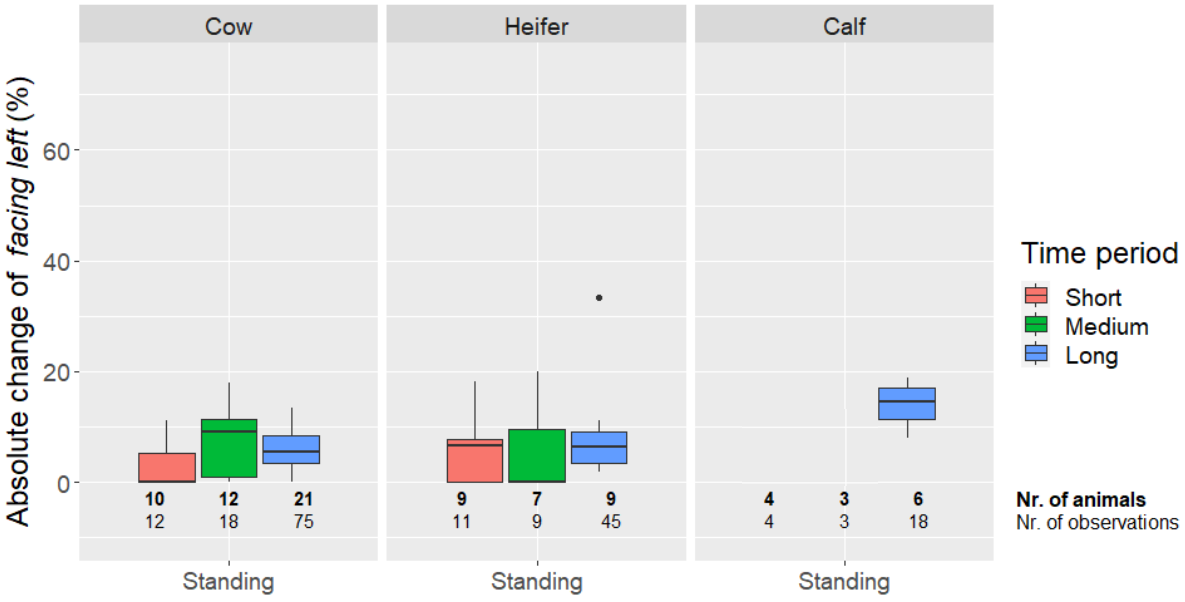

Figure 26 **Absolute changes of *facing left, standing.*** Changes of *facing left* are given as absolute values describing the change in occurrences of *facing left* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

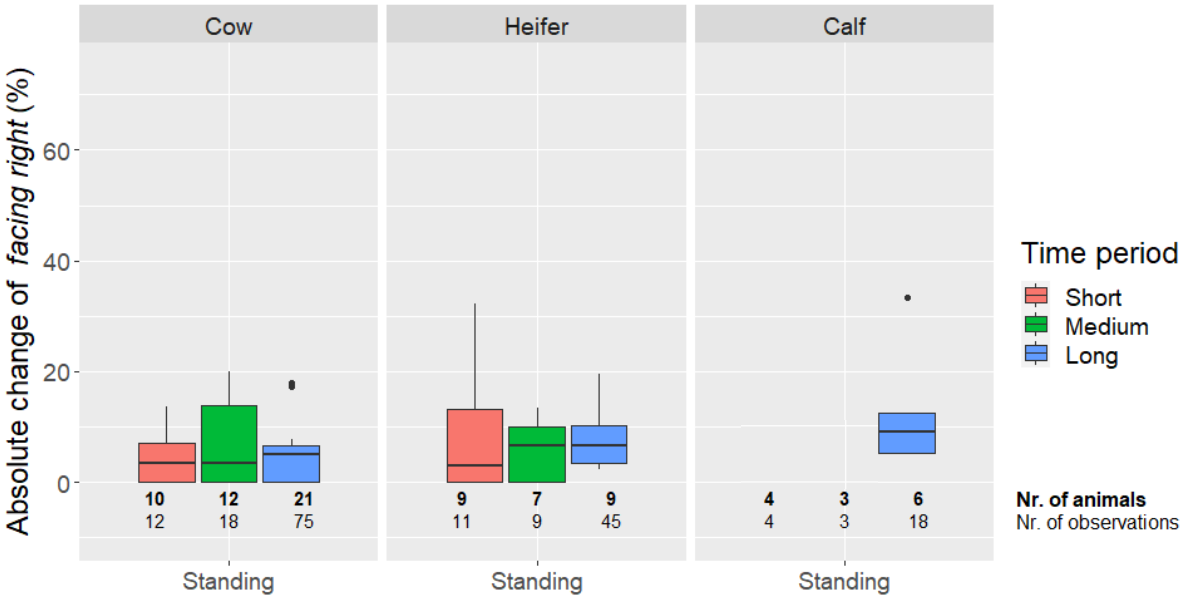

Figure 27 **Absolute changes of *facing right, standing*.** Changes of *facing right* are given as absolute values describing the change in occurrences of *facing right* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

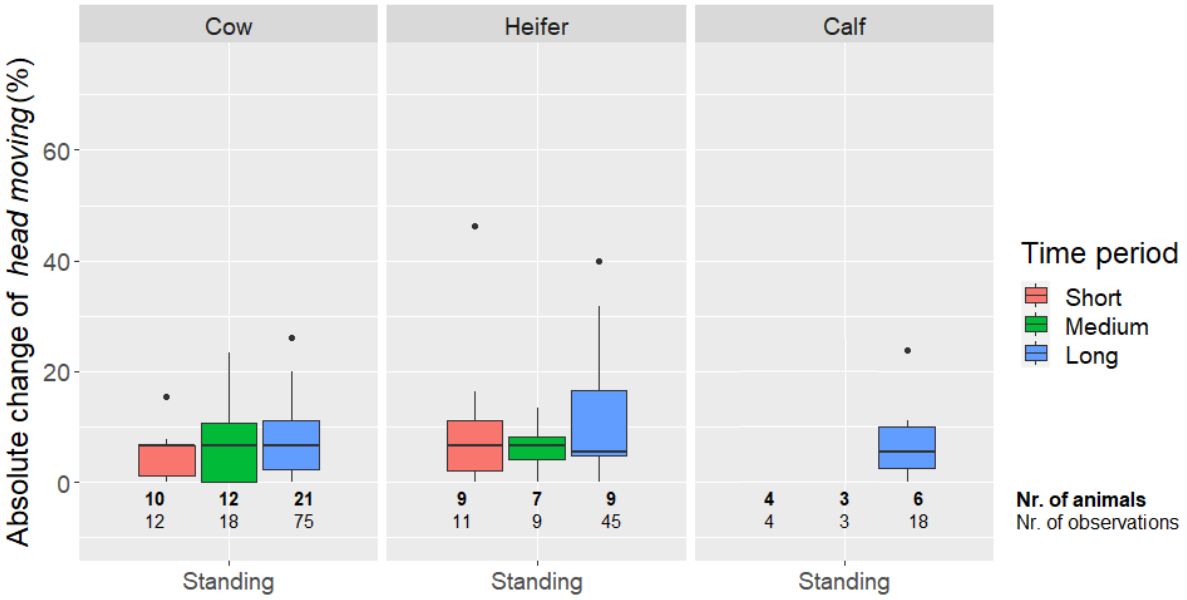

Figure 28 **Absolute changes of *head moving, standing*.** Changes of *head moving* are given as absolute values describing the change in occurrences of *head moving* for the three age categories (cow, heifer, calf) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

3.2.6. Head contact

The head of lying animals was mostly not in contact with the floor or any other obstacle/animal (mean *no contact* lying= 85.4%; mean *no contact* standing = 96.8%). If in contact, then slightly more often the head was in contact with the floor than with an obstacle or animal. Overall, lying cows were most often in contact with the floor or obstacles/animals, followed by lying calves and lying heifers (mean cows =10.1% heifers =5.0% calves =6.8%). The younger the age category of standing animals, the more the head was in contact with an obstacle/animal (Figure 29).

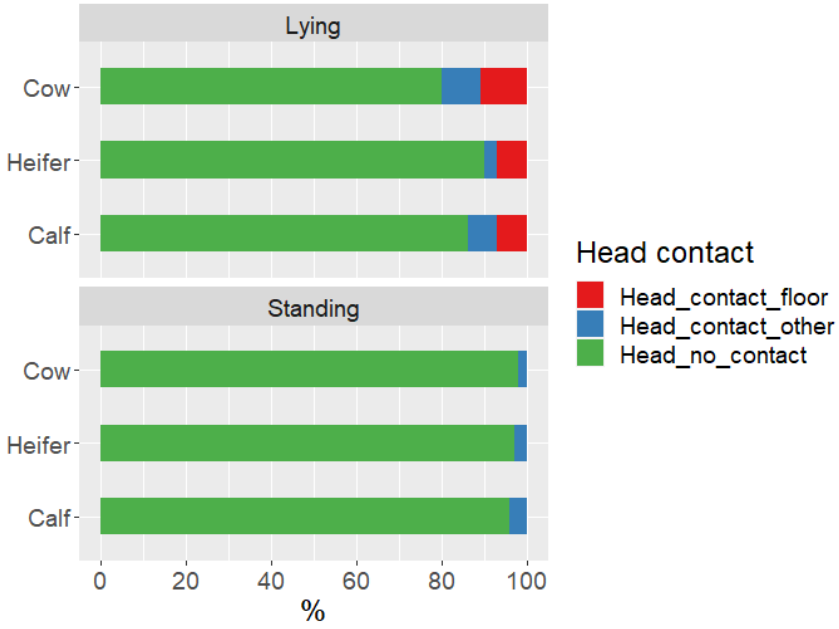

Figure 29 **Head contact.** The occurrence (%) of head contacts is given per basic body position (lying, standing) and age category (cow, heifer, calf).

Changes in *no head contact* were greater in lying animals, than in standing animals across all age categories. No clear pattern regarding time periods could be identified. Lying cows and calves showed a greater change than heifers. Since *head contact floor* mostly only occurred when lying, little data for standing animals were visible. Lying cows showed the greatest change in *head contact floor* posture over time. Head in *contact with other* (obstacles/animals) occurred in all age categories and basic body positions but showed the highest variability in change in lying cows (Figure 30- Figure 32).

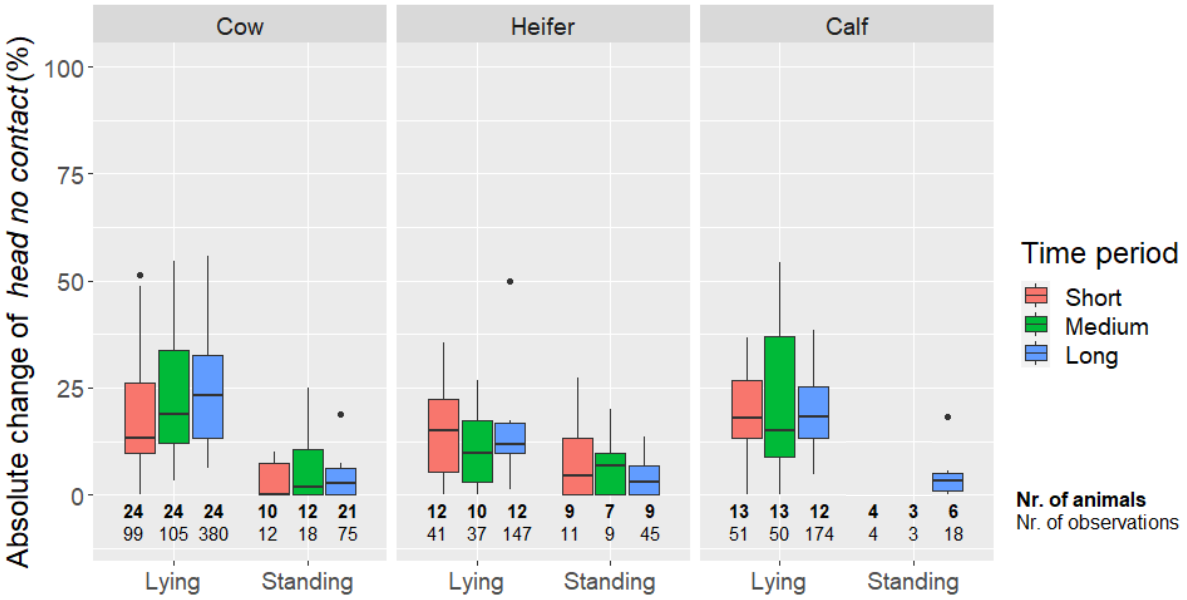

Figure 30 **Absolute changes of *head no contact*.** Changes of *head no contact* are given as absolute values describing the change in occurrence of *head no contact* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

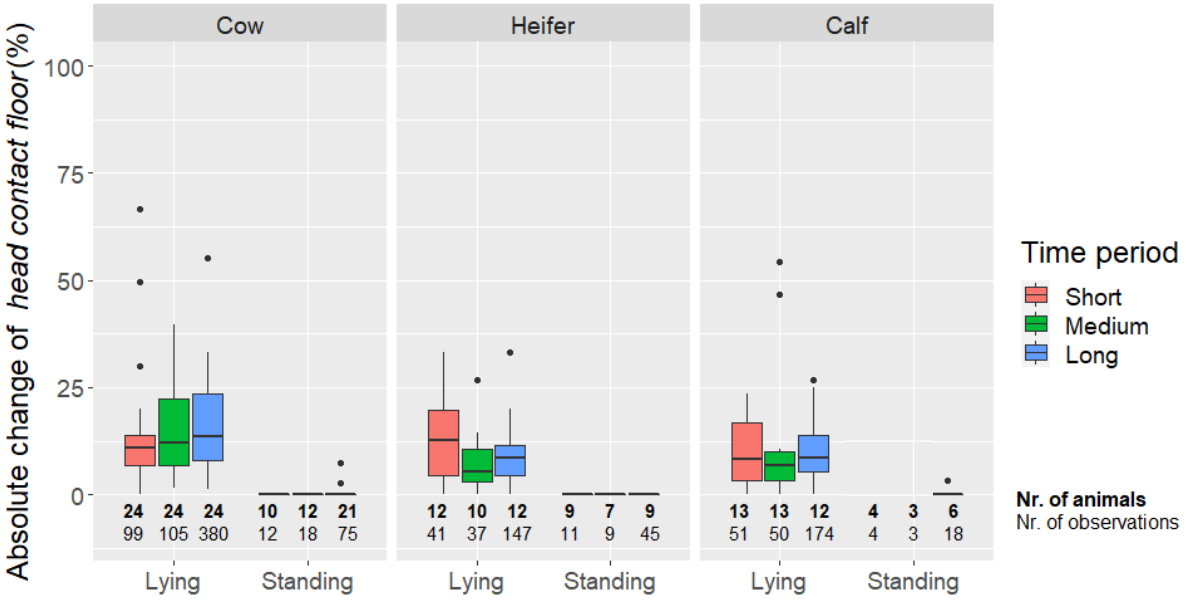

Figure 31 **Absolute changes of *head contact floor*.** Changes of *head contact floor* are given as absolute values describing the change in occurrence of *head contact floor* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

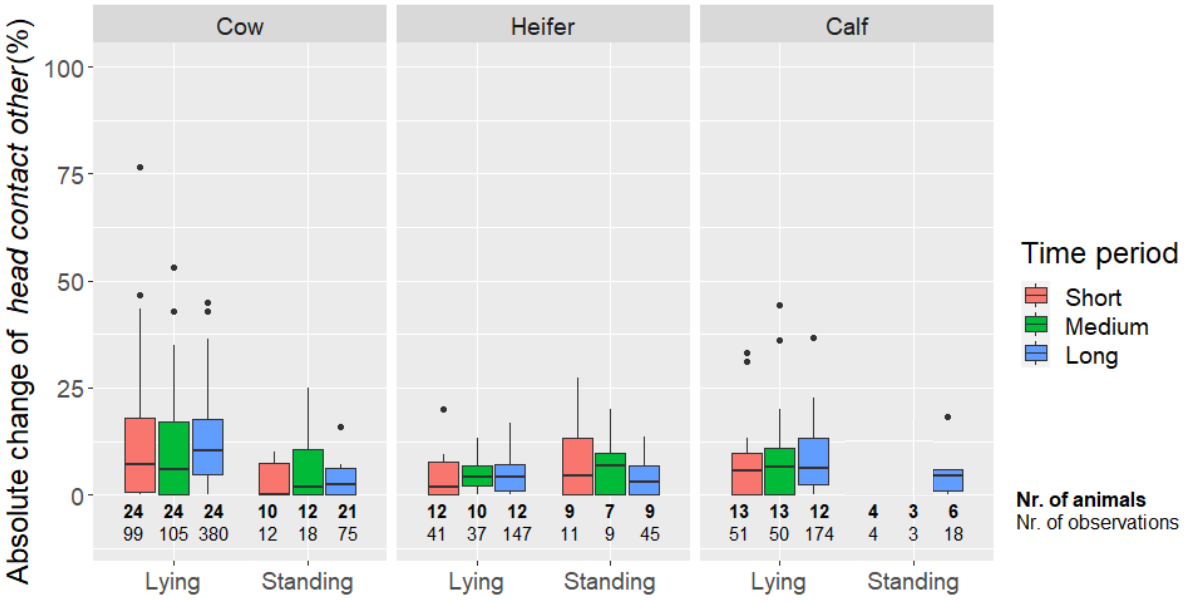

Figure 32 **Absolute changes of *head contact other*.** Changes of *head contact other* are given as absolute values describing the change in occurrence of *head contact other* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

3.2.7. Facial Expressions

3.2.7.1. Ear postures

Comparing different basic body positions, the percentage of occurrence of the different ear postures differed between standing and lying animals. The most frequent ear posture across all age categories for lying as well as for standing animals was ears *backwards* (mean lying= 65.2 ±6.0%; mean standing = 42.6 ±8.11%). When lying, ears *axial* was the second most occurring ear posture (mean = 16.7 ±5.5%), *ears forwards* when standing (mean =21.3 ±14.2%. More *moving*, *forwards* and *asymmetric* ear postures were observed in standing animals than in lying animals. This pattern was seen in all age categories, but strongest in calves where ears *forwards* (mean=37.2%) occurred more frequently than ears *backwards* (mean=34.0%) in standing animals. *Drooping* ears were generally seen rarely, and if, then mostly in lying cows (overall mean=0.4 ±0.8%; lying cows mean=2.0%). Comparing animal categories with each other, in both basic body positions (standing and lying) calves showed a higher occurrence of different postures than heifers and cows (Figure 33).

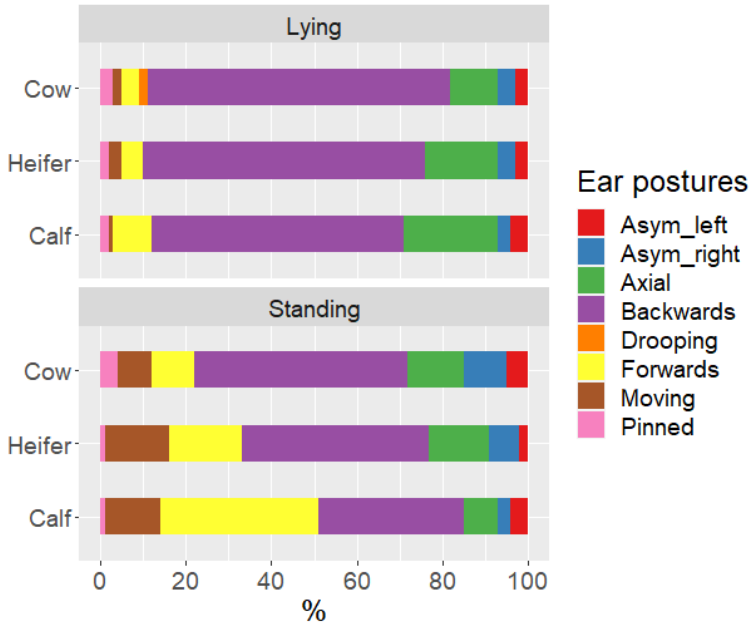

Figure 33 **Ear postures**. The mean occurrence (%) of ear postures is given per basic body position (lying, standing) and age category (cow, heifer, calf).

For ears *forwards* and *ears moving* lying cows and heifers showed less variability in change across all time periods than standing animals. Furthermore, the mean absolute change was similar or higher in standing animals. Other ear postures did not show this pattern. A result for calves was not available, since only a low number of standing calves were observed. When lying, calves and heifers showed a similar or higher absolute change than cows for ears *forwards,* ears *backwards* and ears *axial* across all time periods. Since ears *drooping* nearly only occurred in cows, little change in occurrences could be displayed for heifers and calves (Figure 34 - Figure 41).

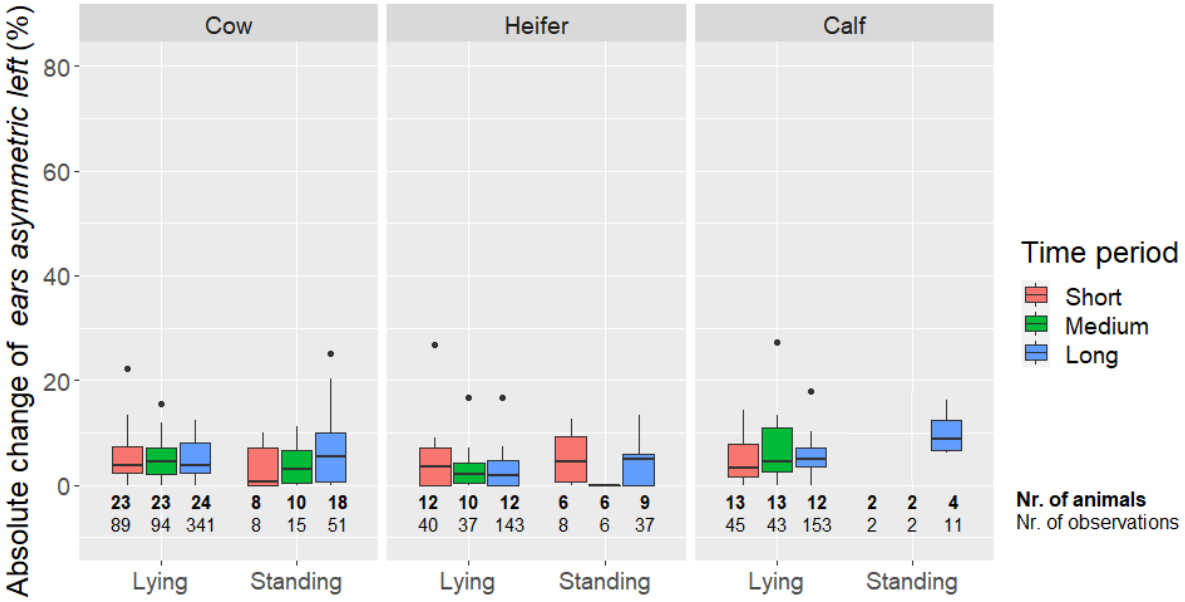

Figure 34 **Absolute changes of *ears asymmetric left*.** Changes of *ears asymmetric left* are given as absolute values describing the change in occurrences of *ears asymmetric left* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

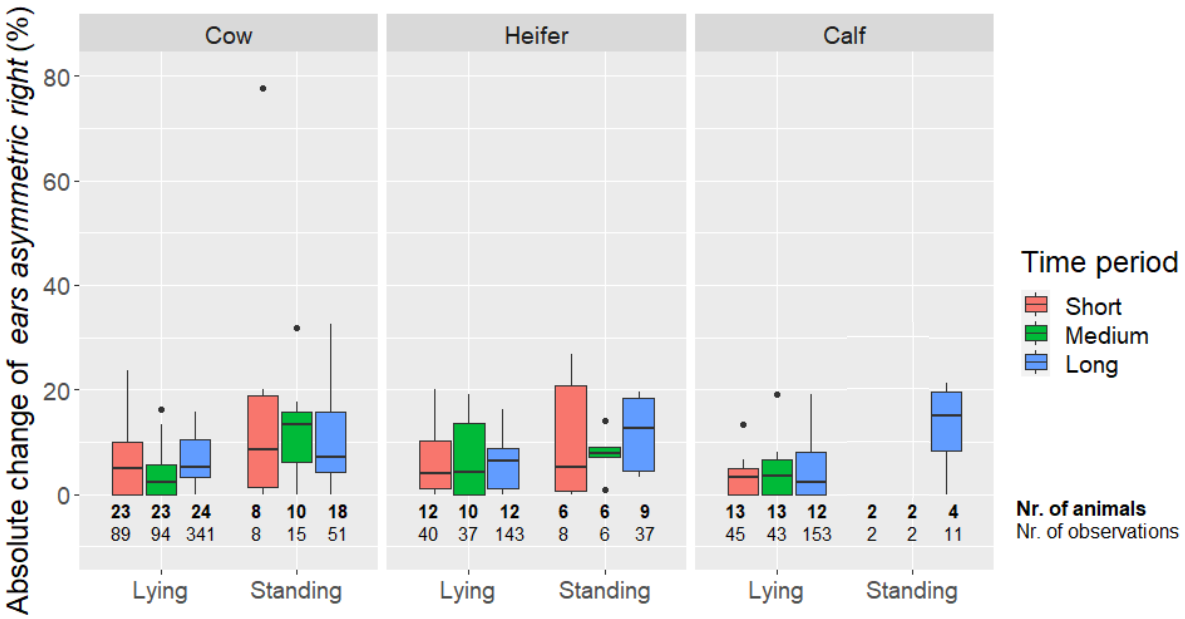

Figure 35 **Absolute changes of *ears asymmetric right*.** Changes of *ears asymmetric right* are given as absolute values describing the change in occurrences of *ears asymmetric right* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

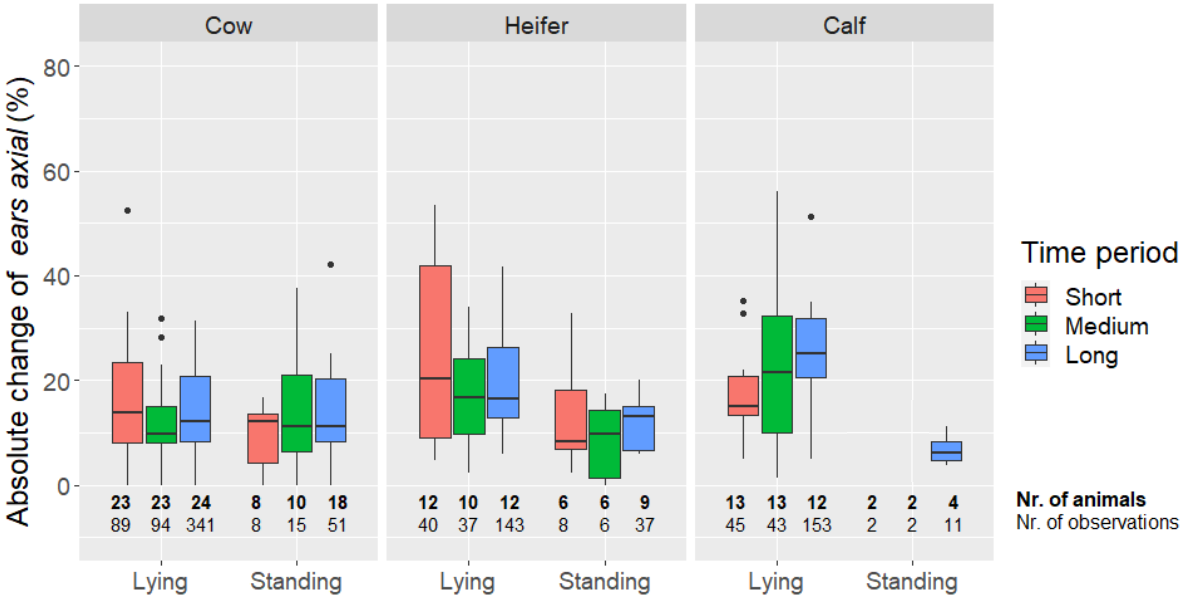

Figure 36 **Absolute changes of ears axial.** Changes of *ears axial* are given as absolute values describing the change in occurrences of *ears axial* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

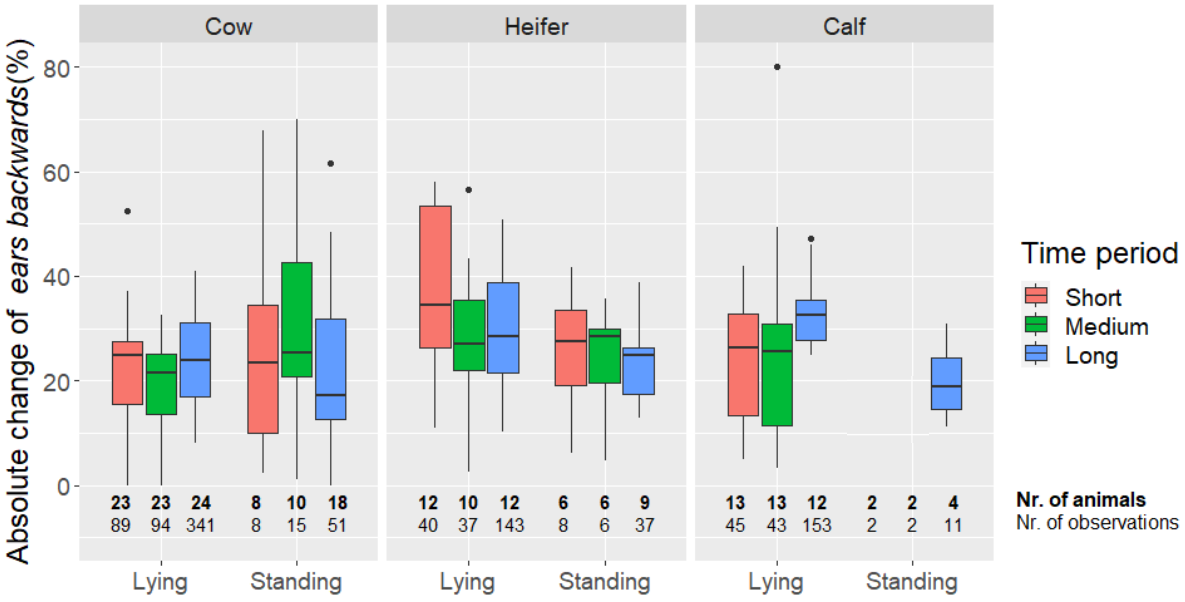

Figure 37 **Absolute changes of ears backwards.** Changes of *ears backwards* are given as absolute values describing the change in occurrences of *ears backwards* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

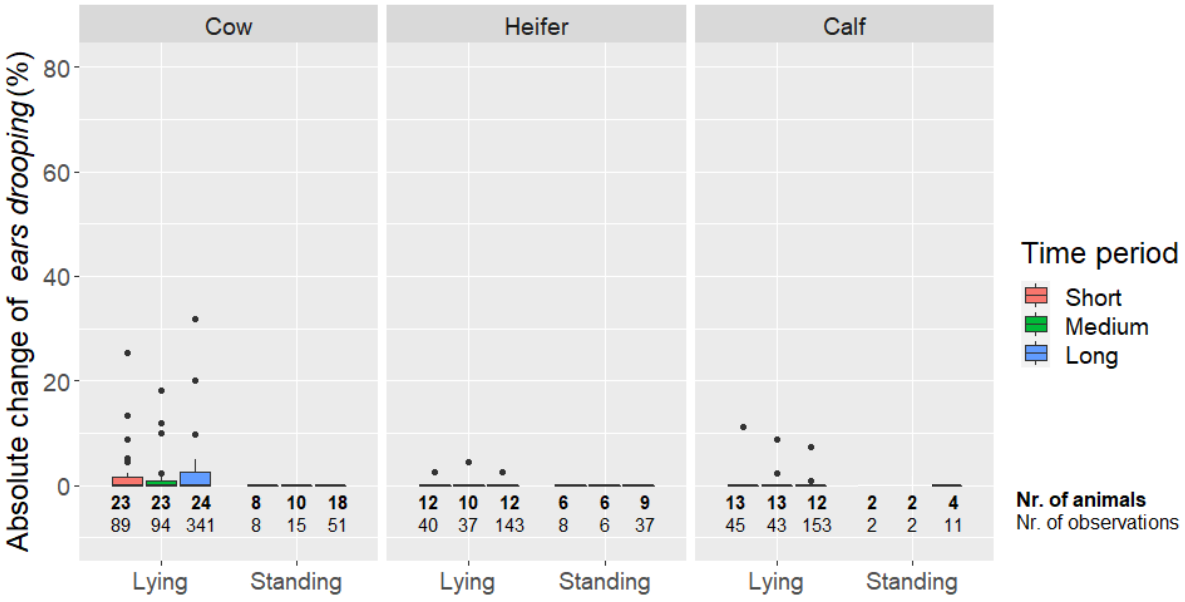

Figure 38 **Absolute changes of ears drooping.** Changes of *ears drooping* are given as absolute values describing the change in occurrences of *ears drooping* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

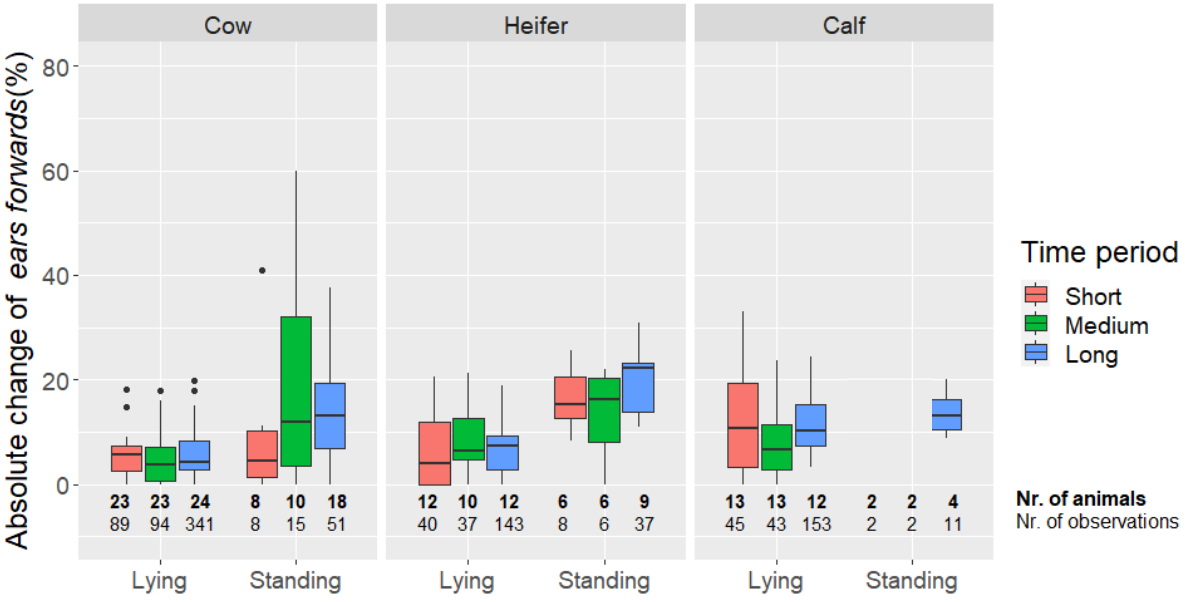

Figure 39 **Absolute changes of ears forwards.** Changes of *ears forwards* are given as absolute values describing the change in occurrences of *ears forwards* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

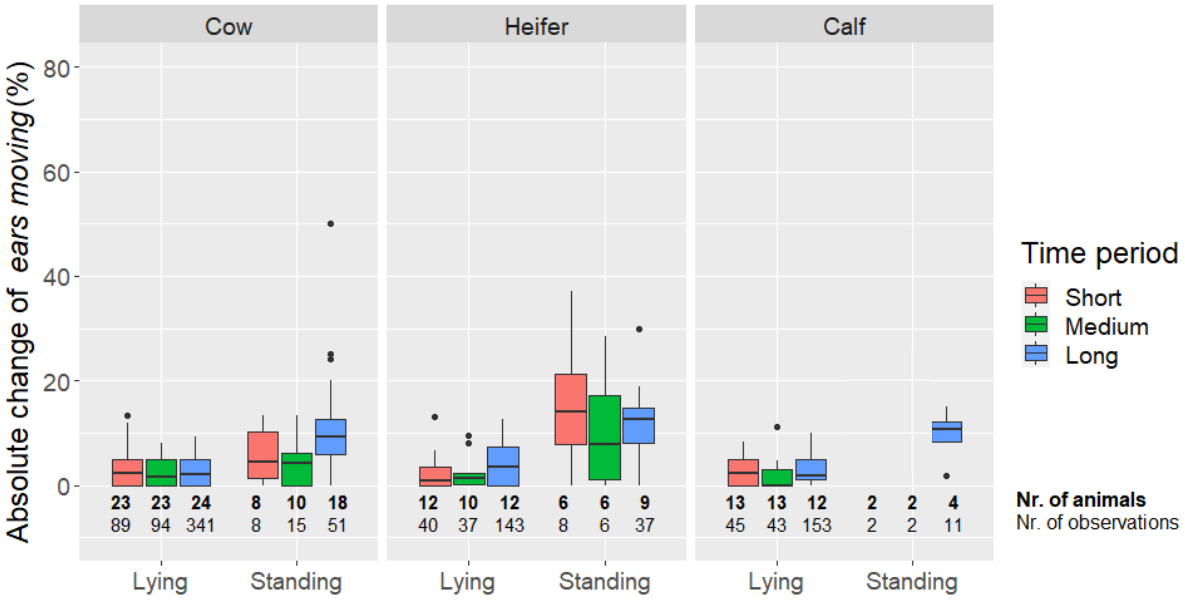

Figure 40 **Absolute changes of ears moving.** Changes of *ears moving* are given as absolute values describing the change in occurrences of *ears moving* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

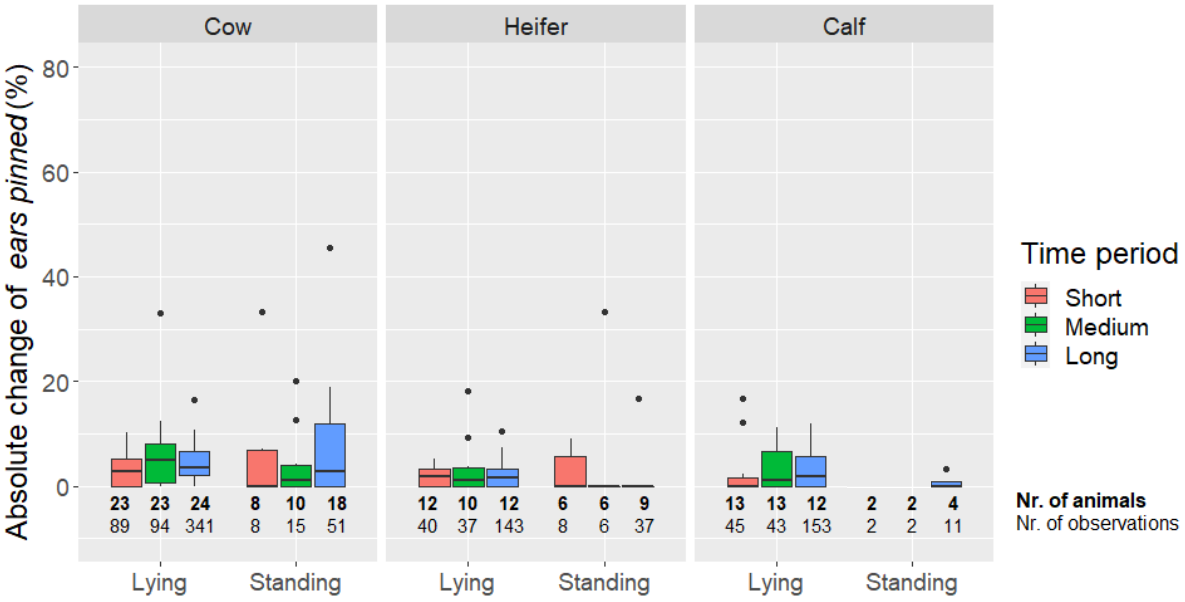

Figure 41 **Absolute changes of ears pinned.** Changes of *ears pinned* are given as absolute values describing the change in occurrences of *ears pinned* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

3.2.7.2. Eye closure

Closed eyes were almost never seen in standing animals, regardless of age category. Slight differences per age category were observed for *closed eyes* in lying animals. Cows showed *closed eyes* slightly more often than heifers and calves (mean cows = 9.0%, mean heifers=7.4% mean calves=7.0%) (Figure 42).

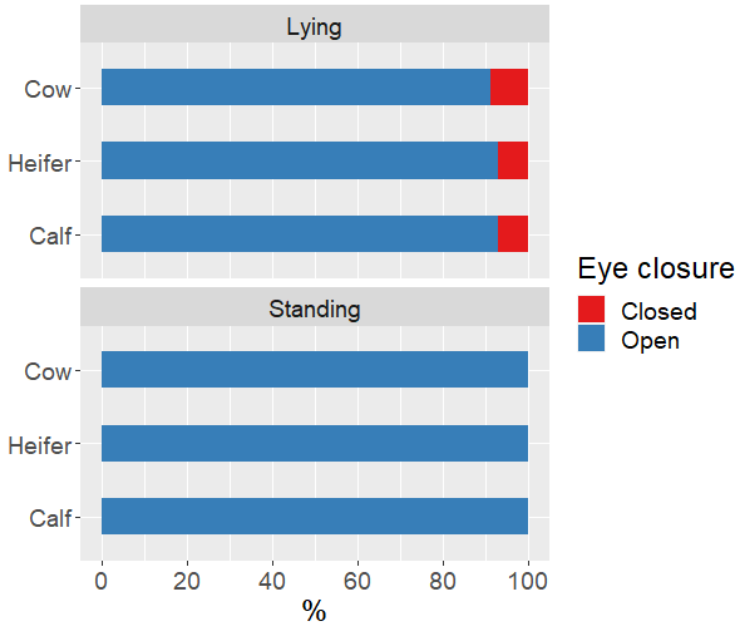

Figure 42 **Eye closure.** The mean occurrence (%) of eye closure (i.e. *eyes open* and *eyes closed*) is given per basic body position (lying, standing) and age category (cow, heifer, calf).

A change in eye closure rarely occurred when animals were standing since their eyes were mostly *open*. Therefore, small or no boxes are shown for standing animals. Across all time periods, lying cows showed a greater range of absolute changes of eye closure than lying heifers and calves. No clear pattern could be distinguished between short, medium or long time periods of time regarding eye closure. Animals within one age category were similarly likely to change eye closure i.e. from *open* to *closed* or vice versa, from one day to the next as to, for example, three days or two weeks later (Figure 43).

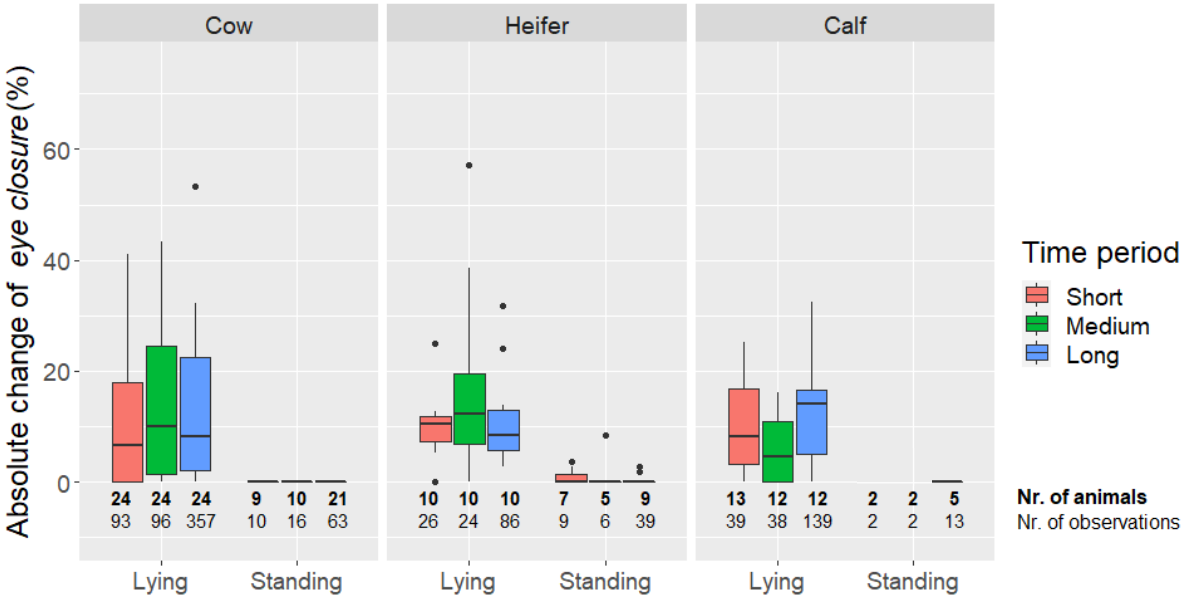

Figure 43 **Absolute changes of eye closure.** Changes of eye closure are given as absolute values describing the change in occurrence of eye closure for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

3.2.8. Tail postures and lifts

3.2.8.1. Tail postures

Lying animals mainly showed the posture *tail tucked between legs. Tail hanging* off the cubicle was seen in less than 10% of all observations across age categories. If the tail was *wagged* when lying, then predominantly rather by cows (2.7%) than heifers and calves. *Tail wagging* was seen more often in standing animals than in lying animals. Here the younger the animal, the less often *tail wagging* was observed (mean cows= 15.6%; mean heifers=12.8%, mean calves=7.0%). If shown, then t*ail tucked between legs* when standing was seen predominantly in calves, seldomly in heifers and cows (mean cows=1.5%, mean heifers=0.1%, mean calves=8.6%) (Figure 44).

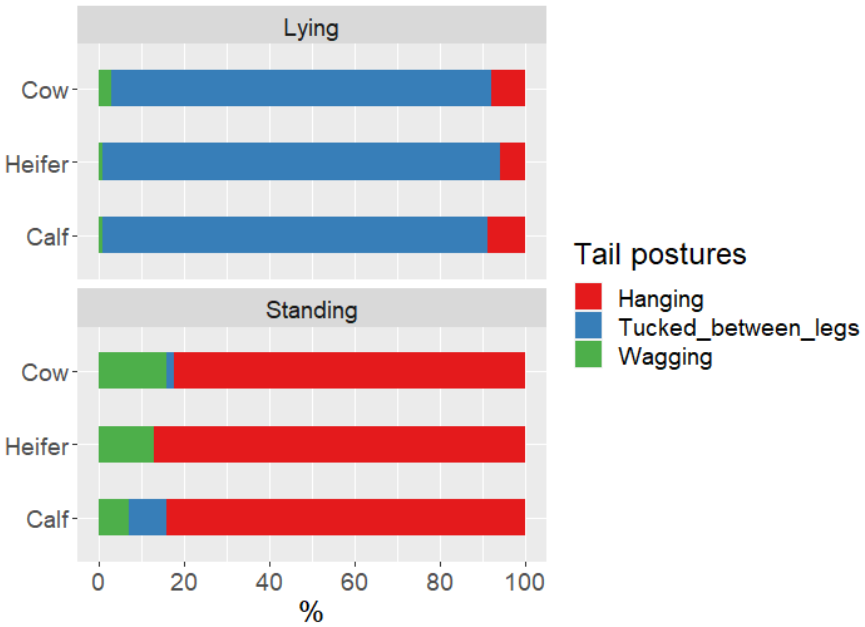

Figure 44 **Tail postures.** The occurrence (%) of tail postures is given per basic body position (lying, standing) and age category (cow, heifer, calf).

Calves in standing position were rarely observed in general, therefore changes in *tail hanging* posture were given for standing cows and heifers only. Absolute changes of *tail hanging* and *tail wagging* were generally greater in standing animals. For *tail wagging*, changes were small in lying animals and greater in standing animals. Changes over time in *tail tucked between legs* posture were rarely documented in standing animals. Lying cows however showed a greater change regarding *tail tucked between legs* when lying than heifers and calves. In addition, they showed less variation in change which was indicated by a smaller height of the boxes-and-whisker graph (Figure 45- Figure 47).

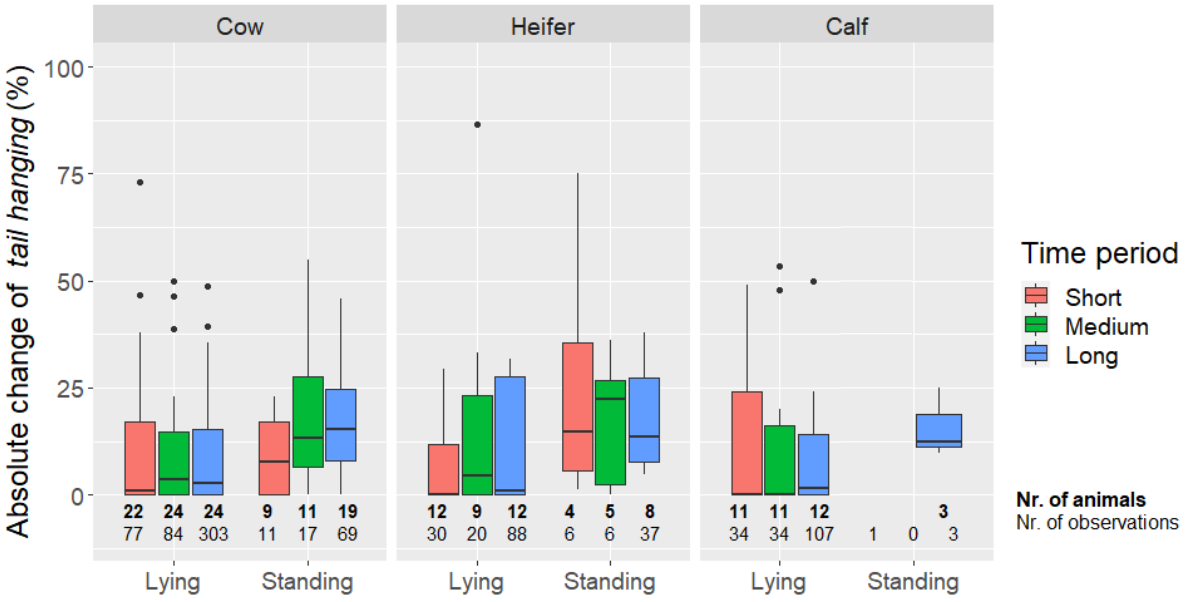

Figure 45 **Absolute changes of *tail hanging.*** Changes of *tail hanging* are given as absolute values describing the change in occurrences of *tail hanging* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

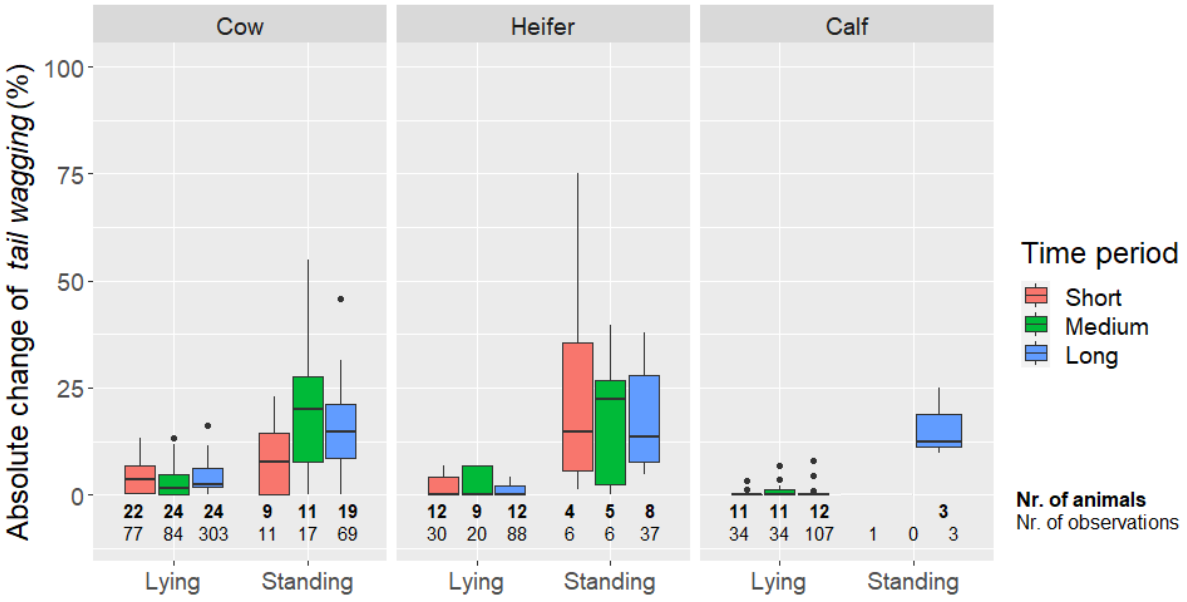

Figure 46 **Absolute changes of *tail wagging.*** Changes of *tail wagging* are given as absolute values describing the change in occurrences of *tail wagging* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

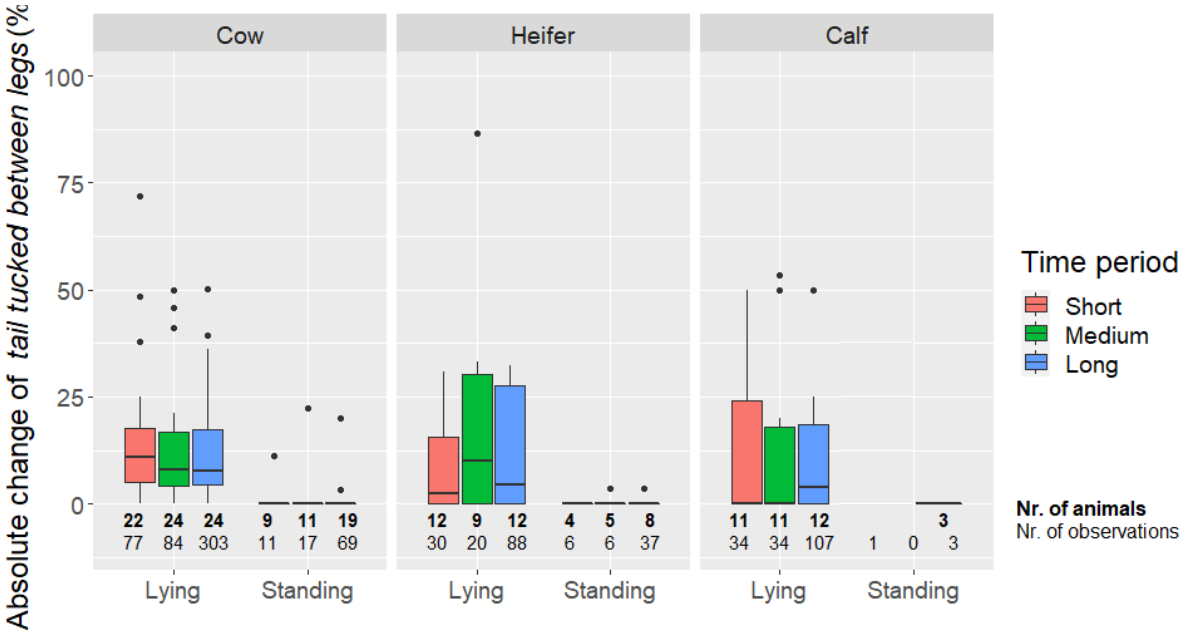

Figure 47 **Absolute changes of *tail tucked between legs.*** Changes of *tail tucked between legs* are given as absolute values describing the change in occurrences of *tail tucked between legs* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

3.2.8.2. *Tail lifts*

Tails were almost never *lifted* when lying. Standing animals showed *lifted tails* in 10.2 ±3.3% of observations (Figure 48).

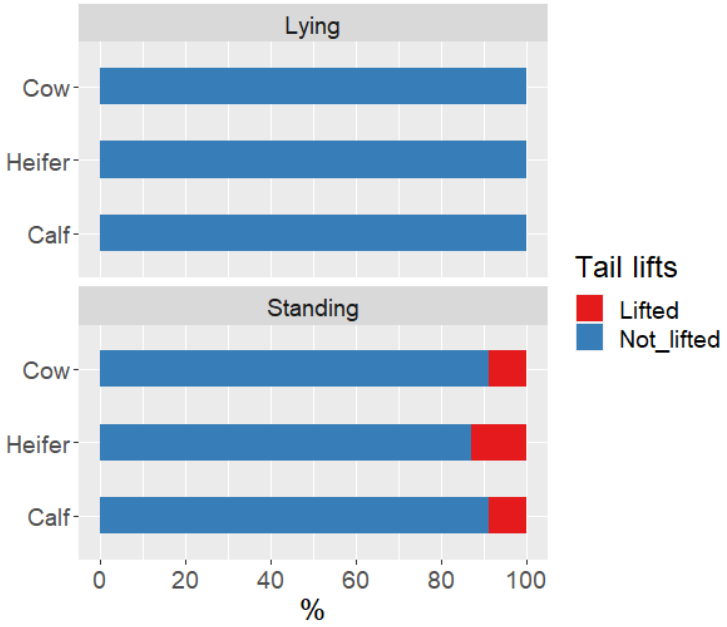

Figure 48 **Tail lifts.** The occurrence (%) of tail lifts is given per basic body position (lying, standing) and age category (cow, heifer, calf).

Accordingly, changes of tail lifts could only be seen for standing animals. Cows showed slightly less absolute change in tail lifts than heifers; calves were not observed often enough to draw a conclusion (Figure 49).

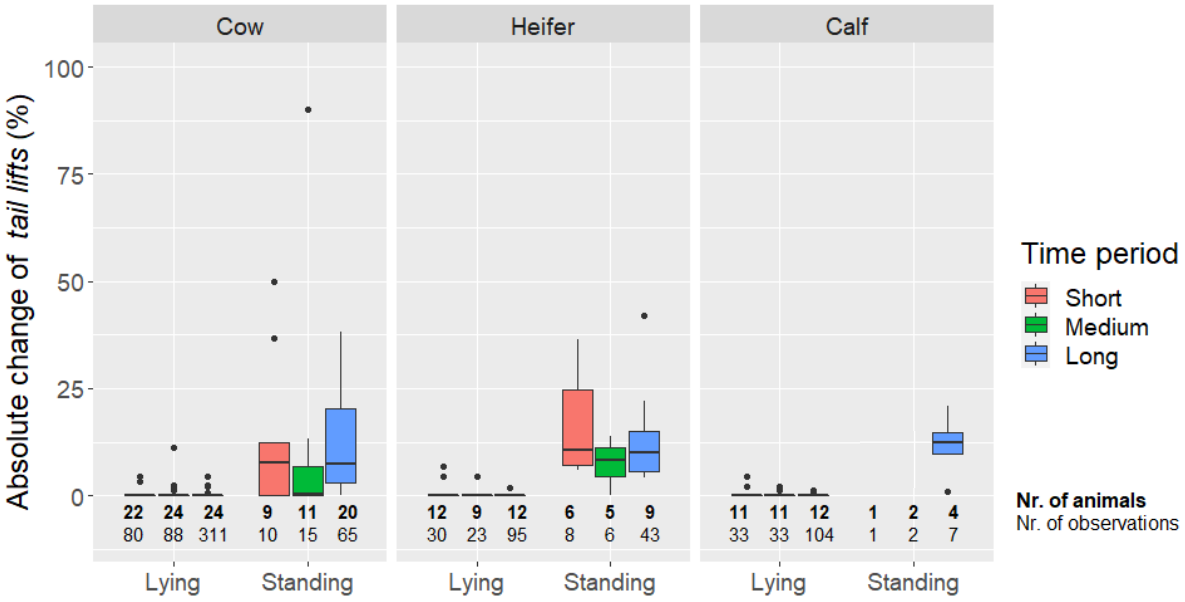

Figure 49 **Absolute changes of *tail lifts.*** Changes of *tail lifts* are given as absolute values describing the change in occurrences of *tail lifts* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the number of animals and total number of observations underlying each boxplot.

3.2.9. Specific postures of lying animals

3.2.9.1. Lying sides

On average across all age categories animals were lying on their left in 53.7% of all observations. Cows were lying on their left more than half of the observations; heifers chose the right side more than half of the observations. Calves were lying on their left in more than 60% of the observations (Figure 50).

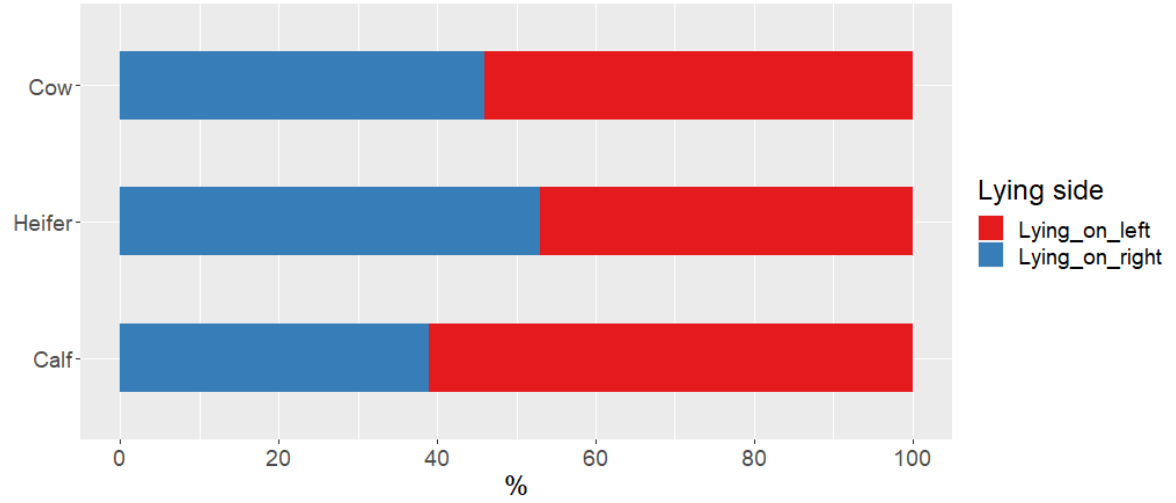

Figure 50 **Lying sides.** The occurrence (%) of lying sides is given per basic body position (lying, standing) and age category (cow, heifer, calf).

Across all age categories the variability of change in lying side was smallest for a long period of time (Figure 51).

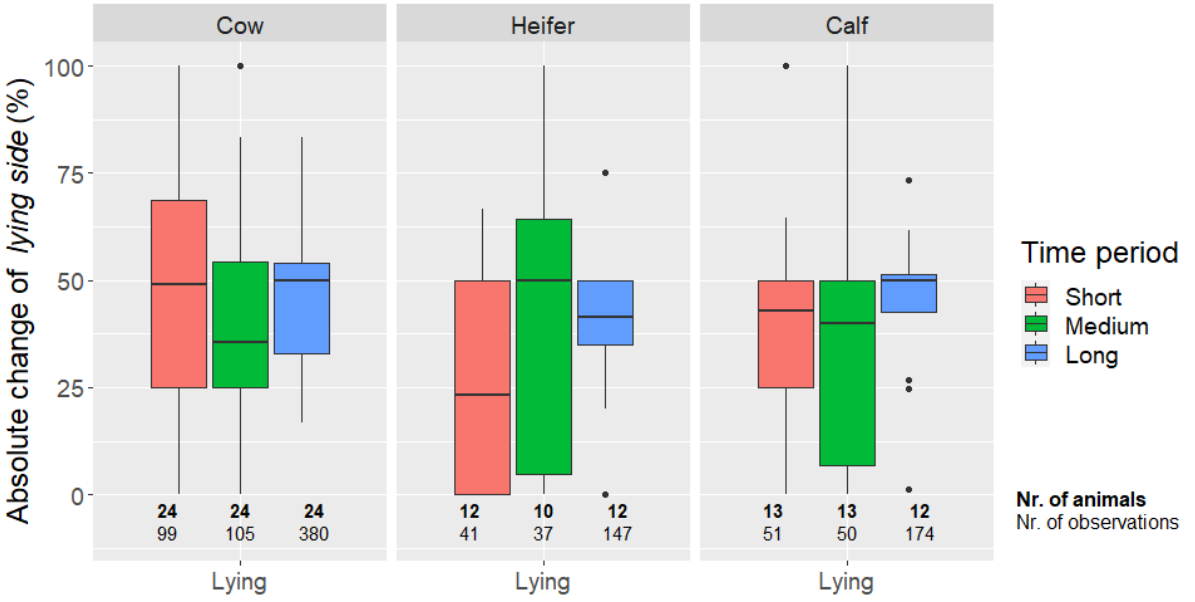

Figure 51 **Absolute changes of lying side.** Changes of *lying side* are given as absolute values describing the change in occurrence of *lying side* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

3.2.9.2. *Lying posture*

The animals were almost never seen *lying flat* on their side. A *chest prone* position was observed on average in 99.8% of all observations across age categories (Figure 52). Accordingly, no great change in occurrences over time can be displayed (Figure 53).

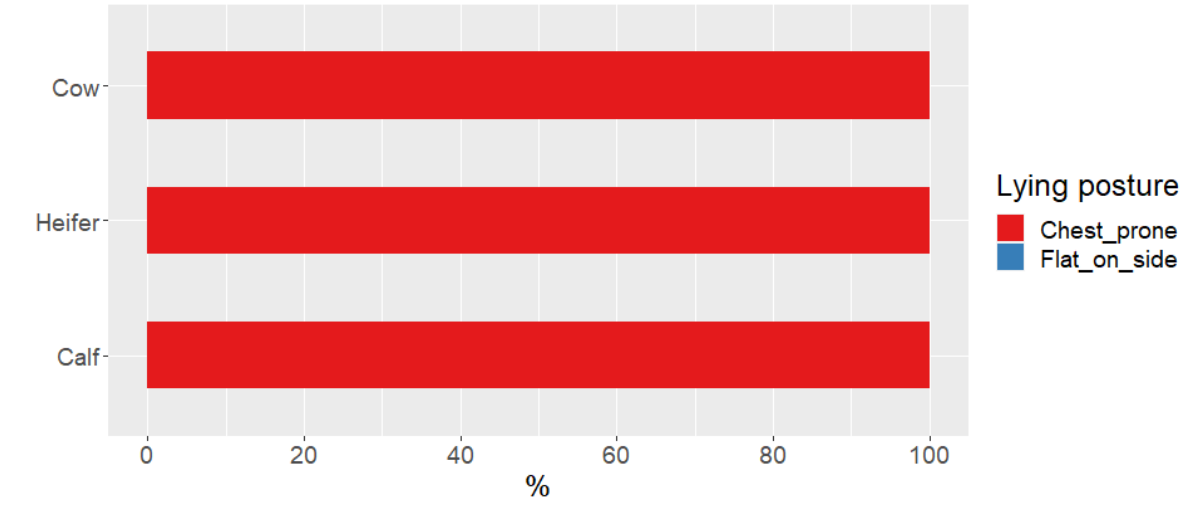

Figure 52 **Lying postures.** The occurrence (%) of lying postures is given per basic body position (lying, standing) and age category (cow, heifer, calf).

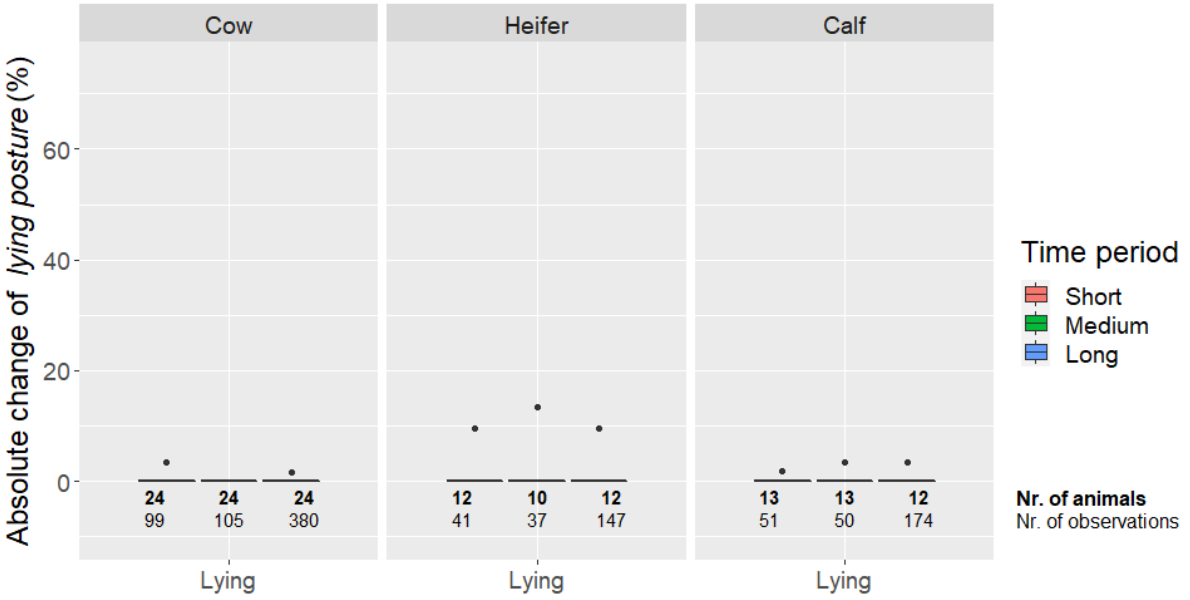

Figure 53 **Absolute changes of lying posture.** Changes of *lying posture* are given as absolute values describing the change in occurrence of *lying posture* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

3.2.9.3. *Front leg postures*

The dominating front leg posture when lying was *front legs tucked,* across all age categories (mean=81.2%). Heifers and calves showed *one front leg stretched* more often than cows (cows=11.5%,

heifers=21.7%, calves=17.6%). *Both front legs stretched* occurred rarely across all age categories (mean=1.9%) but most often in calves (calves =2.5%) (Figure 54).

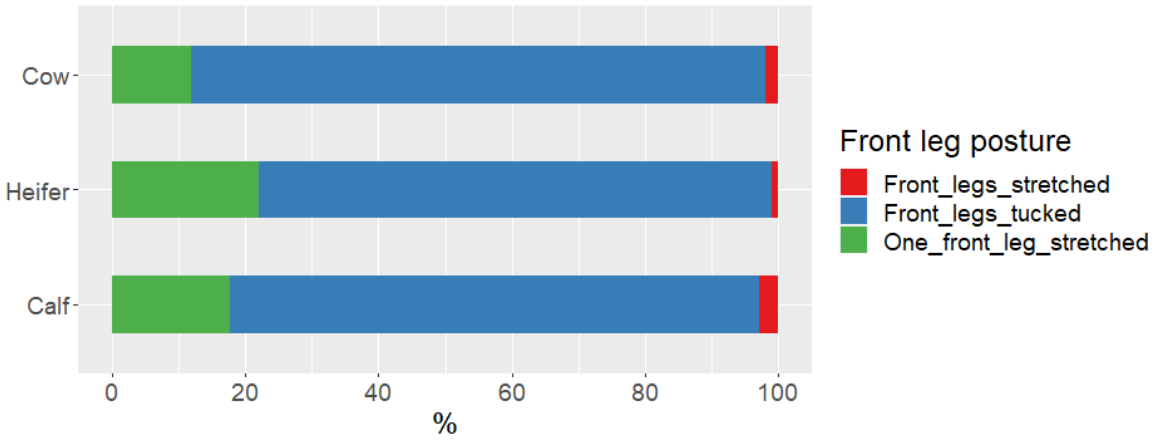

Figure 54 **Front leg postures.** The occurrence (%) of front leg postures is given per basic body position (lying, standing) and age category (cow, heifer, calf).

A change in *front legs tucked* and *one front leg tucked* could be seen in all age categories and across all time periods. Changes were generally higher for both postures in heifers and calves than in cows. Changes for f*ront legs stretched* were small for all time periods and age categories (Figure 55-Figure 57).

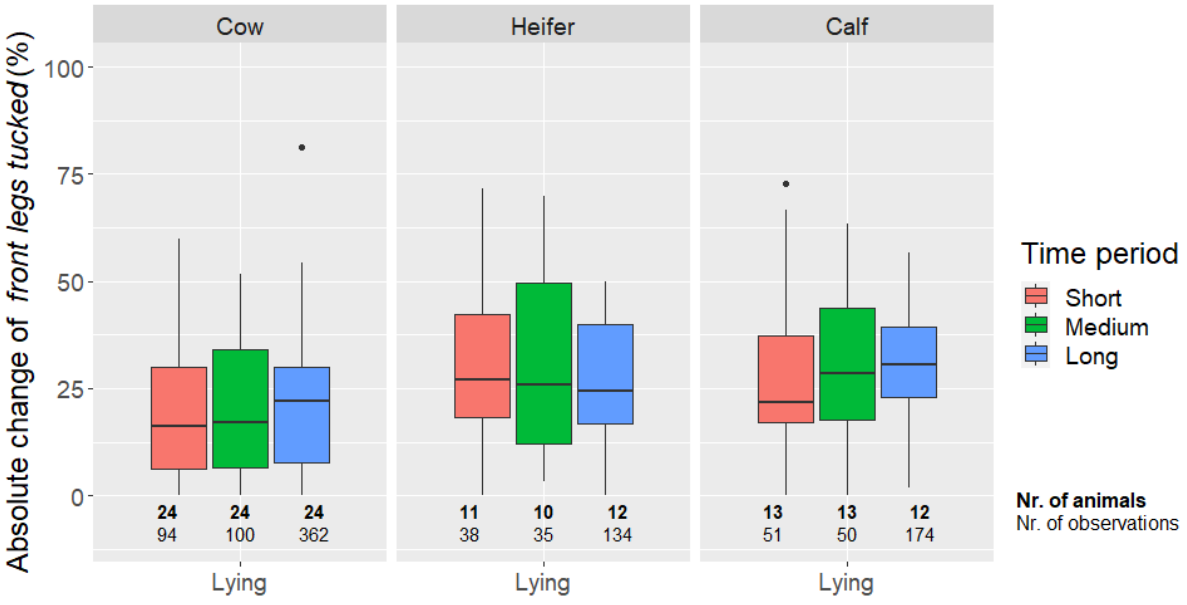

Figure 55 **Absolute changes of front legs tucked.** Changes of *front legs tucked* are given as absolute values describing the change in occurrence of *front legs tucked* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

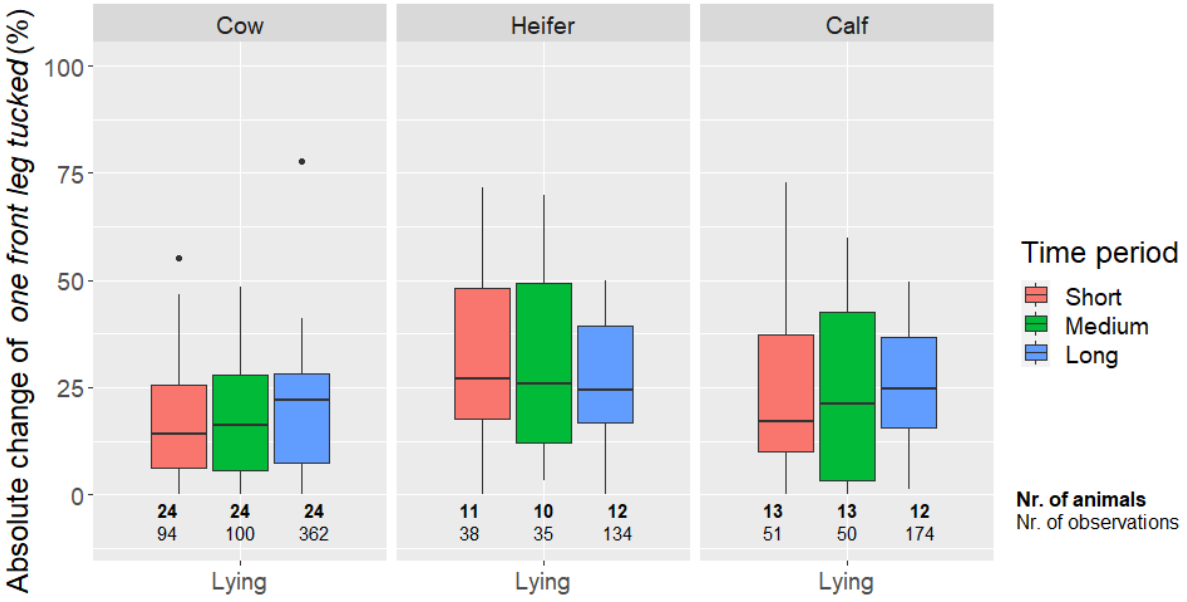

Figure 56 **Absolute changes of one front leg tucked.** Changes of *one front leg tucked* are given as absolute values describing the change in occurrence of *one front leg tucked* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot.

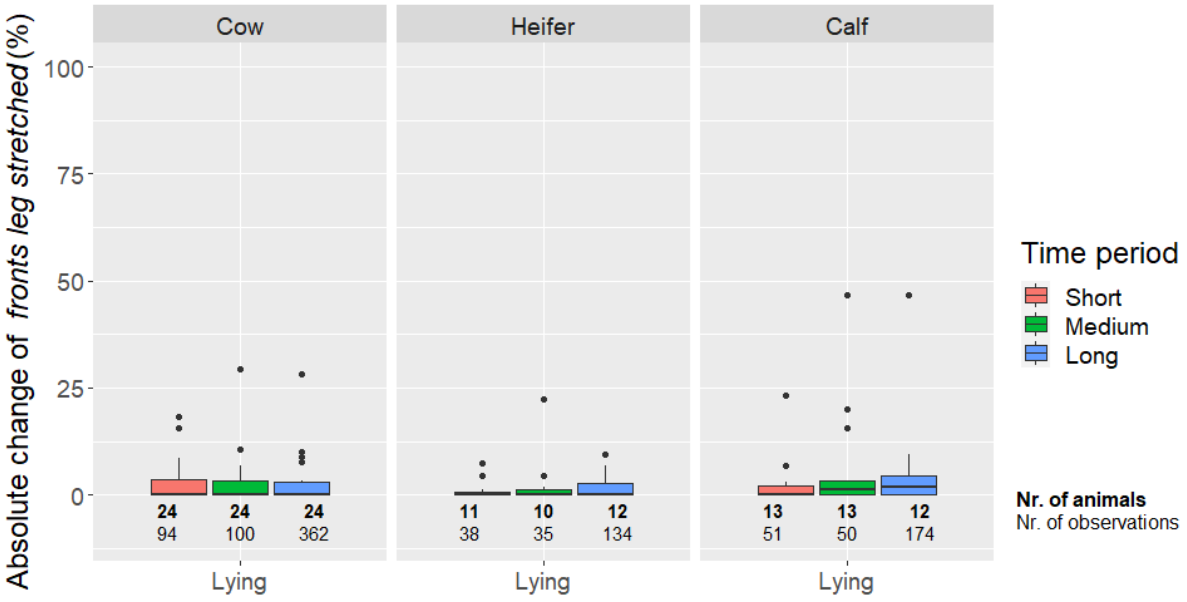

Figure 57 **Absolute changes of front legs stretched.** Changes of *front legs stretched* are given as absolute values describing the change in occurrence of *front legs stretched* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot

3.2.9.4. Rear leg postures

Rear legs were almost equally distributed between *tucked* (mean=50.1%) and *stretched* (mean=49.9%) on average over all age categories. Calves held their *rear legs tucked* more often than heifers and cows (59.3%) (Figure 58).

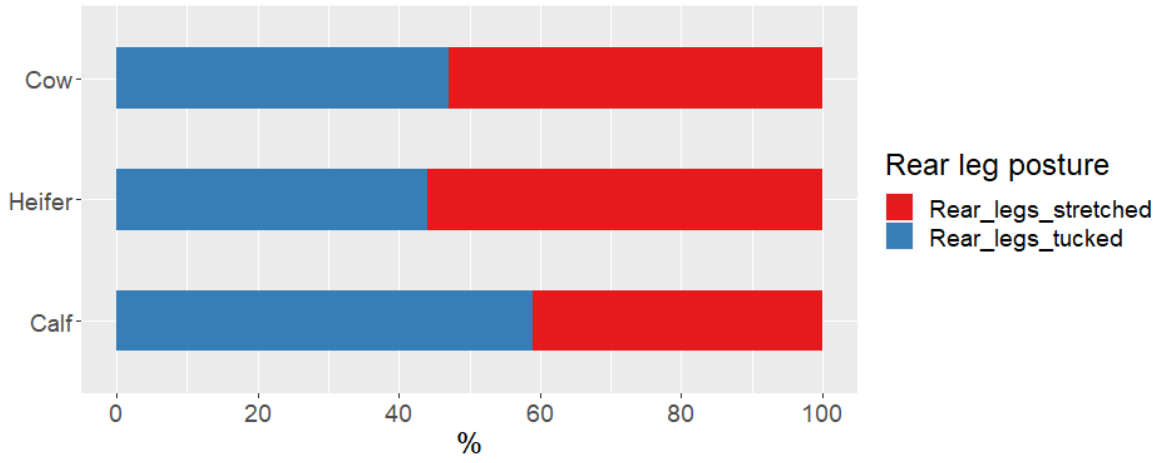

Figure 58 **Rear leg postures.** The occurrence (%) of front leg postures is given per basic body position (lying, standing) and age category (cow, heifer, calf).

The longer the time period, the smaller was the variability in change of rear leg posture, across all age categories (Figure 59).

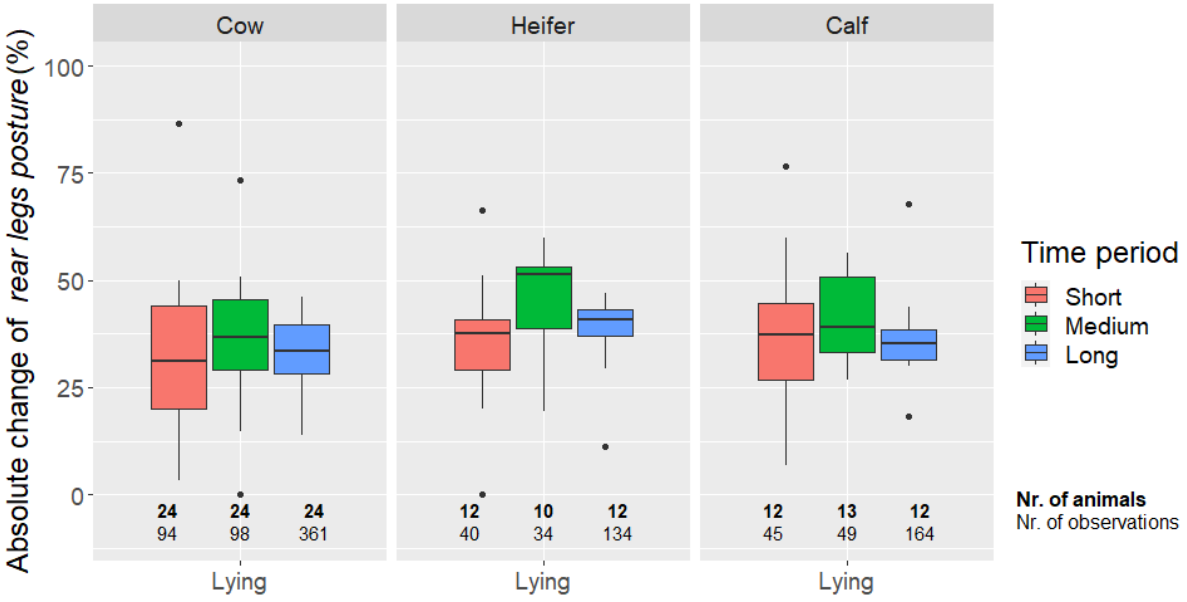

Figure 59 **Absolute changes of rear legs posture.** Changes of *rear legs posture* are given as absolute values describing the change in occurrence of *rear legs posture* for the three age categories (cow, heifer, calf), two basic body positions (lying, standing) and three time periods between observations (short, medium, long). The digits below the chart indicate the total number of animals and number of observations underlying each boxplot

3.3. Occurrences and consistency of body language on herd level

While chapter 3.2 described absolute occurrences and consistencies per animal and posture for different age categories, this chapter addresses occurrences and consistency of postures on herd level, meaning across all age categories per day. For this purpose, the standard deviation as well as coefficient of variation (CV) are given (CV=SD/MEAN). The coefficient of variation allows the absolute standard deviations of different absolute means to be compared to each other (Table 7).

Some postures, in lying or standing position, showed low CVs which indicated a stable occurrence and therefore higher consistency across different days. Regardless of the exact day the herd was observed, it was for example likely to see approximately 98% of lying animals *inactive*. From one day to another the individual level of *(in)activity* when lying could change, but overall, approximately 98% of the herd were *inactive* when lying.

Postures with the lowest CV and therefore relatively stable percentages of occurrence across days were *inactivity* (lying), *eyes open* (lying, standing), *ears backwards* (lying), *facing neutral* (lying), *facing straight* (standing), *tail tucked between legs* (lying), *tail not lifted* (lying, standing), *head no contact* (lying, standing), *no abnormal oral behaviour* (lying, standing), *chest prone* (lying), *front legs tucked* (lying).

Table 7 **Occurrences of postures on group level.** Mean occurrences (%) per posture and day on herd level, standard deviation (SD) and coefficient of variation (CV) are given per basic body position (lying, standing)

		Lying animals			**Standing animals**		
		MEAN (%)	**SD (%)**	**CV (%)**	**MEAN (%)**	**SD (%)**	**CV (%)**
Activity	Inactive	98.2	0.9	1	56.5	9.8	17
	Active	1.8	0.9	52	43.5	9.8	22
Rumination	Ruminating no	41.0	11.9	29	46.5	20.5	44
	Ruminating yes	59.0	11.9	20	53.5	20.5	38
Abnormal oral behaviour	No abnormal oral behaviour	99.9	0.2	0	99.3	1.4	1
	Nose pressing	0.0	0.2	500	0.5	1.1	237
	Tongue rolling	0.0	0.1	347	0.2	0.8	440
Neck postures	Middle	50.0	14.9	30	47.5	14.9	31
	Above horizontal	33.0	10.1	31	11.3	7.3	65
	Below horizontal	4.9	2.4	49	31.0	14.6	47
	Down	11.2	5.6	50	7.5	5.5	74
	Neck moving	0.8	0.7	92	2.8	3.0	106
Head orientation (standing)	Facing straight	n.a.	n.a.	n.a.	83.1	7.8	9
	Facing left	n.a.	n.a.	n.a.	5.2	3.9	75
	Facing right	n.a.	n.a.	n.a.	4.7	4.4	94
	Head moving (standing)	n.a.	n.a.	n.a.	7.1	4.0	57
Head orientation (lying)	Facing neutral	81.7	5.3	6	n.a.	n.a.	n.a.
	Facing in	8.2	3.7	46	n.a.	n.a.	n.a.
	Folded	8.3	4.7	56	n.a.	n.a.	n.a.
	Facing out	0.7	1.1	149	n.a.	n.a.	n.a.
	Head moving (lying)	1.1	0.7	62	n.a.	n.a.	n.a.
Head contact	Head no contact	84.8	7.5	9	97.0	3.2	3

	Head contact floor	8.2	4.6	56	0.3	0.9	330
	Head contact other	7.0	5.1	73	2.7	2.8	103
Ear posture	Asymmetric left	3.6	3.2	88	3.7	2.4	65
	Asymmetric right	4.2	2.5	60	8.4	6.7	80
	Axial	15.9	4.5	29	12.0	6.1	51
	Backwards	64.3	9.4	15	46.4	11.6	25
	Drooping	1.1	1.6	144	0.1	0.8	529
	Forwards	6.3	5.6	89	16.5	7.3	45
	Moving	2.0	1.0	51	10.4	4.5	43
	Pinned	2.7	1.8	69	2.6	2.8	110
Eye closure	Closed	7.8	5.3	68	0.1	0.4	417
	Open	92.2	5.3	6	99.9	0.4	0
Tail postures and movements	Hanging	8.6	6.9	81	78.8	17.1	22
	Tucked between legs	88.9	6.7	8	1.1	2.7	242
	Wagging	2.5	3.3	135	20.1	17.3	86
Tail lifts	Lifted	0.2	0.3	147	9.9	8.3	84
	Not lifted	99.8	0.3	0	90.1	8.3	9
Lying posture	Chest prone	99.8	0.4	0	n.a.	n.a.	n.a.
	Flat on the side	0.2	0.4	238	n.a.	n.a.	n.a.
Lying side	Lying on the left	54.2	14.3	26	n.a.	n.a.	n.a.
	Lying on the right	45.8	14.3	31	n.a.	n.a.	n.a.
Front leg postures	Front legs tucked	83.6	9.0	11	n.a.	n.a.	n.a.
	One front leg stretched	14.6	8.3	57	n.a.	n.a.	n.a.
	Both front legs stretched	1.8	2.0	111	n.a.	n.a.	n.a.
Rear leg posture	Rear legs stretched	50.6	8.5	17	n.a.	n.a.	n.a.
	Rear legs tucked	49.4	8.5	17	n.a.	n.a.	n.a.

4. Discussion

By describing the occurrences of postures, which could be largely reliably assessed, patterns become apparent depending on basic body position and age categories, i.e. it depends on the basic body posture and the individual body part, e.g. head or tail, whether older or younger animals show higher or lower occurrences.

Standing cows, heifers and calves showed generally a higher *activity*. Their tails were *lifted* more often than when lying, ears tended to show *asymmetric* and *forwards* postures or *movement* more often and for cows, *ears pinned* showed higher occurrences in standing than in lying animals. In the rare cases in which *nose pressing* was seen, this occurred almost exclusively in standing cows.

Age had an influence on the occurrence of different postures with cows differing from heifers and calves. While lying as well as standing, cows mostly showed more of the postures presumably indicating rest than heifers and calves. E.g. when lying, cows rather than heifers or calves showed more *inactivity* and more *eyes closed, ears backwards* and *ears drooping*, *head folded, neck middle*, *down* or *below*, *head in contact with floor and front legs tucked*. Exceptions are *rumination* which was seen less in cows than in heifers or calves when lying as well as *tail wagging* which cows performed more often than heifers and calves when lying and standing. For some postures, patterns of occurrence differed between heifers and calves as heifers showed more of the "resting" postures than calves (e.g. higher occurrence in heifers for *inactivity*, *neck middle* and *neck below* (lying), less *movement* of *ears*, *head* and *neck*).

Regarding consistency of body language shown by different age categories across time, no general pattern emerged. However, for individual postures and body parts, differences in consistency can be seen for either lying or standing animals or between age categories. Data for standing calves were generally rare, therefore only long periods of time could be evaluated for calves.

The research questions which will be answered in the following chapter focusing on the reliability of observers during live observations, the overall occurrence of body postures of different age categories of dairy cattle in different basic body positions as well as on the consistency of these postures over different time periods (short i.e. from one day to the next, medium i.e. 2-3 days later, long i.e. 11-17 days later). Furthermore, consistency on herd level will be discussed and the methodological approach taken in this study will be critically reflected.

4.1. Inter-observer reliability

Research question 1 asked to describe the reliability of multiple observers recording the body language of dairy cattle during live observations.

Overall, this study showed that 53 out of 55 postures could be observed at least moderately reliably (Kappa > 0.4) by three different observers during live observations. This indicates that, after training, reliable on-farm observations performed by multiple observers are possible. Values for inter-observer reliability regarding several postures and movements assessed in this study have not been stated before in literature, therefore our results provide knowledge on the feasibility of body language as indicator for animal welfare assessments.

The two indicators that could not be observed reliably were *eyes closed* (Fleiss' Kappa = 0.09) and *ears pinned* (Fleiss' Kappa = 0.39). They were difficult to test for inter-observer reliability (IOR) since they occurred rarely during testing. One reason for the rare occurrence could be, that during IOR testing all three observers were observing the same animal simultaneously and the distance to the animal was rather small. This might have led to a high level of attention or stress in the animal, reducing the

occasions where she closed her eyes completely, since *closed eyes* were seen more often in relaxed contexts such as being on pasture (Battini et al. 2019). Testing IOR on video footage instead of live observations on-farm might avoid this problem. However, even for the observers who did see *eyes closed* and *ears pinned* no sufficient reliability was given. In addition to low occurrence, the postures *ears pinned* and *eyes closed* might not have been defined and calibrated enough between observers. In both indicators (*eyes closed; ears pinned*) pairwise Cohens' Kappa was higher for individual pairs of observers than the overall Fleiss' Kappa. This indicates that with further training and calibration across all observers higher Fleiss' Kappa values could be achieved in future experiments.

Comparing our results for IOR with results stated in the literature, studies using photos or video footage have to be taken into account, since live observations are rather scarce. Some studies with multiple observers using video or photo footage did not provide detailed information on inter-observer reliability (Proctor and Carder 2014; Battini et al. 2019). Procter and Carder (2014) stated a minimum of 95 % agreement among observers before observations of ear postures were performed on video footage, but no Fleiss' Kappa value for comparing multiple observers with each other was given. Lange et al. (2020) provided a Cohen's Kappa above 0.78 for ear postures and Cohen's Kappa above 0.89 for all other behaviours observed on video footage. These values are similar or occasionally higher than the reliability achieved in this study. However, video footage can be replayed and photos can be rewatched, which is not possible during live observations on-farm. This has to be considered when comparing inter-observer reliability between studies.

Using live observations, Gleerup et al. (2015) reported a weighted Kappa coefficient of 0.68 for two observers applying a *Cow Pain Scale*, including facial expressions, ear postures, head and back position as well as the animal's attention towards surroundings and the response to approach. The values match the substantial inter-observer reliability shown in this study. A comparable study using multiple observers for behavioural observations live and on-farm did not state Fleiss' or Cohens' Kappa values to prove inter-observer reliability (Oliveira and Keeling 2018). Hence no detailed comparison can be made. However, it might not have been the main research aim of those studies to provide inter-observer reliability information for the ethogram applied, like in this study.

4.2. Occurrences of body language

Research question 2 addressed the description of the overall occurrences of different body postures of different age categories of dairy cattle (cow, heifer, calf) in different basic body positions (lying, standing). Occurrences of body postures differed between age categories as well as basic body positions (lying vs. standing). An overview of the occurrence of different postures is important to assess changes over time. Such changes may lead to valuable statements about animal welfare states once the behavioural indicators have been validated regarding their meaning for positive or negative animal welfare.

Depending on whether lying or standing animals are observed, the body language pattern can differ regarding occurrences of postures. For on-farm assessments, a separate investigation of both lying and standing animals is useful for a broad picture of body language. The variety of postures occurring was often higher in standing animals than in lying animals, usually across all age categories. On the one hand this could be due to a generally reduced behavioural spectrum in animals which are lying down, an assumption which, however, would need to be proven in future studies. On the other hand, in total more observations were available for lying animals than for standing animals (Table 6). This could also lead to less variability of postures seen since outliers were levelled by calculating means. However, the fact that there were more than twice as many observation cycles for lying animals compared to

standing animals enhances the reliability of the results seen for lying animals. This is especially relevant for calves where only 18% of all available observation cycles were recorded in standing position.

Age had an influence on the occurrence of different postures. When lying as well as when standing, cows mostly showed higher occurrences of the "resting" postures than heifers and calves e.g. more *inactivity*, *eyes closed, ears drooping*, *head folded*. Some postures also show a clear distinction in patterns of occurrence between heifers and calves by heifers showing more of the "resting" postures than calves. These results may be explained by a naturally reduced behavioural agility and activity as animals grow older. However, no references could be found for this assumption.

Focusing on only one age category in an on-farm animal welfare assessments relying on occurrences and consistency of body language over time, does not deliver sufficiently comprehensive information about a dairy farm, if the whole herd which usually comprises multiple age categories should be addressed. Observations of different age categories are suggested in this case.

Discussion of (in)activity, postures and behaviours

In the following paragraph individual postures investigated in this study are compared to information on occurrences given on the same or at least similar postures in other studies. Not all postures investigated in this study are considered.

(In)activity

Specific forms of inactivity have been suggested as indicator for positive or negative animal welfare (Fureix and Meagher 2015; Hintze et al. 2020). In this study, inactivity increased with older age categories, at least for standing animals (+10.4% from heifers to cows; +17.2% from calves to heifers). Since the experimental animals in this study were of good health, inactivity due to illness can be excluded. Absolute values of *inactive* animals were rather high in our study compared to a similar study on fattening cattle. Hintze et al. (2020) described inactivity and body postures in fattening cattle in different husbandry systems on group level and individual animal level. They concluded that the more intensive the farming system is, the more inactivity was observed on group level and individual level. For the intensive farming system, the share of inactivity on group level was 50.9% ±29.2 of which 57.9% ±39.6 were lying animals. Combining both basic body positions, we found occurrences of 77.4% for *inactivity* on herd level including all age categories, which is higher than the values stated by Hintze et al. (2020).

Instead of female Austrian Fleckvieh fattening cattle in intensive housing system on fully slatted floor pens, we observed Swedish Red and Holstein-Friesian female dairy cattle in a loose housing system, as one of the first studies on (in)activity in dairy cattle. Hence differing results regarding (in)activity levels can be attributed to the differing contexts of the studies. Still, we showed high occurrences of *inactive* animals on herd level (lying: mean > 98%, standing: mean > 56%). Being housed in a highly intensive housing system could support the thesis of Hintze et al. (2020), that with intensity of housing systems, inactivity levels increase.

Since Hintze et al. (2020) only observed and described animals defined as *inactive* regardless of their basic body position, a detailed comparison with the occurrences for individual postures given in this study is difficult, since we did not separate our data according to (in)activity but according to basic body positions. Hence, we can only give information about the occurrence of postures for all animals, regardless of whether they were classified as *active* or *inactive* (Table 8). Nonetheless, in total we observed more animals in lying position than in standing position (Table 6) and more *inactive* animals than *active* animals (Figure 5), which again supports the results of Hintze et al. (2020), seeing less activity in more intensive housing systems. Though slatted floor pens as well as free stall cubicles can both be classified as intensive housing systems, they still differ in several aspects e.g. space availability per animal. This has to be considered when comparing the results of Hintze et al. (2020) and this work.

Table 8 **Comparison of mean occurrences (%) per posture and day on herd level between this study (Landvogt) and Hintze et al. (2020)**

Authors	**Hintze et al. (2020)**	**Landvogt (2022)**	**Landvogt (2022)**	**Landvogt (2022)**
Context	**Intensive housing (inactive animals)**	**Lying and standing (inactive and active)**	**Lying animals (inactive and active)**	**Standing animals (inactive and active)**
Posture	**Occurrence of posture MEAN (%)**	**Occurrence of posture MEAN (%)**	**Occurrence of posture MEAN (%)**	**Occurrence of posture MEAN (%)**
Inactivity	100 (only inactive animals included)	77.4	98.2	56.5
Ears asymmetric (left and right)	12.9	10.0	7.8	12.1
Ears backwards	50.9	55.4	64.3	46.4
Ears forwards**	33.8	16.4	6.3	26.4
Ears low/Ears drooping	2.4	0.6	1.1	0.1
Eyes open	88.1	96.1	92.2	99.9
Tail hanging (i.e. tucked and hanging)**	> 99.0	88.7	97.5	79.9
Tail wagging **	< 1.0			
Front legs tucked	88.3*	83.6	83.6	n.a.

*across all housing systems, not only intensive housing system

**postures are only partly comparable due to differences in the ethograms

In Hintze et al. (2020), the combination of postures observed most often in intensive and semi-intensive husbandry systems was "lying, *eyes open, head up and ears backwards*", which is similar to the most occurring postures determined in this work for lying animals (*eyes open, facing neutral, neck middle*, *head no contact, ears backwards).* Additionally, for lying animals the *chest-prone* position with *both front legs tucked* under the body was observed for most of the time, which matches the most occurring postures for lying animals in our study.

Rumination

Several authors support the fact that cows spent their rumination time rather lying than standing (Paudyal 2021; Hörning 2003). In our study the age category of cows, however, was seen *ruminating* in slightly less than half of the observation cycles when lying and more than half of the observation cycles when standing (Figure 7). Lying time and rumination pattern can be influenced by breed, season, housing system, bedding material, stocking density, diet, age and animal-based factors such as e.g. milk yield (Norring et al. 2012; Paudyal 2021; Tucker et al. 2021). To explain the comparably high occurrence of rumination in standing position in this study, a detailed comparison of multifactorial circumstances would be needed, which exceeds the scope of this work.

Neck postures

Calves and heifers showed a higher occurrence of the posture *neck above horizontal* when lying and standing compared to cows. One reason for this pattern might be the overall higher activity of younger animals (Figure 5), which could result in more changes of head and neck postures.

In future studies, associations between neck posture and arousal level could be analysed, since the posture *neck above horizontal* observed in cows could possibly be associated with states of higher arousal. This is a first hypothesis which evolved from working with the animals on-farm during live observations but was not investigated further. The same neck posture observed on calves however did not seem to reflect states of high arousal. A calf could for example be seen with a combination of seemingly low arousal postures such as lying *inactively*, *ruminating* with *ears back*, but she would additionally show a *neck above* posture. Hence the interpretation of neck postures regarding valence and arousal levels should be studied separately for different age categories.

Ear postures

Several studies focused on validating ear postures as indicator for animal welfare (Battini et al. 2019; Proctor and Carder 2014; Lambert and Carder 2019; Keeling et al. 2021). Information about occurrences of the individual postures per animal, day or herd is scarce. Hintze et al. (2020), studying fattening cattle of Austrian Fleckvieh breed in three housing systems of different intensity, stated *ears backwards* (50.9% ±27.8) as the most occurring ear posture followed by *ears forwards* (33.8% ±27.5), in intensive housing systems. This corresponds with the most occurring ear postures in this study across all animal categories and basic body positions, being *ears backwards* (55.4%) and *ears forwards* (16.4%9). However, *ears forwards* was not observed as often as stated in Hintze et al. (2020), who recognised a decrease in *ears forwards* posture with increased intensity of the housing system. *Low* or *drooping ears* were observed less often in this study (0.6%) than in the intensive housing system in Hintze et al. (2020) (2.4% ±8.4), who again recognised a decrease in *low ear* posture as housing systems become more intensive (e.g. ears *low* on pasture: 16.2% ±28.9). Compared to other less intensive housing systems e*ars asymmetric* was seen most often on fattening cattle in intensive housing systems (12.9 % ±14.3) (Hintze et al., 2020) which is a similar result as for our dairy cattle in loose housing systems (10.0%).

The basic information about ear postures given in this work could be a useful reference for future studies on this topic. If, as stated in Keeling et al. (2021), for example "*ears backwards*" or "ears hanging" (i.e. "*drooping*" or "*low*") proves to be a valid indicator of positive low arousal states in multiple contexts and when clinical pain can be excluded, comparing occurrences of ear postures across housing systems could help to assess individual farms regarding (positive) animal welfare.

Tail postures

Cows were *wagging* their tails more often than heifers and calves in both lying and standing body position (Figure 44). *Tail wagging* did therefore not follow the pattern of lower activity in the age category of cows compared to heifers and calves. One possible reason for higher occurrences of tail *wagging* could be a higher occurrence of flies in the cow pen than in the pens for heifers and calves. Compared to the occurrence of *tail hanging* in Hintze et al. (2020) we documented a lower share in dairy cattle than for fattening cattle in intensive housing systems (Table 8). To draw conclusions regarding possible correlations, fly assessments should therefore be added to future on-farm assessments.

Lying posture

For fattening cattle which were observed lying and classified as *inactive*, a share of not more than 2% for animals *lying laterally* was documented across different housing systems (Hintze et al. 2020). In our study we documented an average of 0.02% of the observation cycles in *lateral* position. Hörning (2003) found an increase in animals *lying flat* on their side with housing systems offering more space (pasture vs. loose housing system). As we observed dairy cattle in a loose housing system and not fattening

cattle on slatted floor pens, differences might be explained by breed, age, space or structure of lying areas available in the individual housing systems.

Lying side

A slightly biased distribution between *lying on the right* (46.3%) and *lying on the left side* (53.7%) could be observed in this study. Studies have shown, that some groups of cattle show a tendency to *lying on the left side*, but this is no general pattern (Tucker et al. 2009). As reviewed in Hörning (2003) the preferred lying side evens out across animals on herd level, even though individual preferences can exist. Our results support the information given in the literature.

Front and rear leg posture

A wide range of shares for *one* or *two front legs stretched* were stated in the literature (15% to 35%, depending on the housing system (Hörning 2003). Hintze et al. (2020) found a share of less than 20% across three husbandry systems for fattening cattle. In our study for dairy cattle, we documented an occurrence of 1.9% for *both front legs stretched* and 16.9% for *one front leg stretched*, which lies within the range of shares stated in the literature.

Most of the time in our study the animals were lying with *both front legs tucked* under the body (81.2%). Values above 80% of the observation cycles spent with *both front legs tucked* were stated for fattening cattle (Hintze et al. 2020), across intensive-, semi-intensive and pasture systems. Different housing systems can have an influence on leg posture occurrences. However, the results shown in this study range among similar results presented in the literature.

For rear legs, we had differing definitions of *stretched* and *tucked*, therefore no comparison to the work of Hörning (2003) can be made. Hintze et al. (2020) did not assess rear leg posture.

As a first step, we described the occurrences of postures of front and rear legs as part of the body language of dairy cattle. Whether useful and valid information for animal welfare assessments can be drawn from leg postures has yet to be validated.

4.3. Consistency of body language

4.3.1. Guide to interpreting graphic information

Barcharts

The stacked bar charts show the occurrence of different postures and their shares of the animals' postures per body part on average across all observation days. Some body parts are binary indicators, where the absence of one posture results in the presence of the other. Binary indicatory are: *(in)activity, eye closure, rumination, tail lifts, lying side, lying posture, rear leg posture*. For other body parts multiple postures were possible, as e.g. eight options for ear postures.

Boxplots

Instead of displaying absolute changes of occurrences from one day to another we could have displayed relative changes. This, however, could be misleading, as an increase in occurrence of one posture from 1% to 2% would be rated as an increase of 100% whereas an increase of another posture from 50% to 100% occurrence would also be rated as a 100% relative change. Relatively seen they are the same, on an absolute level, however, important information would be lost about absolute occurrences. Therefore, absolute changes are displayed in the results. A schematic graph disentangling the two options is displayed in Figure 60.

To draw conclusions on the absolute changes of postures as displayed in box and whiskers graphs, those must be put in relation to their absolute percentage of occurrence as displayed in stacked bar

charts. For example: absolute changes of less than 5 % in *(in)activity* (Figure 6) which occurs in more than 90% of observations available for lying cows, could be rated as relatively consistent indicator (Figure 5). On the contrary, *ears backwards* occurred in 70.6% of all observations of lying cows (Figure 33) but had a range of absolute change between 20% to 30% over different time periods (Figure 37), which indicates a comparably lower consistency. Accordingly lying cows show *ears backwards* less consistently than they show *(in)activity* over time.

Relatively consistent postures within an individual are such, which show low means of absolute change as e.g. *(in)activity* for lying animals (Figure 6). Low absolute changes, however, can be interpreted in two ways. Either did the individuals of one age category, basic body position and time period, show the same or similar occurrence of a posture from one day to another day, e.g. mostly above 95% for *(in)activity* (Figure 5), or it indicates, that a posture was very rarely seen and therefore no changes could be measured. The posture was mostly absent, as seen for *ears drooping (*Figure 38*)*.

Nevertheless, if these particular postures occur, they could still indicate substantial changes in behaviour and possibly animal welfare e.g. *drooping* ears as a possible sign of positive, low arousal states in cattle (Proctor and Carder 2014).

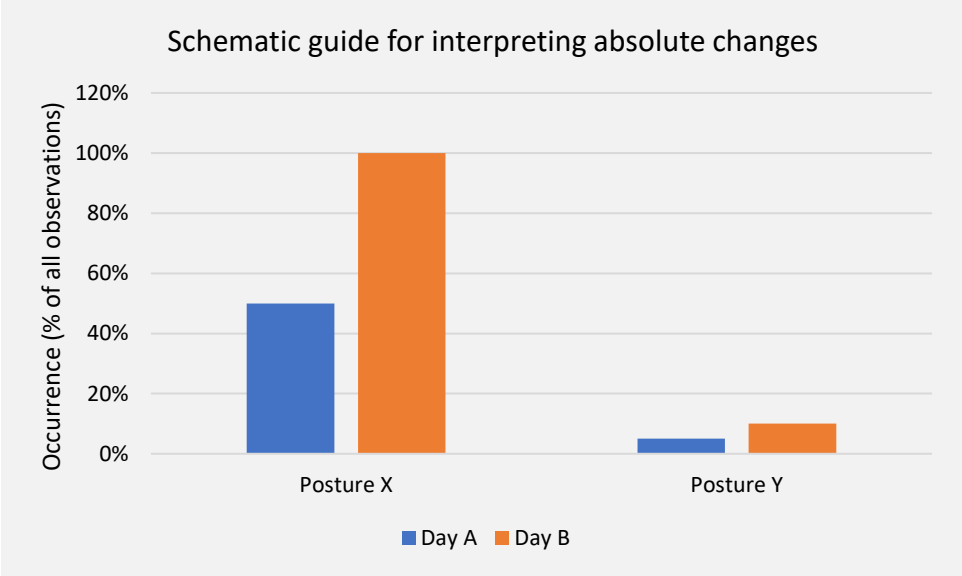

Figure 60 **Schematic guide to interpreting absolute changes.** Posture X and posture Y show the same relative change from day A to day B (100%) but different absolute changes as well as different occurrences (50% vs. 5%).

Relatively consistent postures across individuals are such, which show a low variability in change. Low variability in change i.e. small boxes and short whiskers, indicate that within a group of the same age, basic body position and time period, all animals were likely to be observed with a similar absolute occurrence of the posture on one day as on the other. A higher variability in change signals higher individual differences between animals regarding the occurrence of the posture from one day to another (e.g. *tail wagging* in lying animals vs. standing animals).

Binary postures comprise only two possible options, whereas other postures might have multiple options according to the ethogram. More postures to choose from might have increased the possibility to see changes in postures. Still, this bias is only of relevance when comparing the consistency of postures with each other. However, our approach of analysing graphically visible patterns is not suitable for giving a hierarchical order of most and least consistent indicators. We investigate relative consistency within one indicator comparing individuals and groups of animals within the framework of age categories basic body positions and time periods. Therefore, the number of available options per posture does not influence the results regarding consistency.

4.3.2. Consistency on individual animal level

Research question 3 consisted of two parts:

- How consistent is body language within individuals of different age categories of dairy cattle (cow, heifer, calf) in different basic body positions (lying, standing) and over different time periods (short, i.e. from one day to the next, medium, i.e. 2-3 days later, long, i.e. 11-17 days later)?
- How consistent is body language in dairy cattle on herd level in different basic body positions (lying, standing) across a period of seven weeks? (See chapter 4.3.3)

In the following paragraphs it is discussed whether reoccurring patterns were found regarding consistency of body language across different time periods depending on age category and basic body position. Furthermore, possible explanations for the existing or non-existing patterns are given and possible consequences for on-farm animal welfare assessments in the future are discussed.

At least certain components of body language including facial expressions are known to be influenced by the affective states of an individual (Paul et al. 2005; Mogil et al. 2020). Consequently, consistency of body language over time may serve as an indicator of stable affective states of the animal whereas inconsistency may reflect changes in affective states.

However, body language is not only affected by affective states and it is thus paramount to identify which expressions and postures reflect underlying or changing affective states and the role of other factors possibly influencing the occurrence. In this study, such other factors, e.g. housing system, management practices, temperature, group composition, health status, feed composition and quality stayed relatively unchanged. Also, the selected calves were relatively old calves, so rapid developmental changes in the early life of dairy cattle happened before the investigation started.

Consistency of body language regarding different time periods

One might assume that a high occurrence of a posture correlates with a low mean absolute change, i.e. high consistency, because the probability to see a change in postures should be lower when fewer different postures are shown. This pattern was shown e.g. across all time periods for *(in)activity* and *head no contact* in lying animals and for *front legs tucked, ears forwards* and *ears backwards* for lying animals only over a long time period of time. Several other postures did not follow this pattern or even showed a contrary result, where a high occurrence resulted in a high mean absolute change, i.e. low consistency, in *neck above horizontal* for lying and standing animals, *ears axial* and *ears forwards* in lying animals across all time periods. Hence, a general association between a high occurrence of a posture and a high consistency was not found.

Independent of the individual level of (in)consistency per posture, the investigation of different time periods did not show an increase or decrease of consistency over time in most of the postures and behaviours. Most postures were as consistent/inconsistent from one day to another as within a week or two weeks later. For example, *eye closure* in lying heifers changed roughly by 10% in absolute occurrences across all time periods.

Exceptions were *rumination* and *rear leg posture, for which* variability in change was lower the longer the time periods were. Both were binary indicators and showed average occurrences of roughly 50% of each possible posture i.e. *rear legs tucked* or *rear legs stretched* or of each possible behaviour i.e. *rumination* or *no rumination*. The longer the time period, the more pairs of observation days were available to calculate changes between days. It is possible, that the pattern of reduced variability for longer time periods in *rumination* and *rear leg posture* arose from more observations being available, eventually resulting in an average change of occurrences of roughly 50% (Figure 8). Hence statistical reasons may underly this exceptional pattern for the two indicators.

Nevertheless, for on-farm (welfare) assessments the results with respect to (in)consistency imply that a timespan of six weeks does not systematically affect (in)consistency of a behaviour. A meta study on repeatability of behaviour concluded that behaviour is more repeatable when time intervals of observations are short i.e. less than one year (Bell et al. 2009, p. 780). This supports the unchanged (in)consistency seen in our study over the timespan of several weeks. However, this review comprised various types of behaviour for various taxonomic classes of which only a few were mammalia. Additional studies on consistency of body language in dairy cattle over time periods longer than one year could provide further information to classify the level of (in)consistency investigated in this work.

Based on the results of this study, on-farm animal welfare assessments applying individual postures and (in)activity as indicator could be spread over different days without being confounded because inconsistency or consistency of the individual postures did not systematically change over time. This was the case across all basic body positions and age categories. We want to emphasise, that due to continued inconsistency of many postures the pattern of body language differs from day to day. Hence, it does not make a difference on which days on-farm observations are performed i.e. short, medium or long time periods between observation days. For valid and reliable data on body postures representing the general welfare state of dairy cattle in future on-farm animal welfare assessments, multiple observation days should be considered, to even out possible outliers or unusual circumstances on the farm on single days.

Consistency of body language regarding different basic body positions

The graphical comparison showed similar or less absolute changes and less variability in change in lying animals compared to standing animals, mostly regardless of age categories and time periods. Thus, a higher consistency exists for some postures in lying animals compared to standing animals. This was the case for *(in)activity, ears asymmetric right, ears moving, neck below horizontal*, *tail wagging* and *tail lifted*. For calves only long periods of time could be analysed since data were lacking for short and medium periods of time. For the latter four postures *ears forwards, tail hanging, neck moving* and *rumination* similar tendencies could be identified. However, for these postures lying animals compared to standing animals did only show less absolute change and less variability in change in some age categories and time periods. No general pattern could be identified.

Overall, lying animals seem to show less behavioural variety than standing animals. On the one hand the spectrum of possible movements and postures is physically limited for animals lying down in a cubicle. On the other hand, activity was generally lower in lying than in standing animals (Figure 5). This could also be the result of a generally calmer state of mind when an animal is lying down, which again is an assumption which has yet to be proven by scientific investigations.

Lying animals might be more suitable to reliably detect changes in consistent behaviour if the postures *ears asymmetric right, ears moving, neck below horizontal* and *tail wagging* or *lifted* are of interest. If, for example, further research shows that a change in e.g. occurrence of ear movement indicates positive affective states, this change might be more significant in lying animals than in standing animals. Generally, it can be recommended, to assess lying and standing animals separately, as patterns of occurrences differ between the two basic body positions.

Consistency of body language regarding different age categories

Regardless of time periods, cows showed less or similar absolute change and less or similar variability in change than heifers and calves during lying position for *(in)activity, ears forwards, front legs tucked* and *one front leg tucked.* For *ears axial* and *ears backwards*, this was only true for medium and long time periods.

In standing animals, the pattern of cows showing less absolute change and less variability in change than heifers (across all time periods) and calves (only long time periods) can be seen for *ears moving*,

facing straight, neck moving (not for variability), *neck above* (except medium time period) and *below horizontal* (only compared to heifers), *tail hanging* as well as *tail wagging* and *tail lifts* (not for long periods).

Other postures did not follow this pattern or showed only little changes across age categories, too small for comparison. The pattern is not bound to certain body parts, but a random selection, of single postures per body part. Among the postures are some, which are currently treated as indicators for animal welfare (e.g. *(in)activity, ear posture*) and some which need validation first (e.g. *leg posture*).

One explanation for the different consistencies of postures regarding age categories might be a correlation between absolute occurrences and absolute changes, as discussed earlier. A high occurrence could possibly correlate with a low mean absolute change of a posture, because when fewer different postures are seen, the probability to see a change in postures is lower. However, there is no general pattern. Only in some postures cows showed the lowest mean change and lowest variability in change of a posture additionally to the smallest occurrence of the posture (lying animals: *(in)activity, ears forwards, one front leg tucked, ears axial /* standing animals*: ears moving, neck moving, neck above horizontal, tail hanging, tail lifted*). On the contrary other postures showed the highest occurrences in cows, even though they still showed smaller mean changes and less variability in change than heifers and calves (lying animals: *front legs tucked, ears backwards*; standing animals: *facing straight, neck below horizontal, tail wagging*). Another reason for the different consistencies of postures regarding age categories might be a naturally reduced "behavioural plasticity" and activity as animals become older.

Hence when creating an on-farm animal welfare assessment protocol using behavioural indicators to detect changes in welfare, the different age categories present on the farm should be considered. An animal welfare assessment based on only one age category is not representative of a whole farm which often comprises multiple age categories. Older or younger animals might be more or less suitable to reliably detect changes in body language and behaviour, at least for specific postures. To support this tendency, a study showed, that personality traits such as *boldness* and *exploration* are consistent before and after puberty but could not detect any consistency during the sexual maturation phase (Neave et al. 2020). Consequently, intervals between observations on-farm should be adapted to the consistency of the postures of interest as well as to the age category of the focal animal. A sub-sampling of different age categories for assessing animal welfare was also suggested by Haskell et al. (2012).

4.3.3. Consistency on herd level

To compare the consistency of body language over time on herd level, the coefficient of variation (CV) was calculated. A low CV value indicates that it was very likely to see the same percentage of individuals in the herd performing a posture on one day as on any other day. It may have been different animals performing the posture from day to day, but with a low CV value on herd level a consistent number of animals was recorded performing the posture over time. A wide range of CV values was found across all postures and behaviours (0% - 529%).

Especially interesting is the posture *ears backwards*, which had a CV of 15% when lying and 25% when standing. Despite ears being a fast changing "volatile" body part, a relatively high consistency on herd level across days was found. In contrast, *nose pressing* (CV standing =273%; CV lying =500%) or *lying flat on the side* (CV=238%) showed relatively high CV values. *Ears backwards* as well as other postures with relatively low CV values might by the "default" postures of dairy cattle under normal routine housing conditions. These postures encompass *inactivity* (lying), *eyes open* (lying, standing), *ears backwards* (lying), *facing neutral* (lying), *facing straight* (standing), *tail tucked between legs* (lying), *tail not lifted* (lying, standing), *head no contact* (lying, standing), *no abnormal oral behaviour* (lying, standing), *chest prone* (lying), *front legs tucked* (lying).

Merging the information obtained from individual animals within one herd can provide additional information on animal welfare. Hence if the occurrences of relatively consistent postures change significantly on herd level during several on-farm assessments, this could indicate a shift to increased or decreased animal welfare and signal a need to take action to adapt animal welfare practices. In addition to changes of consistent postures, the appearance of rare and therefore rather inconsistent postures or behaviours such as *abnormal oral behaviour* or *drooping ears* may also give valuable information about the welfare state regardless of (in)consistent occurrence. Still, further work on validation of the individual postures and behaviours is needed for this approach to be applied.

4.4. Critical reflection of methods

This work only draws an individual picture of body language of the selected animals and their preferences in the context of the specific research farm. Other animals on other farms could show different occurrences for each posture due to differences in individual preferences and farm structure. The experimental animals were generally used to humans as the farm is a research institute and veterinary students are present on a regular basis. Animals less used to human interaction might show different percentages of occurrence per posture.

To assess animal welfare, the combination of multiple indicators is advisable (Keeling et al. 2021), which is why we assessed multiple body postures (see 2.4.1). However, only those body parts and postures which could be observed during live observations on-farm in our experimental setup were included in the ethogram of this study. Hence, more detailed facial expressions besides ear posture and eye closure were not included (e.g. eye white, half closed eyes). More complex behaviours like allogrooming, self-grooming or other social interactions were additionally recorded as important indicators for positive animal welfare but were analysed only in the context of the broader Swedish study project. Integrating these indicators exceeded the scope of this work, which mainly focused on the behaviours shown by focal animals, rather than analysing group behaviour.

Specific circumstances in the farm work such as loud noises, defect milking robots resulting in lack of milking capacity of cows or construction of new lighting in the farm were not specifically noted, neither were the data of these days excluded from the dataset. It was avoided to observe the animals when a disturbance was happening, but disturbances of several hours could not be circumvented. Single outliers are expected to be compensated by the averaging of all observation cycles available. Daytime as well as routine husbandry procedures could be a possible driver of consistency by e.g. triggering rumination right after feeding hours. Initially the experimental setup was controlled for daytime, but this limited the possible timeslots for observations, leaving us with insufficient data. Hence, no controlled schedule for morning or afternoon observations was possible. Instead, we applied an observation round of 15 observation cycles per day, which was feasible for the observers but is likely too short to fully reflect the animals body language of a whole day. Future research should consider increasing the observation time per animal and day. Due to the requirement of observing the animals in both lying and standing basic body position on certain days, a lot of time on-site was used for finding animals in the right basic body position. Without this restriction, more observations per animal and day would have been possible. Additionally, observation rounds of calves and heifers were often cancelled after few observation cycles before reaching the minimum of 8 observation cycles because the animals became *active* by e.g. grooming, being groomed or walking and the time limit of 30 minutes was thus exceeded before the animal became *inactive* again. Consequently, data for calves in standing position were scarce. Therefore, the consistency of body language of calves could only be investigated for long periods of time, i.e. 11 to 17 days after day x. In future studies the higher activity of younger animals should be considered when setting time limits for observations.

Even though a distance of several meters was kept to the animal she still noticed the observer and often paid at least some attention to her. Occasionally animals avoided direct eye contact by shifting their head slowly and slightly, so the eyes would be covered by a metal bar or another obstacle. This occurred with cows, but less obviously with calves. Since this behaviour was not recorded or measured in any way, it remains a hypothesis, which could be analysed in future research projects. Accordingly, we never observed animals in a completely undisturbed state. This influence on behaviour could be avoided by video recording. It might also be an option to stand very close to the animal instead of several meters away. They might feel less disturbed and threatened when the observer is very close than looking at them from a distance. Up close, direct eye contact can be avoided but the observer has a detailed view of all relevant body parts. This was an impression by working with animals on a research farm, being used to humans in their pen. On the contrary, the attention animals pay to the disturbance of an observer being present might be even higher in farms, where animal-human interaction is usually rare.

4.5. Future research

Most of the literature on animal behaviour as indicator for animal welfare that was mentioned in the introduction assessed behaviour during experimental situation. The animals were faced with a specific task or a circumstance (being in pain, being stroked by a human etc.) to assess and validate their behaviour accordingly. Battini et. al. (2019) for example state, that eye white and ear postures were significantly influenced by the context i.e. during feeding, resting, being on pasture or while undergoing an avoidance distance test. Furthermore, the long-term mood is an important part of positive animal welfare which should be assessed (Mendl et al. 2010). An underlying hypothesis for this study is, that if the animal is involved in activities like feeding, drinking, queuing to be milked, being milked, brushing or walking, short-term emotions induced by these stimuli might bias the body language immediately, overwriting the animals' underlying long-term mood. Hence, we only observed animals in "neutral" situations where the body language is relatively "undisturbed" by short-term events.

A possible utilisation of the approach given in this work could be to detect shifts from "usual" to "unusual" patterns of occurrences of posture of dairy cows within individual farms. If, after validation, certain postures proved to indicate negative or positive animal welfare, then changes in patterns of occurrence for this indicator are of interest to measure and track welfare. This could be done via video observation and deep-learning camera vision algorithms identifying postures on a routine basis. Changes in patterns of occurrence can be tracked, analysed and eventually interpreted regarding positive or negative animal welfare. A similar approach has been taken for detecting insufficient pig welfare (Alameer et al. 2020), identifying and classifying discrete cattle behaviour (Fuentes et al. 2020) or processing facial expressions of cattle and pigs (Neethirajan 2021) using deep-learning based object detection mechanisms. Advantages of this approach are a non-invasive and non-disturbing collection of data via cameras on-farm, an automatic classification into different behaviours and the analysis of shifts from usual to unusual patterns of distribution, possibly in real-time speed. The level of detail in ethogram differentiation still seems to be higher when classified by humans. Further research, however, could improve the level of detail in detecting different behaviours and even increase reliability of classification when done by algorithms compared to multiple human observers. Inevitable for this approach is a validation of body language and the unusual shifts of occurrences of behavioural patterns, to then interpret the data regarding positive or negative animal welfare, with the goal to ultimately improve husbandry systems.

5. Conclusion

- Animal behaviour can provide relevant information on an animal's welfare, including its affective state and is thus of interest in animal welfare science. If future research on animal welfare focusses on behaviour as indicator for positive and negative animal welfare, a basic understanding of occurrences and consistency in behaviour is essential to improve animal welfare. Therefore, we described occurrences of several detailed body postures and behaviours on individual animal as well as herd level over time. To the best of our knowledge this has not been done before. This work may serve as a reference for further investigation of occurrences and shifts in animal behaviour as a potential indicator for animal welfare. If, in future, some of the studied postures prove to be valid welfare indicators, then future studies can be compared to the information about occurrences and consistency we found in our study. Many studies about cattle behaviour in the context of animal welfare focused on complex behaviour such as for example play, anticipatory behaviour or allogrooming (see Keeling et al. (2021) for a review). Often the studies were performed in test setups to validate the behaviour as indicator for positive or negative animal welfare. In this study we took a step back and focused on assessing consistency in body language by describing individual postures of body parts e.g. ear, neck and tail posture, since not many studies have drawn attention to consistency of body language as one aspect of cattle behaviour.
- Overall, it is possible to reliably observe a wide range of detailed body postures in dairy cattle during on-farm live observation with multiple observers. Individual postures demand increased training and calibration between observes. Future research can focus on improving specifically those postures with weak reliability.
- We provided information on occurrences of individual body postures and behaviour in dairy cattle over six weeks by daily live observation rounds of 15 minutes per animal. Detailed information for two basic body positions (lying standing) as well different age categories (cows, heifers, calves) were given. Patterns of occurrences of multiple postures and behaviours differed across age categories and basic body positions. Accordingly, in future on-farm animal welfare assessments, a sub-sampling of age categories and basic body positions is recommended. Standing animals showed a higher *activity* overall. Their tails were *lifted* more often than when lying, ears tended to show *asymmetric* postures, *forwards* or *movement* more often and for cows, *ears pinned* showed higher occurrences in standing than in lying animals. In the rare cases that *nose pressing* was seen, this occurred predominantly in standing cows. When lying as well as when standing, cows mostly showed more of the "resting" postures than heifers and calves. E.g. when lying, cows showed more *inactivity* and more *eyes closed, ears backwards* and *ears drooping*, *head folded, neck middle*, *down* or *below*, *head in contact with floor and front legs tucked*. Exceptions are *rumination* which was seen less in cows than in heifers or calves when lying as well as *tail wagging* which cows performed more often than heifers and calves when lying and standing. For individual postures, a clear distinction in patterns of occurrence between heifers and calves could be seen (e.g. higher *inactivity*, *neck middle* and *neck below* (lying), less *movement* of *ears*, *head* and *neck*).
- Consistency of some postures seen in dairy cattle differs between age categories (cow, heifer, calf) as well as basic body position (lying or standing). Consistency across time periods of different length (1, 2-3, 11-17 days between first and second observation) is not systemically different. Future studies should investigate consistency across longer time periods and focus on further possible influences on consistency of behaviour.
- Future on-farm animal welfare assessments may be adapted regarding frequency of observations, intervals between observation days, age of the animal and their basic body positions. A representative sample of relatively consistent postures requires less frequent observations across a time period than relatively inconsistent postures. Due to unchanged (in)consistency of many postures over different time periods it is irrelevant for those postures on which days on-farm observations are done, i.e. short, medium or long time periods

between observation days. For valid and reliable data however, multiple observation days should be considered.

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7. List of figures

8. List of tables

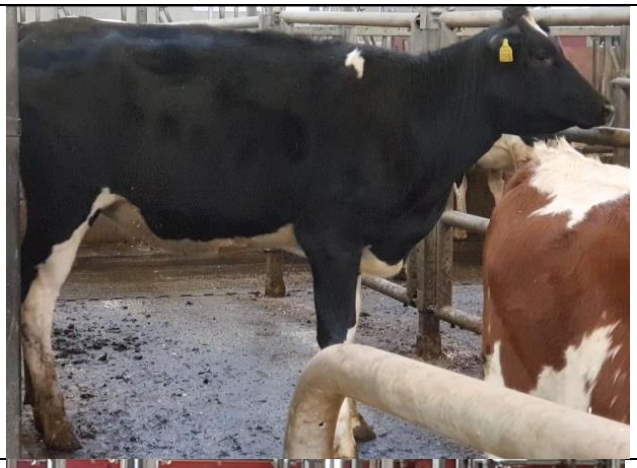
9. Appendix

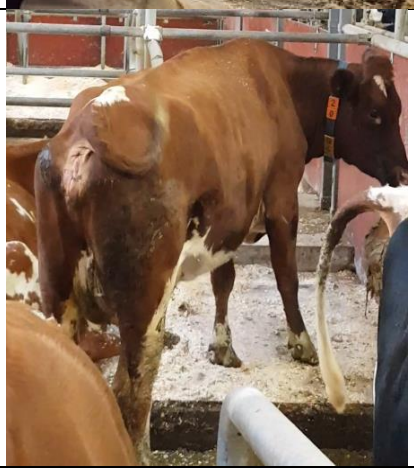
9.1. Ethogram with pictures

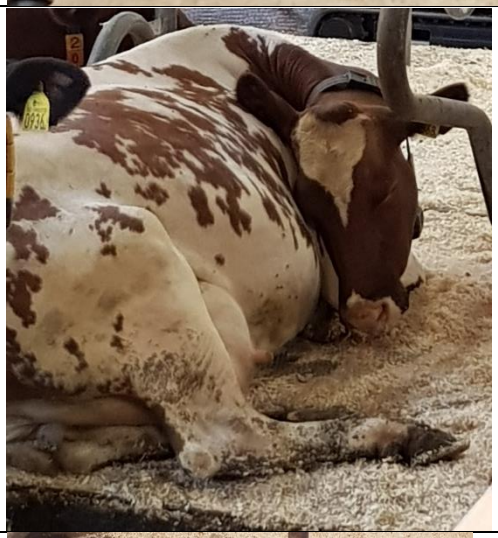
Table 9 **Ethogram with pictures.**

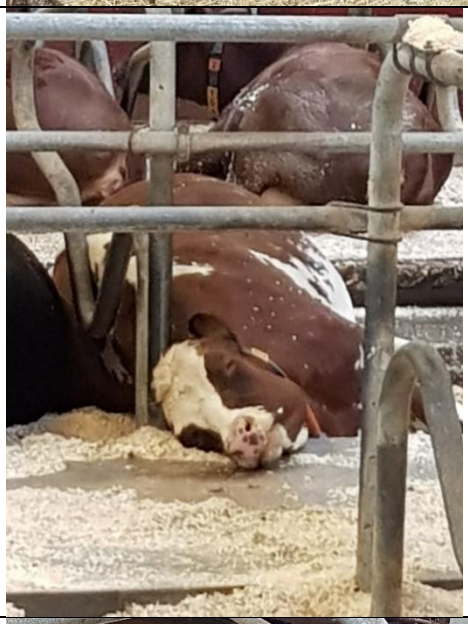
Body Part	**Posture**	**Picture**
(In)activity		n.a.
Basic body position	Standing	
	Lying	

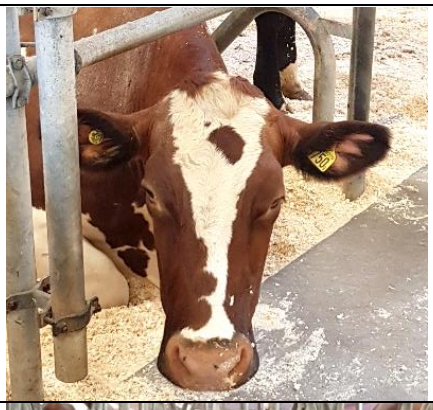
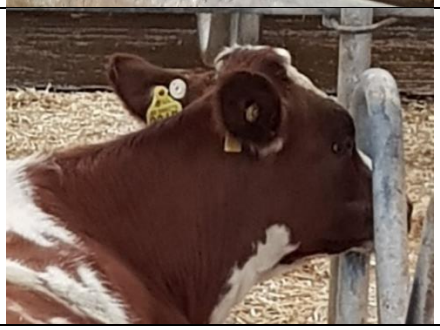
Rumination	Ruminating yes	
	Ruminating no	n.a.
Abnormal oral behaviour	No abnormal oral behaviour	n.a.
	Tongue rolling	n.a.
	Nose pressing	
Neck posture	Middle	

	Above horizontal	
	Below horizontal	
	Down	
	Moving	n.a.
Head orientation (standing)	Facing straight	

	Facing left	
	Facing right	
	Head moving	n.a.
Head orientation (lying)	Facing neutral	

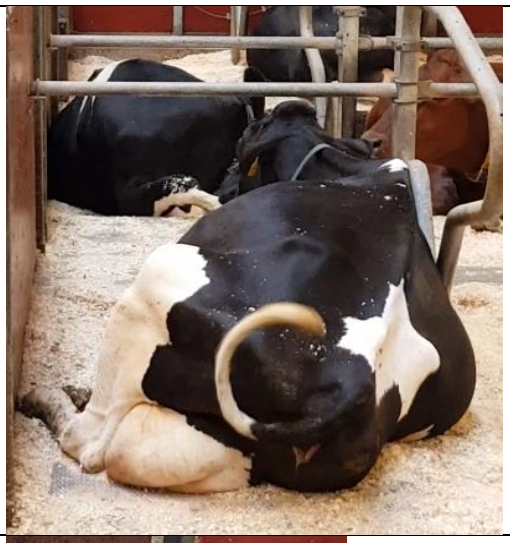
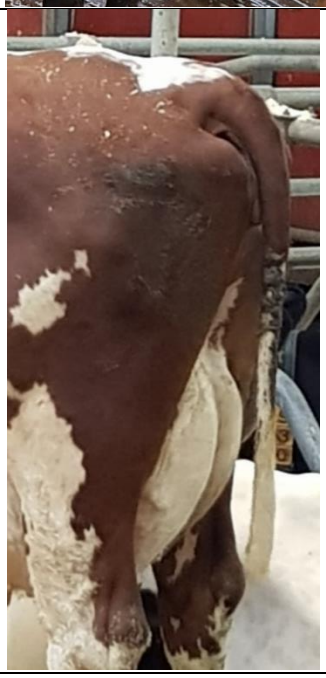
	Facing in	
	Folded	
	Facing out	
	Head moving	n.a.

Head contact	No contact	
	Head contact floor	
	Head contact other	

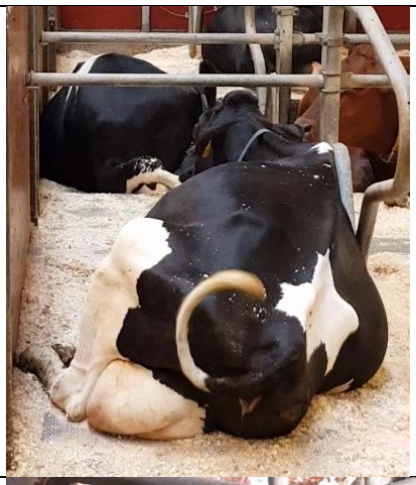
Ear postures	Axial	
	(Pushed) forwards	
	Backwards	
	Ears pinned	n.a.
	Asymmetric left	
	Asymmetric right	

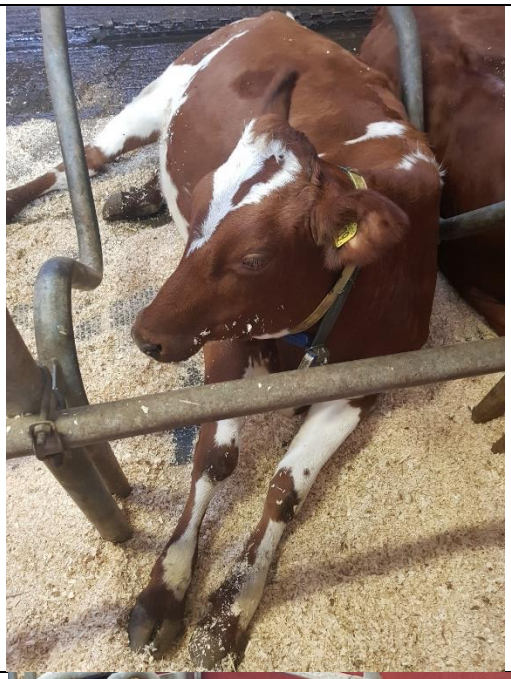
		
	Drooping	
	Ears moving	n.a.
Eye closure	Eyes open	
	Eyes closed	

Tail postures and movements	Hanging (standing)	
	Hanging (lying)	n.a.
	Tail tucked between legs (standing)	n.a.
	Tail tucked between legs (lying)	

	Tail wagging	
Tail lifts	Lifted	
	Not lifted	

Lying posture	Chest prone		
	Flat on the side		
Lying side	Lying on the left		

	Lying on the right	
Front leg posture	Front legs tucked in	
	One front leg stretched	

	Both front legs stretched	
Rear leg posture	Rear legs tucked	
	Rear leg stretched	

9.2. Additional information

Figure 61 Cow number 552 with *Bilateral convergent strabismus with exophthalmus (BCSE).*