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Naïve dairy cattle do not produce less milk in response to brown bear (*Ursus arctos*) fecal odor

Master thesis

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This thesis is worth 60 study points

Preface

This master thesis is part of the Master degree program at the Department of Natural Sciences and Environmental Health at University College of Southeast Norway, Bø in Telemark, Norway. I have followed the guidance of the journal of Applied Animal Behaviour Science, but with some exceptions. First of all, I am very grateful to my supervisors, Associate Professor Andreas Zedrosser, and Professor Frank Rosell, for giving me the opportunity to work with a field that I find very interesting and engaging, i.e. conflict between human and large predators. I appreciate the help they have offered through their time, and guidance, and most important, the encouragement and patience I have received when motivation were low, and frustration was high. Next, I want to thank Professor Jon E. Swenson in The Scandinavian Brown Bear Research Project, for addressing the possible problem connected with milk production in dairy cattle and an increasing brown bear population in Norway, and for his financial support. I am forever grateful for his patience. I also want to express my gratitude to Professor Øystein Holand at the Department of Animal and Aquacultural sciences at NMBU for his help and financial support. I want to thank the IT department at Norwegian University of Life Sciences (NMBU) for their assistance, the crew of 2013 at the Animal Production Experimental Center (SHF) at Ås for the help I received during the experimental period, especially Kai Ole Hänsch for his valuable help, and to Tore Bendos for providing information and answering all my questions up until today. Thanks to The Scandinavian Brown Bear Research Project for providing bear fecal samples to this experiment, and to Sigtryggur Thôr Benediktsson at Dagsrud Deer farming AS for his time and help at collecting deer fecal samples on a rainy day. Last but not least, I want to thank my family and friends for their support and motivation.

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Abstract

Predators can have direct effects on prey by killing individuals, but may also have indirect effects caused by fear. Indirect effects may represent costs that can affect the individual fitness of prey by reducing growth, survival or reproduction, and on a population level predator-induced fear may cause effects in prey that can be more substantial than the direct effect of predation. Olfaction is an important sensory modality in mammals, which aids in the detection and avoidance of predators. In addition to direct encounters with a predator, the odor of the predator itself may act as a strong stressor, potentially eliciting behavioral - and physiological responses in the prey. Although costly anti-predator behaviors may be reduced or lost in the domestication process of animals, predator stimuli may still elicit similar responses in domestic species as in wild mammals. Conflict between large carnivores and livestock owners in Norway are severe. Brown bear (*Ursus arctos*) depredation on domestic sheep (*Ovis aries*) is considered to be one of the main problems for the conservation of brown bears, and sheep farmers are encouraged to shift to other income sources, such as dairy farming. An increasing bear population in the future may lead to more conflicts with dairy farmers as encounters between cattle and bears may increase, and bears can potentially cause negative indirect effects on cattle caused by fear. Farmers in Scandinavia have reported reduced milk production in cattle as a consequence of bear presence, which represents a risk of income loss for farmers. Here, I tested the hypothesis that milk production in naïve dairy cattle would be affected when experimentally exposed to brown bear odor (feces). I predicted that (i) milk yield would be lower when cattle are exposed to bear odor in comparison to when cattle are exposed to a herbivore odor (red deer (*Cervus elaphus*)), or a blank control (i.e., no odor), and (ii) that milk yield would be lower during experimental periods, when cattle are exposed to odor treatments, in comparison to before or after experimental periods. I found no support for these predictions as (i) no significant difference in mean milk yield among treatments was found (i.e., bear: 24.8±4.4 (SD), red deer: 24.2±4.6, blank: 24.4±5.1) and (ii) cattle produced significantly less milk before an experimental period, and produced significantly more milk during an experimental period when compared to after an experimental period (i.e., before: 22.8±5.1,

during: 24.5±4.6, after: 24.6±4.9). Reasons for the lack of response in cattle to bear odor may be complex, but the lack of experience (e.g., depredation events) with bears is likely an important factor. Further research with dairy cattle experienced with bears are needed to gain a deeper understanding on how free-ranging cattle will respond physiologically to bear odor.

Keywords: *Brown bear, dairy cattle, olfaction, indirect effects, physiological stress, milk production.*

1. Introduction

Predation is a strong selective force that influences and shapes the behavior, morphology, and life-history traits of prey individuals (Lima & Dill 1990; Brown 2003). Predators can have direct effects on prey populations by killing individuals, but can also have indirect (i.e., non-lethal) effects (Boonstra et al. 1998; Creel & Christianson 2008) on prey caused by fear (Altendorf et al. 2001), causing changes in habitat use, vigilance, foraging, or physiological stress (Matassa & Trussel 2014). Such indirect effects represent costs that may affect the individual fitness of prey by reducing growth, survival or reproduction (Creel & Christianson 2008; Matassa & Trussel 2014), and on a population level predator-induced fear may cause effects in prey that can be more substantial than the direct effect of predation (Altendorf et al. 2001).

Predator effects are mediated by the prey's ability to detect the predator (Blumstein et al. 2002), and the sensory modality an animal uses for predator detection is species-specific (Apfelbach et al. 2005). Olfaction plays a crucial role in the ecology and behavior of almost all mammals, as this sensory modality enables animals to orient and navigate through their environment, mediate inter- and intraspecific interactions (Rajchard 2007), locate food (Nams 1997; Wyatt 2014), protect themselves from parasites and pathogens (Kavaliers et al. 2005), and it aids in the detection and avoidance of predators (Kats & Dill 1998; Wyatt 2014; Rajchard 2007; Bytheway et al. 2013). Throughout their territories or home ranges, all mammals leave behind urine, feces, and glandular secretions (Hegab et al. 2015). Prey is able to detect and respond to predator odor (Parsons & Blumstein 2010), and such heterospecific discrimination (Staples et al. 2008) has been investigated in several studies (Kats & Dill 1998; Blanchard et al. 2003; Apfelbach et al. 2005). For instance, Eurasian beavers (*Castor fiber*) are able to distinguish between odors from a predator and a non-predator (Rosell & Sanda 2006), and western grey kangaroos (*Macropus fuliginosus*) can discriminate between cues from a sympatric and a novel predator (Parsons et al. 2007).

Anti-predator behaviors increase survival (Apfelbach et al. 2005), but may also be costly in terms of energy and time (Brown 2003; Nonacs & Blumstein 2010). Therefore, animals often trade-off time and energy allocated to predator avoidance with other fitness-related activities, such as feeding, territorial defense and courtship (Lima & Dill 1990; Brown 2003; Nonacs & Blumstein 2010). It is expected that costly anti-predator adaptations will be used only when prey has an accurate assessment of the predation risk (Kats & Dill 1998; Kavaliers & Choleris 2001), and many animals use chemical cues from predators to assess the risk of predation (Kats & Dill 1998). In addition to the stressful situation of a direct encounter with a predator, just the odors of a predator may act as a strong stressor (Hegab et al. 2014a, 2014b, 2015), causing the sympathetic nervous system to release catecholamines (Buchanan 2000) and glucocorticoids into the circulatory system by activation of the hypothalamic-pituitary-adrenal axis (Fletcher & Boonstra 2006). Glucose uptake is inhibited in tissues, and as a result, energy stores are released. This energy mobilization may help the animal to cope with the stressful stimulus, and are used in the display of behavioral and physiological responses (Monclús et al. 2009; Hegab et al. 2014a). Responses to predator stimuli are not always expressed through observable behaviors, however, usually physiological responses can be measured (Monclús et al. 2006).

Prey animals may lose their predators and predation pressure during the domestication process due to relaxed natural selection (Price 1999). As a consequence, costly anti-predator behaviors may be reduced or lost (Eggen 1995; Blumstein 2006; Blumstein et al. 2006), as behaviors crucial for survival in nature (e.g., predator avoidance) lose their adaptive significance (Price 1999). Predator-naïve prey can be less sensitive to stimuli that reveals the presence of predators (Berger et al. 2001), and may fail to respond with appropriate behavior to avoid predation (Sand et al. 2006). However, several studies have shown that predator stimuli often still elicit similar responses in domestic species as in wild mammals (Hansen et al. 2001; Welp et al. 2004; Shrader et al. 2008; Kluever et al. 2009). For instance, domestic cattle (*Bos taurus*) avoided feed bins contaminated with fecal odor from red fox (*Vulpes vulpes*), coyote (*Canis latrans*), cougar (*Puma concolor*), and American black bear (*Ursus americanus*) (Pfister et al. 1990), or displayed behavioral responses to dog feces (i.e. increase in sniffing air, and increased stretched locomotion, such as lifting and putting down at least three legs, head slowly stretched forward and downward, hoofs hardly loosing contact with floor) (Terlouw et al. 1998).

Brown bear (*Ursus arctos*) depredation on free-ranging and unattended domestic sheep (*Ovis aries*) is considered to be one of the main problems for the conservation of brown bears in Norway (Sagør et al. 1997; Dahle et al. 1998). Conflicts between livestock owners and large carnivores are severe, and as a consequence, several farmers, especially in large carnivore conservation zones, have been advised to abandon sheep husbandry (Zimmermann et al. 2003). In comparison, in neighboring Sweden depredation on sheep is not considered an important topic in bear management and conservation due to differences in the husbandry system (i.e., sheep are usually not free-ranging but kept in fenced enclosures close to farms) (Swenson & Andrén 2005; Steyaert et al. 2011). Zimmermann et al. (2003) suggested farming of free-ranging cattle as a good alternative to sheep in brown bear conservation zones in Norway. During the last 10 years (i.e. January 2006 – January 2016) 11 cattle have been killed by bears in Norway (data from www.rovbase.no; accessed on November 15, 2016). Reports from Sweden show that 18 cattle were killed by bears between 2005 and 2015 (data from www.viltskadesenter.se; accessed on November 15, 2016). Steyaert et al. (2011) showed that direct encounters between brown bears and cattle are not common in Sweden, due to differences in the spatial resource selection of the species, and because the cattle husbandry system creates a temporal mismatch in the activity pattern of the two species, i.e., cattle are mainly day active while bears are most active during early morning and evening hours (Moe et al. 2007).

Dairy cattle farmers argue that bears are not just problematic due to the threat of direct depredation, but that there may be severe indirect effects on cattle due to increased stress levels caused by the mere presence of bears in the same area, despite the lack of direct encounters (Steyaert et al. 2011). Farmers have claimed that the presence of bears, advertised by odor from bear feces, urine or tracks, causes behavioral changes and lowers both quality and quantity of milk in dairy cattle (Zimmermann et al. 2003; Bengtson 2004). Reduced milk production in dairy cattle due to such indirect effects of bear presence could lead to income loss for famers (Steyaert et al. 2011). Physiologically, such a stress response of cattle to a predator would be caused by the release of stress hormones, such as glucocorticoids, via the blood stream into the mammary glands, where milk production would be reduced

(Jouan 2006). Several studies have investigated responses to predator odors in domesticated animals (Pfister et al. 1990; Arnould & Signoret 1993; Weldon et al. 1993; Terlouw et al. 1998; Christensen & Rundgren 2008; Shrader et al. 2008; Kluever et al. 2009). To my knowledge, the only studies that have investigated changes in milk production in response to predator stimuli, found that domesticated animals can show predator-avoidance behavior towards humans (Forkman et al. 2007), and that cattle's fear of humans has been associated with reduced milk yield (Rushen et al. 1999; Breuer et al. 2000; Waiblinger et al. 2002).

Research investigating indirect effects of predators on livestock is important to reduce human-wildlife conflicts and for the conservation of carnivores (Kluever et al. 2009). Here, I tested the hypothesis that milk production in naïve dairy cattle (hereafter referred to as cattle) would be affected when experimentally exposed to brown bear (hereafter referred to as bear) odor (feces). To control if cattle respond to a novel odor rather than the odor of a predator (Christensen et al. 2005), I included odor (feces) from a non-predator (red deer (*Cervus elaphus*)) and a control (blank, i.e., no odor) into the experiment. Because milk yield in cattle is highly affected by food intake and age (Grant & Albright 2001), I controlled for these variables in the analyses. Specifically, I predicted that: (i) milk yield (measured in liter (L)) would be lower when cattle are exposed to bear odor in comparison to when cattle are exposed to control odor (red deer) or no odor (blank), and (ii) milk yield would be lower during experimental periods, when cattle are exposed to odor treatments, in comparison to before or after experimental periods.

2. Materials and methods

2.1. Study animals

Cattle used in this study were 37 lactating and pregnant individuals of the breed Norwegian Red Cattle, with a mean age of 3.7±1.5 (SD) ranging from 2-7 years. Cattle were located at the Faculty of Veterinary Medicine and Bioscience at the Norwegian University of Life Sciences, Ås, Norway. Cattle had no experience with the odor of bears or red deer prior to the experiment, and were naïve to depredation events by carnivores. Cattle were milked twice per day, in the morning at approximately 6:30, and in the evening at approximately 15:30 (Figure 1). Milking took place in a milking parlor inside the barn, which had 10 milking boxes, and were performed by milking machines (Delaval 2x5 tandem parlour) that were attached by barn employees, and which automatically loosened when cattle were done milking. Milk yield was automatically recorded and stored. All cattle wore a collar with an ID chip. When cattle entered a milking box the ID was automatically registered, and cattle were provided individual amounts of grain feed (measured in kg) from an automatic feed dispenser. Cattle also had access to silage hay in the waiting area before milking.

2.2. Study area and period

The experiment was conducted in 4 one-week study periods during June-August 2013 at a summer pasture at the Faculty of Veterinary Medicine and Bioscience, Ås, Norway, and were performed in two 25x25 meters enclosures with electric fencing on a large (>5ha) pasture. The enclosures were spatially separated by at least 150 m to decrease odor transfer. Due to grass depletion inside the enclosures, new experimental enclosures had to be established every day.

2.3. Odor donors and collection of odor

Bear feces were collected from six captive animals in Orsa Predator Park, Dalarna, Sweden, during August 2010. I chose to use captive bear feces rather than wild bear feces, because the food content in captive bear feces likely was more similar, and thus more standardized, than among wild bears. The animals consisted of three males (two 3.5-year-old subadults, and one 10.5-year-old adult) and three females (two 2.5-year-old subadults and one 8.5 year adult). As part of another study (Elfström et al. 2013), the bears were fed with either berries (a mixture of bilberry *Vaccinium myrtillus* and lingonberry *V. vitis-idaea*) or animal carcasses (either domestic pig (*Sus scrofa*), cattle, horse (*Equus caballus*), European rabbit (*Oryctolagus cuniculus*), or semi-domestic reindeer (*Rangifer tarandus*). Feces of the individual bears was collected from enclosures where a bear was kept separate from its conspecifics for up to 24 hours (Elfström et al. 2013), and stored in zip lock plastic bags at -20°C until further use. Samples were defrosted overnight at room temperature before use in an experiment. Due to the experimental design used by Elfström et al. (2013), each bear feces sample consisted of only berries or only carcasses. Feces from the same individual bear comprised of the different food items were mixed together and used as treatment during the odor experiments. I chose to use a mixture of food items to avoid the possibility that cattle may react more strongly to feces containing carcasses only (Rosell et al. 2013).

Red deer feces were collected in May 2013 at Dagsrud Deer Farming AS, Telemark, Norway. The sampling was carried out by collecting fresh feces from two enclosures where a total number of 40 red deer were kept. All samples were collected during one day. Age and sex of the individuals the feces originated from were unknown. Since I only collected fresh feces, and because gut retention time in red deer is assumed to be 14 hours (Steyaert et al. 2009), I found it likely that they originated from different individuals. Feces were collected in 40 ml glass vials with Teflon-lined caps, and stored at -20°C until further use. Samples were defrosted overnight at room temperature before use in an experiment.

2.4. Design of odor experiments

The four weeks of experiments were divided into cycles, periods and days. An experimental cycle lasted for four days, and an overall of four cycles were carried out. Each cycle was divided into two periods lasting two days each (Figure 2, see also Table 1). The first period in each cycle lasted from Tuesday to Wednesday, and the second period from Thursday to Friday (Figure 2).

For each period, one group of 10 cows was randomly selected and divided into two subgroups of five cattle each. In general, all random selections within this experiments were carried out with the random number generator in Microsoft Excel®. An individual cattle could thus be selected for both periods within a given cycle. After the morning milking, these subgroups were placed in the two experimental enclosures in the morning of day 1 and morning of day 2. Each subgroup was then randomly assigned one of three possible odor treatments: bear feces, red deer feces, or a control treatment (blank, i.e., no odor). The only non-random requirement was that at least one of the subgroups on either day 1 or day 2 had to be exposed to bear feces (Figure 1).

Odor samples were placed on petri dishes (PS 90x14.2mm, no vent, sterile, VWR, Brisbane, Australia). An empty petri dish was used as control treatment, i.e., as blank. For presentation in the enclosures, petri dishes were placed in a container that allowed odors to evaporate (a transportation cage for pets with air holes; Trixie Pico, 30x21x23cm). The air holes on top of the container were closed with adhesive tape to prevent the feces samples from being dried in direct sunlight or washed away in rain. The containers were cleaned with chlorinated water every morning before use. The container with the odor treatment was randomly placed in the enclosure, where it was fixed to the ground with tent pegs to prevent the cattle from moving it around. Random placement was achieved by dividing the enclosure into a grid of 16 cells. The only requirement for the random location of the odor treatment was that it could not be placed in the same grid cell as the tank containing drinking water for the cattle. The odor treatment was placed in the middle of a selected cell in the morning, where it remained until cattle were collected for milking the morning after (i.e. 24h). Cattle participating in an experiment were separated from the main herd after morning milking of day 1 in an experimental period, and remained in their subgroups until they were joined again with the main herd after morning milking the day after an experimental period (Figure 1).

Production of milk was measured four times during an experimental period (i.e., two days): in the evening of day 1, in the morning and evening of day 2, and in the morning the day after an experimental period. Milk measures from the morning of day 1 were not included since cattle were not exposed to the odor treatments until after morning milking. In addition, I also received data on the amount of milk produced on the two days preceding (i.e., in the morning of day 1, in the morning and evening one day prior and in the evening two days prior to an experimental period), as well as the two days following (i.e., in the evening the day after, in the morning and evening two days after, and in the morning three days after) an experimental period (Figure 3).

2.5. Data analysis

I used parametric statistics for preliminary and exploratory analyses (Sokal & Rohlf 1995). I used a generalized linear mixed model (GLMM) (Zuur et al. 2009) to evaluate whether milk production was affected by the odor experiments. As dependent variable, I used the amount of milk produced by a cattle during a given day of a period (i.e., 2 following milk yield registrations after the cattle had been exposed to an odor, i.e., evening on day 1 + morning of day 2, and evening on day 2 + morning on day 3) (Figure 3). As independent variables I used “odor treatment” (as factor variable with the levels “bear”, “red deer” and “blank”; the level “bear” was set to 0), “period” (as factor, with level 0 for period/week 1, level 1 for period/week 2, and level 2 for period/week 3), “age” (in years), and “grain feed” (the amount of grain feed in kg cattle received on a given day). The variable “cattle ID” was used as random effect in the analysis.

I used a GLMM to evaluate whether the total milk production of a cattle during an experimental period was different from milk production before or after the experimental period. As dependent variable, I used the amount of milk produced by a cattle during an experimental period (i.e., amount of milk produced on day 1 + day 2), before a period (amount of milk produced the two days prior to the start of an experimental period), and after a period (amount of milk produced the two days after the end of an experimental period). As independent variables I used the same variables and random factors as in the previous analysis, i.e., “age”, “grain feed”, “odor treatment”, “period”, and the random effect “cattle ID”. In addition, I used a factor variable denoting if the amount of milk was produced after (level 0), before (level 1), or during (level 2) an experimental period. As significance level I used $p \leq 0.05$, and p-values $0.05 < p < 0.1$ were considered as statistical trend.

3. Results

Because of a data storage problem in the computer registering the amount of milk of every individual cow at each milking, all data from week 4 were lost and are therefore not available for further analyses. Overall, 37 cattle were exposed various times to odor by bears, red deer and a blank, resulting in 236 number of individual milk yields. These cattle yielded on average 14.8±3.4 L milk during morning milking, 9.6±2.2 L during evening milking, and on average 24.5±4,6 L milk per day (Figure 4). A positive and strong correlation between the amount of milk produced in the morning and the total amount of milk produced during a day was found ($r = 0.90$, $p < 0.001$). Correlation between the amount of milk produced in the evening and the total amount of milk produced during a day showed the same positive relationship ($r = 0.90$, $p < 0.001$). A high correlation was found between the amount of milk produced in the morning and the evening ($r = 0.70$, $p < 0.001$) (Figure 4). Therefore, only the total amount of milk produced during a day was used in further analyses.

No difference in milk yield was found among odor treatments (Table 2). Cattle yielded on average 24.8±4.4 L of milk when presented bear odor, 24.2±4.6 L when presented odor from red deer, and 24.4±5.1 L when presented blank (Figure 5a). Cattle produced significantly less milk before an experimental period and produced significantly more milk during an experimental period when compared to after an experimental period (Table 3). Before an experimental period, average milk yield was 22.8±5.1 L, during an experimental period 24.5±4.6 L, and after an experimental period 24.6±4.9 L (Figure 5b). Additionally, milk yield before, during and after experimental periods increased significantly with age, and with total grain feed given, and a reduction in milk yield was found in week 2 and 3, compared to week 1 (Table 3). In week 1 cattle yielded 25.4±3.6 L of milk; in week 2 milk yield was 23.9±4.5 L, and in week 3 milk yield was 24.1±5.5 L (Figure 5c). A significant increase with age and total grain feed given, and a reduction in milk yield in week 2 and 3 compared to week 1 was also found when analyzing milk amounts during experimental periods (Table 2).

4. Discussion

In general, the results did not support my main hypothesis that milk production in naïve dairy cattle would be affected when experimentally exposed to brown bear odor (feces), but rather suggest that bear fecal odor as a predator cue was not a strong enough stressor to elicit a physiological response affecting milk production. My first prediction was rejected, as milk yield did not differ significantly among odor treatments (i.e., bear, red deer, or blank). Also my second prediction was rejected, because milk yield was significantly lower before an experimental period, and significantly higher during an experimental period when compared to after an experimental period.

I did not find a significant difference in milk yield when cattle were exposed to bear odor. Potential explanations could be (i) that I was not able to measure it, or (ii) alternatively, that cattle did not experience the fecal odor of bears as threatening. The time from when stress hormones are released to when levels are normalized are unknown. Other methods are routinely used when measuring stress responses in animals, such as collecting blood samples (i.e., levels of stress hormones) (Monclús et al. 2006; Muñoz-Abellán et al. 2011), or measuring heart rate (Rushen et al. 2001). However, these are invasive methods that may cause stress by themselves due to penetration of the skin or other discomfort (Monclús et al. 2006), and results can therefore be misinterpreted. Measuring milk yield by taking milk from the udder naturally involves a certain disturbance for the animal, however, this activity is part of a dairy cattle's daily routine. Measurement of stress hormones in milk is also considered a non-invasive method, which is known to provide reliable results (Cook 2012). Levels of stress hormones are not included in this experiment, and could be investigated in a follow-up study.

The reason for the lack of response in cattle may be complex, but can likely be attributed to several factors. Which sensory modality an animal uses for predator detection is species specific (Apfelbach et al. 2005; Parsons et al. 2007). As a large ungulate, and a highly gregarious species (Boissy & Neindre 1997), cattle probably depend more on visual cues for predator detection (Apfelbach et al. 2005; Christensen et al. 2005; Sarno et al. 2008), which could be an explanation for the lack of response to bear odor. Odor from different sources from the same predator may elicit different responses in prey (Parsons & Blumstein 2010), and there are indications that fur-derived odors produce the strongest physiological and behavioral effects in prey (Apfelbach et al. 2005). For instance, laboratory rats have shown physiological changes in response to ferret (*Mustela nigripes*) fur/skin, but not to urine, feces or anal gland secretions (Masini et al. 2005), and ungulates have strongly avoided areas with bear pelts (Sahlén et al. 2016). While fur/skin odor is a strong indicator of a predator's presence, odor of feces/anal gland and urine indicate that the predator has left, and thus may not be interpreted as a straightforward danger cue (Blanchard et al. 2003; Hegab et al. 2015). One likely explanation to why cattle did not respond could therefore be that the bear feces, in the absent of other reliable predator stimuli, was not interpreted as a real threat, or as a reliable cue about the predators present, or about the predation risk. A similar explanation was proposed by Fletcher & Boonstra (2006) to why male captive meadow voles (*Microtus pennsylvanicus)* did not mount a hormonal response to ermine (*Mustela erminea*) odor (feces and urine). In addition, Christensen & Rundgren (2008) found that domestic horses showed behavioral (i.e., increased sniffing and vigilance, more behavioral shifts, and decreased eating) but no physiological response (i.e., increased heart rate), when exposed to wolf (*Canis lupus canadiensis*) or lion (*Panthera leo leo*) urine. A physical response was recorded only when the wolf odor (fur) was presented in combination with an additional stimulus (a sudden auditory stimuli). Cattle has earlier shown changes in behavior (i.e., increased vigilance) and foraging (decreased foraging rates) in response to predator (wolf (*Canis lupus*)) stimulus when presented a combination of stimuli (i.e., odor and visual), and a combination of odors (i.e., urine and feces) (Kluever et al. 2009). In my experiment, cattle were presented only with an olfactory stimulus, and from only one odor source (feces). It is therefore possible that fecal odor from bear is not the olfactory source that provokes a physiological response in cattle, and that other sources of bear odor (e.g., urine, fur), or a combination of stimuli (e.g., auditory, visual, olfactory) can evoke a response in cattle, however, this needs further investigation.

Responsiveness to a predator odor may be absent if predator and prey do not share a common evolutionary history (Apfelbach et al. 2005), and anti-predator behaviors may be lost when prey animals are isolated from their predators (Blumstein & Daniel 2005). Further, odors from sympatric predators can elicit stronger responses than odors from non-sympatric predators (Apfelbach et al. 2005). This has been demonstrated in western grey kangaroos (*Macropus fuliginosus*) (Parsons et al. 2007) and domestic cattle (Kluever et al. 2009). In the latter study, cattle responded stronger to the sympatric wolf than to the non-sympatric mountain lion (*Puma concolor*), when exposed to a combination of visual and olfactory stimuli. All domesticated cattle origin from the now extinct wild aurochs (*Bos primigenius*) (Kyselý 2008), which evolved in Eurasia with several large predators, such as wolves and bears (Van Vuure 2005; Kluever et al. 2009). Cattle's more profound response to wolf was suggested to be innate due to predation over the millennia by the sympatric predator (Kluever et al. 2009). Although the wolf probably was a more important predator to aurochs in Europe, bears likely were also a natural predator (Van Vuure 2005), yet no response to bear odor was found in cattle in my experiment. Despite a shared evolutionary history between bear and the domestic cattle's ancestor (Van Vuure 2005), and that brown bears were quite common in Norway until the 19th century (Swenson et al. 1995), cattle and bears in most of Norway usually do not encounter each other nowadays, except from maybe during summer months (during the cattle's dry period), when cattle are grazing untended on open range. This lack of exposure to bears as predators may have resulted in a loss of anti-predator behaviors, or relaxed selection for an innate response to bear odors (Price 1999). Sarno et al. 2008 also found that guanacos (*Lama guanicoe*) did not respond to urine from mountain lion, despite their common evolutionary history, and lack of response has also been revealed in other studies (Apfelbach et al. 2005).

The ability to recognize and respond to a predator or its odor may be innate, or learned through experience (Griffin et al. 2000; Blumstein et al. 2002). An innate response to a predator cue, such as odor, probably results from a coexistence over evolutionary time between predator and prey (Ward et al. 1997). Isolation between predator and prey may lead to the loss of "hard-wired" (i.e. experience-independent) mechanisms underlying an innate recognition or response, and once lost, these mechanisms are likely difficult to restore (Blumstein et al. 2002). Learned responses can result from individual experience (Epp & Gabor 2008), or through social learning (Griffin 2004), and may, with adequate experience, be more easily re-adapted when lost after a loss of predators (Blumstein et al. 2002). Although some studies have indicated an innate recognition and response toward predator odors (Blumstein et al. 2002; Apfelbach et al. 2005), it has been suggested that recognition may generally have to be learned (Blumstein et al. 2002). Berger et al. (2001) found that wolf-naive moose (*Alces alces*) failed to respond to wolf olfactory cues after the two species had been separated for over 80 years, whereas bear-experienced moose in Alaska showed increased vigilance in response to bear olfactory cues. The dairy cattle participating in my experiment have to be considered as predator-naïve, as they had no experience with bears or depredation events. This lack of experience is likely the major reason to why I did not find a response when cattle were exposed to bear odor. To obtain a better understanding of whether lack of experience was a causing factor, it would be helpful to compare my result with experiments on free-ranging cattle residing in the same area as bears, as they are more likely to encounter predators, and therefore may retain some level/degree of anti-predator behavior (Shrader et al. 2008).

I can only speculate as to why my second prediction (i.e., that milk yield will be lower during periods when cattle are exposed to odor treatments in comparison to the periods before or after exposure) was rejected. A contributing factor can be that during exposure, the cattle stayed in a smaller group of five individuals, contrary to staying with the main herd before or after the exposure. Competition over resources such as food and water, affects feeding behavior in cattle (Grant & Albright 2000). Fewer individuals may have decreased competition, and more time could therefore be allocated to feeding. Feed and water intake are important factors influencing milk production (Dado & Allen 1994; Grant & Albright 2001). I have no data of either quality or quantity of grass or other plants that were consumed on the pastures. Botanical composition is an important quality-factor affecting food intake, and thereby cattle performance (Randby et al. 2010). Food digestibility and nutrient content are also very important factors affecting intake and consequently milk production. Increasing digestibility increases food intake and milk yield (Keady et al. 2013). Differences in quality and quantity between pastures provided to the experimental groups and the main herd may therefore have contributed to differences in milk yield before, during, and after experimental periods.

Results also showed that cattle's age affected milk yield positively. This finding correlates with other previous work which have shown that older cattle produce more milk than younger cattle, which is likely related to a difference in feed intake (Grant & Albright 2001). Dado & Allen (1994) found that older cattle had a higher feed intake, consumed larger meals more quickly, had a more efficiently and longer rumination time, and had a higher water intake than younger cattle.

A significant decrease in milk yield in week 2 and 3, compared to week 1 was also found, which was expected as milk yield steadily declines with the advance of the lactation period, until the animal goes dry (Brody et al. 1923) some weeks prior to calving, and next lactation period starts (Annen et al. 2004).

5. Conclusion and management implications

I found that the cattle did not produce significantly less milk in response to bear odor, and that milk yield was significantly higher during experimental periods, than before and after. Reasons for the lack of response in cattle may be complex, and attributed by several factors, but I suggest that the cattle's lack of experience with bears (e.g., depredation events) could be an important factor. Further investigation is needed to get a better understanding on how cattle residing in the same areas as bears may respond to an increasing bear population in Norway.

Population goals for brown bears in Norway has not yet been met (Aarnes et al. 2016) and more bears should reside in the same areas as cattle in the future. As free-ranging cattle may be exposed to a variety of predator stimuli in their surroundings, and may have experienced encounters with bear, they are probably better able to recognize, and interpret bear fecal odor as a threat. Therefore, I cannot rule out that milk yield in more experienced cattle may be reduced when exposed to bear odor. It would be valuable to compare my results with results from an additional experiment using cattle residing in the same area as bears. This could provide a better understanding of the lack of response seen in this experiment, and also provide valuable knowledge that can be helpful in creating a more sustainable management system for cattle, that reduces conflicts with bears.

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Table 1. Description of the experimental setup of the experiment conducted at Ås, Norway during June-August 2013, where cattle (N=37) were exposed to odors (feces) from brown bear, red deer (a non-threatening control odor), or a control (blank, i.e., no odor). Eight groups with 10 individuals were participating in the experiment, and each group was divided into two subgroups (A and B). Experiments were carried out through a four-week period, where one week represented one cycle. One cycle consisted of two periods, and one period represented two days. During a period one group participated in the experiment, and its subgroups were tested in two separate enclosures.

Week		1		2		3		4	
Cycle		1		2		3		4	
Group		1 + 2		3 + 4		5 + 6		7 + 8	
Subgroup		A + B		A + B		A + B		A + B	
Periode 1	Day 1	1 A	1 B						
	Day 2	1 A	1 B						
Periode 2	Day 3	2 A	2 B						
	Day 4	2 A	2 B						
Periode 3	Day 1			3 A	3 B				
	Day 2			3 A	3 B				
Periode 4	Day 3			4 A	4 B				
	Day 4			4 A	4 B				
Periode 5	Day 1					5 A	5 B		
	Day 2					5 A	5 B		
Periode 6	Day 3					6 A	6 B		
	Day 4					6 A	6 B		
Periode 7	Day 1							7 A	7 B
	Day 2							7 A	7 B
Periode 8	Day 3							8 A	8 B
	Day 4							8 A	8 B

Table 2. Factors affecting the total amount of milk produced during an experiment conducted at Ås, Norway during June-August 2013, where cattle were exposed to odors (feces) from brown bear, red deer (a non-threatening control odor), or a control (blank, i.e., no odor). β denotes the parameter estimate, SD the standard deviation, df is degrees of freedom, t is the t-value, and p is the p-value.

Variable		B	SD	df	t	P
Intercept		15.539	1.046	85	14.858	<0.001
Age		0.605	0.253	30	2.394	0.023
Grain feed		2.567	0.241	85	10.651	<0.001
Week						
	Week 1	0	0	0	0	0
	Week 2	-1.621	0.538	85	-3.015	0.003
	Week 3	-3.446	0.560	85	-6.153	<0.001

Table 3. Factors affecting the total amount of milk before, during and after an experiment at Ås, Norway during June-August 2013, where cattle were exposed to odors (feces) from brown bear, red deer (a non-threatening control odor), or a control (blank, i.e., no odor). β denotes the parameter estimate, SD the standard deviation, df is degrees of freedom, t is the t-value, and p is the p-value.

Variable		β	SD	df	t	p
Intercept		16.001	1.215	667	13.180	<0.001
Before experiment		-0.466	0.163	667	-2.855	0.004
During experiment		1.279	0.206	667	6.223	<0.001
After experiment		0	0	0	0	0
Age		1.313	0.317	34	4.135	<0.001
Grain feed		1.095	0.186	667	5.875	<0.001
Week						
	Week 1	0	0	0	0	0
	Week 2	-0.864	0.244	667	-3.545	<0.001
	Week 3	-2.915	0.267	667	-10.926	<0.001

Figure legends

Figure 1.

Graphical description of an experimental period at Ås, Norway, during June-August 2013, when evaluating the effect of bear feces on milk production of dairy cattle. One group of 10 cattle were divided in two subgroups (A and B), consisting of five cattle each, which were tested for two days (i.e., a period).

(1) Cattle were milked. After milking cattle participating in the experiment were separated from the main herd.

(2) Cattle were released on experimental pasture, and exposed to odors (feces) from either brown bear, red deer (a non-threatening control odor), or a control (blank, i.e., no odor). A non-random requirement was that at least one of the subgroups on either day 1 or day 2 had to be exposed to bear feces.

(3) Cattle were milked.

(4) Cattle were released on experimental pasture, and exposed to the odors (i.e., same odors as in the morning). Cattle remained on the experimental pasture until they were joined again with the main herd after milking (at 6:30 am) at day 3.

Figure 2.

Graphical description of the design of an experiment evaluating the effect of bear feces on milk production in dairy cattle carried out at Ås, Norway, during June-August 2013. The experiment was divided into cycles, periods, and days. Experiments were performed over 4 weeks, each week consisting of 1 cycle. A cycle was divided in 2 experimental periods consisting of 2 days each. Cattle participating in the experiments were together with the main herd before and after an experimental period.

Figure 3.

Measurements of the cattle's milk yield before, during and after an experimental period at Ås, Norway, during June-August 2013, when exposed to odors (feces) from brown bear, red deer (a non-threatening control odor), or a control (blank, i.e., no odor). Milk yield measured in the morning (i.e., M) on the first day of experiment, in the morning and evening (i.e., E) 1 day before, and in the evening 2 days before a period represents the milk yield before an experimental period. Measurements from the evening on the first of day of experiment, in the morning and evening of day 2, and in the morning the day after a period represents the milk yield during an experimental period. Measurements from the evening the day after, in the morning and evening 2 days after, and in the morning 3 days after a period represents the milk yield after an experimental period.

Figure 4.

Correlation matrix of the amount of milk (in liter) produced at Ås, Norway, during June-August 2013, in the morning, the evening, and total amount of milk produced during an experimental day (i.e., 2 following milk yield registrations after the cattle had been exposed to an odor, i.e., evening on day 1 + morning and evening on day 2 + morning on day 3). The distribution/histograms of each variable (i.e., milk amount produced in the morning, the evening, and total amount of milk produced during a day) are shown on the diagonal. On the bottom/left side of the diagonal scatterplots of each variable and its corresponding variable are displayed (i.e. milk evening and milk morning, milk evening and milk total, milk morning and milk total). On the top/right side of the diagonal correlation coefficient (*r*) for each variable and its corresponding variable are shown. X- axis: milk amount in liter, Y- axis: frequency.

Figure 5a-c.

Box plots of the amount of milk produced (in liter) during the experiments at Ås, Norway, during June-August 2013, where cattle were exposed to odor treatments (feces) from brown bear, red deer (a non-threatening control odor), or a control (blank, i.e., no odor) (5a), amount of milk produced before (i.e., two days), during, and after (i.e., two days) an experimental period (5b), and milk production during experimental week 1, week 2, and week 3 when cattle were exposed to odor treatments (5c).

Figure 1.

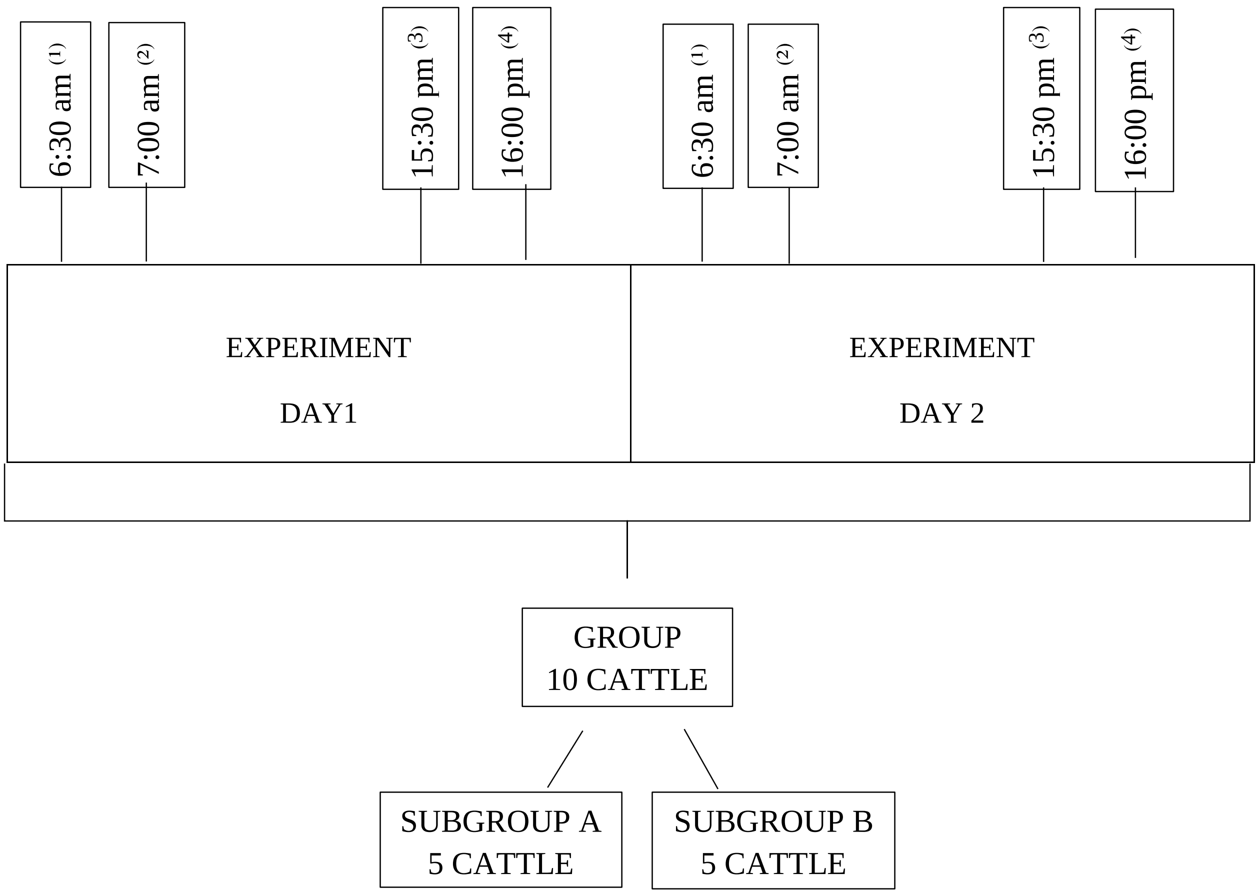

6:30 am (1)
7:00 am (2)
15:30 pm (3)
16:00 pm (4)
6:30 am (1)
7:00 am (2)
15:30 pm (3)
16:00 pm (4)
EXPERIMENT
DAY1
EXPERIMENT
DAY 2
GROUP
10 CATTLE
SUBGROUP A
5 CATTLE
SUBGROUP B
5 CATTLE

Figure 2.

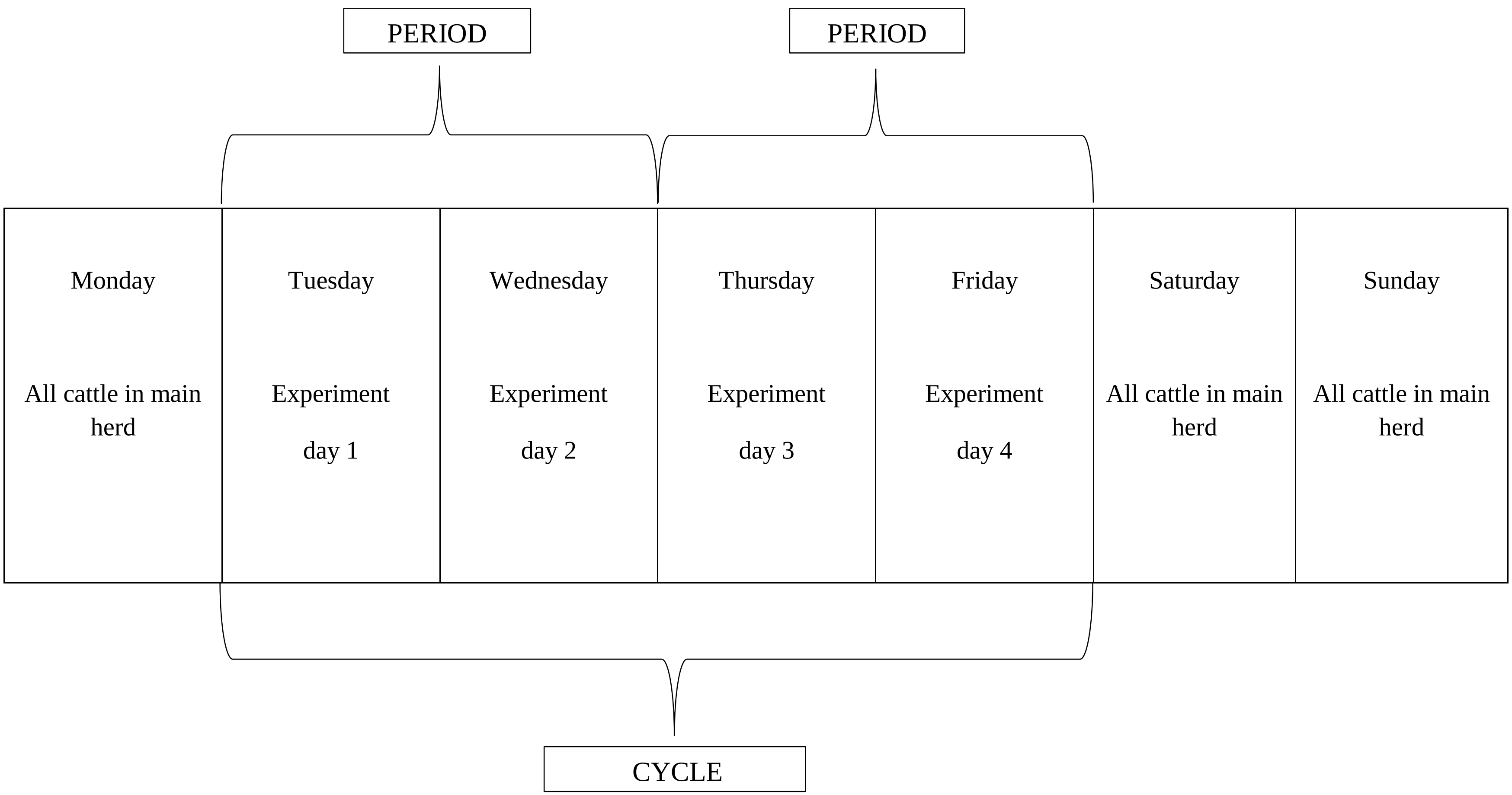
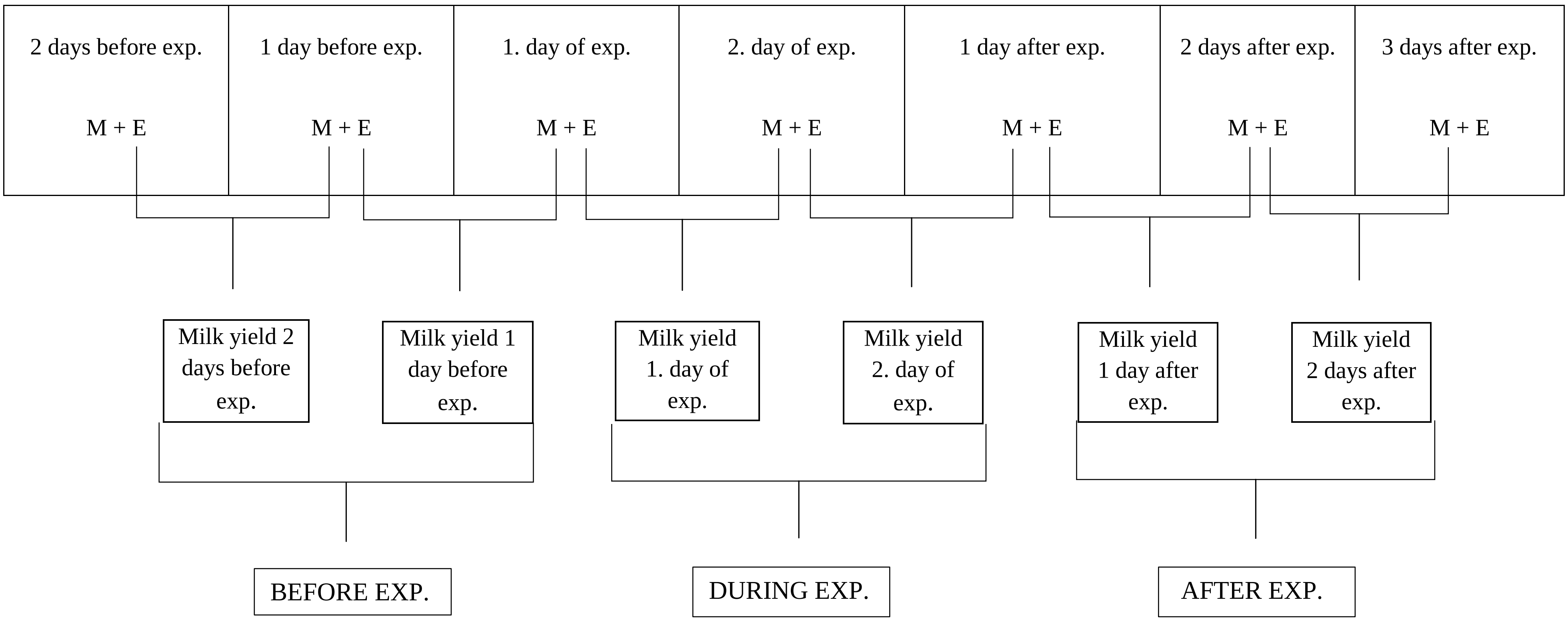
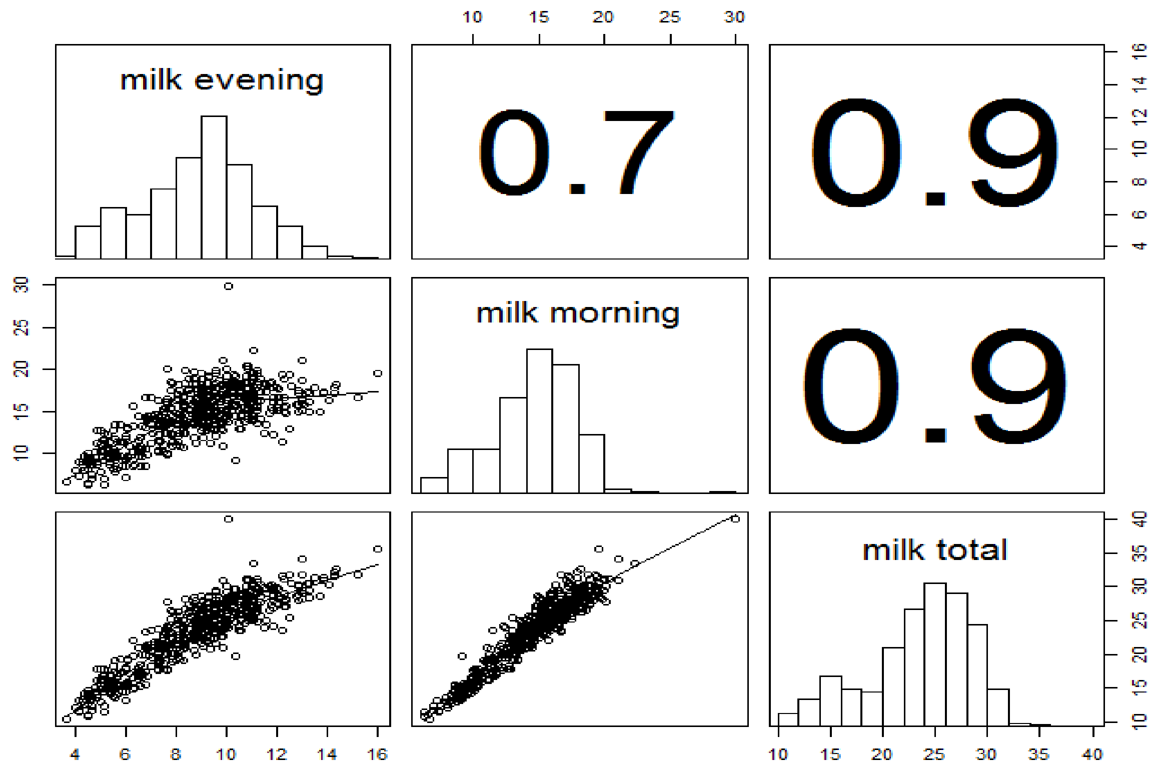

PERIOD
PERIOD
Monday
All cattle in main herd
Tuesday
Experiment
day 1
Wednesday
Experiment
day 2
Thursday
Experiment
day 3
Friday
Experiment
day 4
Saturday
All cattle in main herd
Sunday
All cattle in main herd
CYCLE

Figure 3.



2 days before exp.
M + E
1 day before exp.
M + E
1. day of exp.
M + E
2. day of exp.
M + E
1 day after exp.
M + E
2 days after exp.
M + E
3 days after exp.
M + E
Milk yield 2 days before exp.
Milk yield 1 day before exp.
Milk yield 1. day of exp.
Milk yield 2. day of exp.
Milk yield 1 day after exp.
Milk yield 2 days after exp.
BEFORE EXP.
DURING EXP.
AFTER EXP.

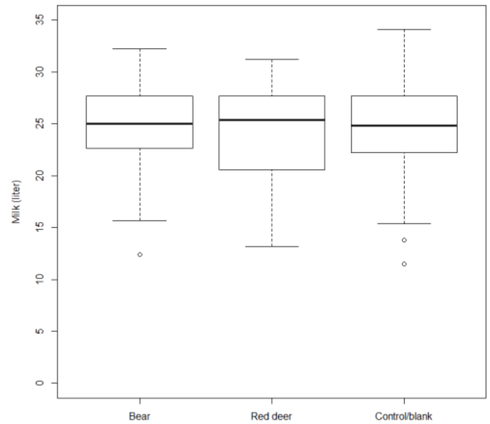
Figure 4.



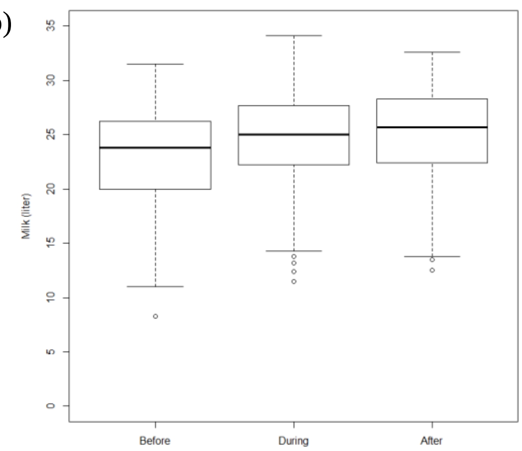
milk evening
0.7
0.9
milk morning
0.9
milk total
10
15
20
25
30
16
14
12
10
8
6
4
30
25
20
15
10
40
35
30
25
20
15
10
4
6
8
10
12
14
16
10
15
20
25
30
35
40

Figure 5 a - c.

(a)



(b)



(c)