Influence of estrus on rumination, activity, feed and water intake of dairy cows

Stefanie Rosemarie Reith

A dissertation submitted to the Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management, Justus Liebig University Giessen, for the degree of Doctor of Agriculture (Dr. agr.)

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Department of Animal Breeding and Genetics, Justus Liebig University Giessen

Supervisor: Prof. Dr. St. Hoy

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Stefanie Rosemarie Reith (M. Sc.)

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Dean: Prof. Dr. Dr. P. Kämpfer

1. Referee: Prof. Dr. St. Hoy
2. Referee: Prof. Dr. H.-P. Schwarz

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II Abbreviations

AI	artificial insemination
BW	body weight
CI	concentrate intake
CL	corpus luteum
cm	centimeter
d	day(s)
DM	dry matter
DMI	dry matter intake
FSH	follicle-stimulating hormone
g	gramm
GnRH	gonadotropin-releasing hormone
h	hour(s)
IFN-τ,	interferon tau
KB	künstliche Besamung
LH	luteinizing hormone
m	meter
min	minute(s)
ml	milliliter
mm	millimeter
NEB	negative energy balance
PGF2α	prostaglandin $F_{2\alpha}$
r	correlation coefficient
RT	rumination time
s	second(s)

SAG sexually active group(s)

SD standard deviation

SE standard error

THI temperature humidity index

WI water intake

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1. General introduction

1.1 The problem of estrus detection

Detection of estrus is one of the most important factors impacting the reproductive efficiency in dairy cattle, especially in farms using AI (Heersche and Nebel, 1994; Sheldon et al., 2006). Reproduction management directly affects the calving-to-conception interval, thus affecting the calving interval and milk production, which impacts profit (Bascom and Young, 1998). However, in several studies, researchers have reported a serious decline in fertility, occurring simultaneously with increased milk yields which can be attributed to the genetic selection for higher milk yields as well as nutritional and management factors (Lucy, 2001; Pryce et al., 2004). The relationship between milk yield and characteristics of estrus has been the subject of numerous investigations (López-Gatius, 2003; Lopez et al., 2004a, b; López-Gatius et al., 2005b; Yániz et al., 2006). Washburn et al. (2002) observed an increase in average d open from 124 d in the late 1970s to 168 d in the late 1990s. Conception rates decreased from 53% to 35% and a lower detection rate of 41.5% was found in 1999 compared to 50.9% in 1985. Additionally, recent reports have demonstrated that variation in cycle length, duration, and intensity of estrus has significantly increased (Van Eerdenburg et al., 1996; Kerbrat and Disenhaus, 2004), especially in modern Holstein cows (Washburn et al., 2002; Cutullic et al., 2009; Sveberg et al., 2015). Friggens and Labouriau (2010) observed that 50% of the cycle lengths were between 19 and 28 d and 20% were longer than 33 d. Dobson et al. (2008) reported that the percentage of animals that stand to be mounted has declined from 80% to 50% and the duration of estrus has reduced from 15 h to 5 h over the past 50 years. In a study of Dransfield et al. (1998), 24% of the estrous periods were characterized by low intensity and short duration. The percentage of cows displaying estrous behavior of short duration (< 8.7 h) and low intensity (< 2.7 standing events/h) was greater in high-yielding cows (53.4%) than in low-yielding cows (32.2%).

Traditionally, estrual cows were identified by visual observation. As herd size increases, visual observation of individual cows is not practical within the available time of the herd manager, resulting in unobserved estrus. Detection efficiency is often below 50% in dairy herds (Senger, 1994; Van Vliet and Van Eerdenburg, 1996; Van Eerdenburg et al., 2002) and failure to identify estrual cows results in remarkable economic losses (Plaizier et al., 1998; Inchaisri et al., 2010). Although poor reproductive performance causes the highest culling rate – in Hesse 23.1% (HVL, 2014) –, few cows are described to be infertile (Bascom and Young, 1998; Seegers et al., 1998). About 90% of the factors for low detection rates can be attributed to management and 10% to the cow (Diskin and Sreenan, 2000). Due to the high variability in duration and intensity of the expressed estrous signs among individuals and the great influence by a number of various factors, detection of estrual cows is still a major problem (Roelofs et al., 2010).

Automated sensor-based technologies that continuously monitor and record detailed information about the cow have been developed to attenuate further reproductive declines. Much research has been conducted to identify physiological and behavioral traits indicating estrus.

1.2 Characteristics of the bovine estrous cycle

From clinical view, the cow's reproductive cycle consists of two phases – the follicular phase (4 - 6 d) and the luteal phase (14 - 18 d) – and is characterized as phases of sexual rest (diestrus) and periods of stress (estrus) (Lyimo et al., 2000), in which the cow is sexual receptive (Beach, 1976). The average cycle length is about 21 d and varies from 19 to 25 d (Savio et al., 1988; Trout et al., 1998; Roth et al., 2000; Friggens and Labouriau, 2010) with tendentially longer inter-estrous intervals in older cows and animals with higher milk production (Gwazdauskas et al., 1983; Sartori et al., 2004). Estrus, usually known as d 0, is characterized by a multitude of physiological and behavioral signs (Esslemont et al. 1980; Diskin and Sreenan, 2000) and can – according to Hurnik et al. (1975) – be divided into proestrus (interval from the first mounting to the onset of true estrus), true estrus (standing estrus, interval during which the cow remains stationary when mounted by others), and metestrus (interval from the end of true estrus to the termination of the mounting period).

Table 1. Mean duration of cow's estrus in dependence on the year and the detection method.

Reference	Mean (h)	Detection method
Marion et al., 1950	18.2 to 21.1	Visual observation
Hall et al., 1959	11.9 ± 6.1 (SD)	Visual observation
Hurnik et al., 1975	7.5 ± 2.3 (SE)	Video camera
Esslemont and Bryant, 1976	14.9 ± 4.7 (SD)	Visual observation
Esslemont et al., 1980	15 ± 2.6 (SE)	Visual observation
Britt et al., 1986	13.8 ± 0.6 (SE)	Visual observation (8 h intervals)
Schofield et al., 1991	13.5 ± 2.3 (SD)	Pedometer
Walker et al., 1996	9.5	HeatWatch
Xu et al., 1998	8.6 ± 0.46 (SE)	HeatWatch
Dransfield et al., 1998	7.1 ± 5.4 (SD)	Mount detector
Lyimo et al., 2000	20.3 ± 10.4 (SD)	Visual observation (30 min every 3 h)
At-Taras and Spahr, 2001	5.83 ± 0.78 (SE)	HeatWatch
Lopez et al., 2004a	8.7 ± 0.6 (SE)	HeatWatch
Roelofs et al., 2005a	11.8	Pedometer
	10.0	Visual observation (30 min every 3 h)
Løvendahl and Chagunda, 2010	8.12 (cows)	Accelerometer system
	9.24 (heifers)	
Valenza et al., 2012	16.1 ± 4.7 (SD)	Accelerometer system
Sveberg et al., 2015	7.1 ± 1.4 (SE)	Video camera

Length of time during which characteristic behavioral signs are expressed and intensity vary considerably between individuals and are significantly influenced by the method used to detect estrus as well as by a number of environmental, cow- and management-related factors (Orihuela, 2000; Roelofs et al., 2010). Previous studies have shown that the duration of estrus has declined. Taking only standing estrus identified by mount detectors into consideration to define estrus, estrus lasts between 5.8 (At-Taras and Spahr, 2001) and 9.5 h (Walker et al., 1996). The duration of expression of secondary signs is substantially longer (25.7 h to 34.6 h) (Yoshida and Nakao, 2005). The frequency increases gradually within 12 h before the onset of the period when standing behavior is exhibited (Sveberg et al., 2011). Esslemont et al. (1985) noted that the duration of all estrous behaviors that were displayed during estrus was almost twice the duration of mounting behavior. Intensity of estrous behavior decreased from 56.3 mounts/estrus (Esslemont and Bryant, 1976) and 11.2 mounts/cow with an average duration of 4.6 s (Hurnik et al., 1975) to 6.7 mounts/estrus (At-Taras and Spahr, 2001) and 7.6 mounts/cow with a mean duration of 3.3 s (Lopez et al., 2004a) during the last years.

1.3 Endocrine regulation of the bovine estrous cycle

Ovarian functions (follicle development, ovulation, luteinisation, and luteolysis) are regulated by endocrine hormones secreted by the hypothalamus (GnRH), anterior pituitary (FSH and LH), ovaries (progesterone, estradiol, and inhibin), and the uterus (PGF2α) (Forde et al., 2011). Knowledge on the hormonal mechanisms is a necessary basis for developing strategies to improve the reproductive management on dairy farms (Allrich, 1994).

1.3.1 Follicular Phase

The follicular phase (proestrus, estrus) is the period following luteolysis of the CL of the previous cycle until ovulation. Ovarian follicle development is characterized by the recruitment of a cohort consisting of 5 - 20 follicles with a diameter ≥ 5 mm (Fortune et al., 1991; Sunderland et al., 1994; Webb and Campbell, 2007) and is stimulated by a transient rise in circulating concentrations of FSH (Webb et al., 2003). Serum concentrations of progesterone, which has an inhibitory effect on the expression of estrous behavior, are very low during the follicular phase (Fabre-Nys and Martin, 1991; Allrich, 1994). The bovine estrous cycle usually consists of two to three waves of follicular growth (Ginther et al., 1989; Sirois and Fortune, 1990; Fortune et al., 1991; Kaneko et al., 1995; Mihm et al., 2000) starting on d 2, 9, and 16 in cycles with three waves or on d 2 and 11 in cycles with two waves (Sirois and Fortune, 1988), thus lasting between 7 to 10 d (Mihm et al., 2000). A single dominant follicle is selected from the cohort to increase in diameter while the subordinate follicles undergo atresia (Savio et al., 1988; Sunderland et al., 1994; Kaneko et al., 1995). Follicle development and survival are dependent on the ability to produce estrogens – the estrogen concentration is higher in dominant follicles compared with other follicles in the cohort (Forde et al., 2011; Rosales-Torres et al., 2012) – and to respond to the FSH surge. In cattle, the primary estrogen is estradiol 17β (Peters and Lamming, 1983). Deviation occurs when the dominant follicle reaches a diameter ≥ 8 mm (Ginther et al., 1996, 2002; Kulick et al., 1999) and secretes large amounts of estradiol and inhibin leading to inhibition of FSH concentrations released from the anterior pituitary gland (Sunderland et al., 1994; Kaneko et al., 1995; Forde et al, 2011). These pre-ovulatory follicles continue to mature in an environment of low FSH concentrations associated with a switch from FSH to LH dependency (Kulick et al., 1999; Webb and Campbell, 2007). The mechanisms of selection are linked to the presence of an enhanced number of LH receptors on the granulosa cells (Webb et al., 2003). Final maturation and ovulation of the pre-ovulatory follicle, 15 to 20 mm in diameter (Dobson et al., 2008), can be realized when the resulting LH surge is of high-frequency and low-amplitude pulses (Forde et al., 2011). Rahe et al. (1980) reported that LH secretion during the luteal phase of the estrous cycle was characterized by low-frequency and high-amplitude pulses that were inadequate for ovulation of the dominant follicle. Thus, these dominant follicles have become atretic followed by a decline in estradiol and inhibin secretion and an increase in FSH release leading to the recruitment of a new follicular wave (Forde et al., 2011).

1.3.2 Estrus and ovulation

The synthesis of follicular estradiol results from the coordinated actions of LH and FSH on theca and granulosa cells, respectively (Forde et al., 2011). Luteinizing hormone binds to membrane receptors on thecal cells. This binding activates the synthesis of androgens that subsequently diffuse through the basement membrane into granulosa cells. The following binding of FSH to its receptors on granulosa cells leads to an increase in aromatase activity, inducing the conversion of androgens to estradiol (Ginther et al., 1996). The initiation of estrus by estradiol (Vailes et al., 1992; Allrich, 1994) and the role of other intra-ovarian factors have been shown in various studies (Mihm et al., 2000; Forde et al., 2011). Elevated concentrations of estradiol secreted by the pre-ovulatory follicle in turn promote a GnRH surge and allow – when progesterone levels are low (Vailes et al., 1992) – the expression of behavioral estrus and the release of LH to cause ovulation. In a study conducted by Valenza et al. (2012), probability of conception was highest when cows were inseminated 7.9 h before ovulation.

1.3.3 Luteal Phase

Following ovulation, the luteal phase (metestrus, diestrus) is characterized by the formation of the CL from luteinized granulosa and theca cells of the pre-ovulatory follicle. Luteinizing hormone is considered to be the major luteotrophic hormone stimulating luteinisation of these cells into luteal cells (Forde et al., 2011). The primary function of the CL is to produce sufficient quantities of progesterone which is required for implantation – progesterone impacts on the endometrium and initiates blastocyst development and elongation to a filamentous conceptus (Spencer et al., 2006) – maintenance of pregnancy and inhibition of GnRH secretion from the hypothalamus (Morris and Diskin, 2007; Lonergan, 2011). Progesterone concentrations increase 6 to 7 d after the onset of estrus and decrease 2 d before onset of the following estrus (Gartland et al., 1975).

If between d 15 and 17 after AI, the maternal recognition of pregnancy, IFN-τ, signaling the presence of the bovine conceptus has not been detected at adequate concentrations (Bazer et al., 1994; Thatcher et al., 1997), luteolysis of the CL occurs by a pulsatile release of endometrial PGF2α at the end of the luteal phase (Tsai and Wiltbank, 1998; Okuda et al., 2002). Physiological processes during this "critical period" are multifactorial and characterized by complex embryo-endometrium interactions which stimulate luteal regression or CL maintenance for establishment of pregnancy (Binelli et al., 2001; Vonnahme, 2012). Hypophysial oxytocin activates synthesis and secretion of PGF2α by binding to specific oxytocin receptors localized on the endometrial membrane (Silvia et al., 1991; Danet-Desnoyers et al., 1994). Prostaglandin F2α induces the luteolytic mechanism via a counter-current transfer between the uterine vein and the ovarian artery, leading to regression of the ruminant CL accompanied by decreased progesterone level during the proestrous period (Kaneko et al., 1995; Forde et al., 2011). Pulse frequency of LH increases followed by a rapid increase in follicular estradiol secretion during the follicular phase of the estrus cycle.

In the presence of an embryo, progesterone concentrations remain high, when the CL does not regress in response to production of PGF2α. Pulsatile release of this hormone is blocked because IFN-τ inhibits the endometrial expression of the oxytocin receptor and the initiation of luteolysis (Mann et al., 1999; Demmers et al., 2001). From d 19 of gestation lasting on average 280 d in cows (Meyer et al., 2000) the process of implantation is initiated with the attachment of fetal cotyledons to caruncles on the maternal uterine epithelium and is completed by d 42 (Hunter, 1980). After parturition, time to first AI depends on the resumption of ovarian cyclicity and the occurrence of silent ovulations associated with difficulties in detecting behavioral signs of estrus (Berka et al., 2004; Peter et al., 2009; Ranasinghe et al., 2010).

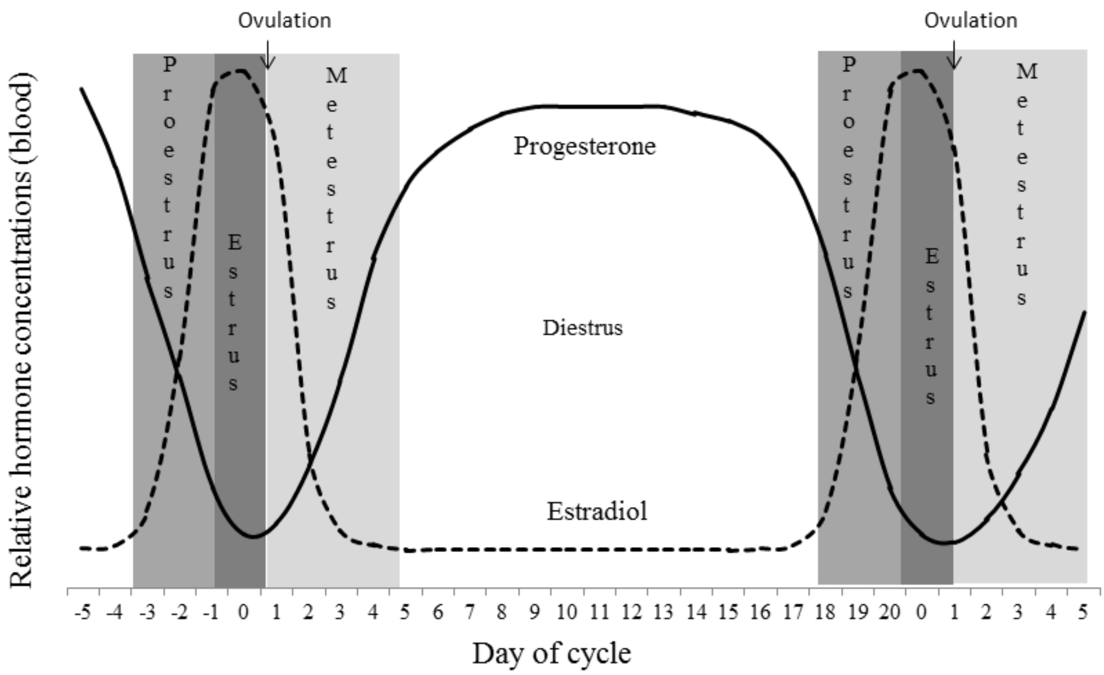


Figure 1. Hormone patterns of cow's estrous cycle, modified from Senger, 2003.

1.4 Physiological and behavioral signs of estrus

Different categories of estrous behavior are proposed in the literature. Beach (1976) differentiates between cow's sexual attractivity, proceptivity, and receptivity. Busch and Waberski (2007) defined sexual attractivity as display behavior contrary to mounting behavior. For most authors (Diskin and Sreenan, 2000; Yoshida and Nakao, 2005; Dobson et al., 2008), estrous behavior can be classified on the basis of primary and secondary signs, and these signs can in turn be divided into visual versus non-visual signs (Foote, 1975) or physiological versus behavioral changes (Negussie et al., 2002; Roelofs et al., 2010).

1.4.1 Physiological signs

1.4.1.1 Hormones

Hormone concentrations, especially changes in estradiol and progesterone have a strong correlation with estrus (see chapter 1.3) and therefore used in various studies as a gold standard to confirm the period of cow's sexual unrest (Redden et al., 1993; Van Vliet and Van Eerdenburg, 1996; Kyle et al., 1998; Kamphuis et al., 2012).

1.4.1.2 Cervical mucus discharge and vulvar swelling

Hormonal changes affect the cow's genital tract by making it tonic, oedematous and highly secretory resulting in cervical mucus discharge. Ezov et al. (1990) observed changes in cell density, fluid volume, and electrolyte content of vulvar tissue. Increases in tissue hydration and blood flow lead to swelling and redness of the vulva and cause changes in tissue electrical resistance (Lehrer et al., 1992; Kitwood et al., 1993).

1.4.1.3 Vaginal electrical resistance

Due to increased hydration and congestion of the vaginal mucus membranes the vaginal electrical resistance decreases at estrus (Gupta and Purohit, 2001). The decrease in vaginal electrical resistance was correlated positively with a decline in plasma progesterone with lowest values occurring in the second half of the estrous period and corresponding with the time of the LH peak on the d of estrus (Gartland et al., 1975; Schofield et al., 1991). Tadesse et al. (2011) reported a decline in electrical resistance from 106.8 ohms during diestrus compared to 82.2 ohms during estrus. High resistance was detectable after ovulation in the luteal phase (Gupta and Purohit, 2001).

1.4.1.4 Milk yield

Cow's milk production also has been reported to be reduced – occasionally – in some individuals on the d of estrus and, especially, when more cows were simultaneously in estrus (Hurnik et al., 1975). In investigations by Britt et al. (1986) and Schofield et al. (1991), milk yield was significantly lower at the first milking near the time of onset of estrus followed by a compensatory increase at the next milking. However, Halli et al. (2015) found no alteration in cows' milk production on the d of estrus. Yet, a slight decrease occurred on the d after estrus.

1.4.1.5 Temperature

The body temperature of cows has a mean level of 38.3 °C with a range of excursion of 1.4 °C (Piccione et al., 2003). High body temperatures of 39.0 ± 0.5 °C were detected during estrus (Suthar et al., 2011). According to Fisher et al. (2008), the vaginal temperature decreased slightly 2 d before the d of estrus followed by an increase at the time of the LH peak. In their study, the average temperature increase was 0.48 °C. The duration of elevated vaginal temperature varied from 6.5 h to 9 h (Clapper et al., 1990; McArthur et al., 1992) with a maximal increase of 0.9 °C ± 0.3 °C (Kyle et al., 1998). Rajamahendran and Taylor (1991) found rises in vaginal and rectal temperature primarily at the onset of standing estrus. Similarly, the milk temperature increased by about 0.4 °C on the d of estrus (McArthur et al., 1992). Piccione et al. (2003) found a larger range of excursion (1.3 °C). Elevations in cow's milk temperature as well as vaginal temperature were detected at only one milking on the d of estrus and mostly associated with a high day-to-day as well as inter-/intraindividual variability (McArthur et al., 1992).

Table 2. Physiological changes during estrus.

Physiological signs of estrus	Selection of further references
Level of hormone concentration	Gartland et al., 1975; Britt et al., 1986; Allrich, 1994; Lyimo et al., 2000; Lopez et al., 2004a
Mucous vaginal discharge	Gartland et al., 1975; Van Vliet and Van Eerdenburg, 1996; Lyimo et al., 2000; Negussie et al., 2002
Electrical resistance	Schams et al., 1977; Aboul-Ela et al., 1983
Swelling and reddening of vulva	Lewis et al., 1989
Milk yield	Lopez et al., 2004a, b
Milk temperature	Fordham et al., 1988
Vaginal temperature	Redden et al., 1993

1.4.2 Behavioral signs

1.4.2.1 Primary sign of estrus

In various studies, standing to be mounted was the primary and most characteristic external sign for determining when a cow is in estrus (Glencross et al., 1981; Dransfield et al., 1998; Negussie et al., 2002; Cutullic et al., 2009) and considered sexually receptive for AI. The estrual cow makes no effort to escape while being mounted by other cows (Hurnik et al., 1975). According to a definition provided by Esslemont and Bryant (1976), cows in estrus mount at least six times or mount another cow and stand to be mounted at least three times. In the literature, a proceeding decrease in the number of cows showing standing estrus is well documented (Stevenson et al., 1996; Dransfield et al., 1998; Walker et al., 2008; At-Taras and Spahr, 2001; Lopez et al., 2002). The number of cows exhibiting standing behavior varied between 8% (Kerbrat and Disenhaus, 2004) and 74% (Britt et al., 1986), with significantly decreasing tendency. In a number of previous studies, less than 50% of the cows stand to be mounted on the d of estrus (Fonseca et al., 1983; Heres et al., 2000; Van Eerdenburg et al., 2002). However, standing estrus was the prevalent sign of estrus (97.8%) in Fogera cows (Negussie et al., 2002). The duration of estrus based on standing mounts averages 8 h to 9 h (Dransfield et al., 1998; Xu et al., 1998) but it could be less than 6 h in some dairy herds (At-Taras and Spahr, 2001). Because not all estrual cows expressed standing estrus (Britt et al., 1986; Heersche and Nebel, 1994; Van Eerdenburg et al., 2002) – in a study conducted by Kerbrat and Disenhaus, standing estrus represented 21.5% of all sexual behavior – Kerbrat and Disenhaus (2004) focused on secondary signs to enhance detection of estrus.

1.4.2.2 Secondary signs of estrus

1.4.2.2.1 Mounting behavior

Mounting or attempting to mount other cows have a high frequency during estrus compared with other d (Esslemont et al., 1980; Kerbrat and Disenhaus, 2004). Mounting behavior was observed in 80% of the cows with an average number of mounts of 2.9 (Van Vliet and Van Eerdenburg, 1996). Front mounts were observed rather infrequently as Britt et al. (1986) found only 3.4% of the cows attempting to mount another cow from the front. In a recent report, the average duration of mounting estrus was 12.9 h (Sveberg et al., 2013). The mean number of mounts was between 6.5 and 8.7 mounts/h (De Silva et al., 1981; Gwazdauskas et al., 1983) and 6.7 and 8.5 mounts/cow (Dransfield et al., 1998; At-Taras and Spahr, 2001; Lopez et al., 2004a), respectively, with an average duration of 3.2 and 4 s (At-Taras and Spahr, 2001; Lopez et al., 2004a; Sveberg et al., 2013). It is well known, that the number of mounts per cow and the length of mounting revealed a significant dependency on housing conditions (Britt et al., 1986), level of milk production (Lopez et al., 2004a) or estrus synchronization, increasing when more cows are in estrus simultaneously (Hurnik et al., 1975).

1.4.2.2.2 Activity

Activity behavior increases markedly in cows approaching estrus (e.g. Liu and Spahr, 1993; At-Taras and Spahr, 2001; Müller and Schrader, 2003; Berka et al., 2004; López-Gatius et al., 2005b, Roelofs et al., 2005a; Peralta et al., 2005; Brehme et al., 2006; Løvendahl and Chagunda, 2010; Neves et al., 2012; Valenza et al., 2012), indicating a reliable prediction of sexual restlessness. Cows were between 2.3 and 6 times (Kiddy 1976; Schofield et al., 1991; Redden et al., 1993; Arney et al., 1994; Brehme et al., 2006; Silper et al., 2015) as active at the time of estrus – mostly defined as d 0 – as when not in estrus. Duration of activity episodes measured by pedometers and neck transponders varied between 10 h and 16.1 h (Kerbrat and Disenhaus, 2004; Roelofs et al., 2005a; Løvendahl and Chagunda, 2010; Valenza et al., 2012). There exist several studies on the incidence of weak estrous signs and silent ovulations, indicating the inability of some cows to express restlessness based on walking activity (Brehme et al., 2006; Ranasinghe et al., 2010). The proportional composition of further daily activities is affected by the estrous stage. Some researchers found changes in time spent eating, resting, and lying (Hurnik et al., 1975; Phillips and Schofield, 1990; Pennington et al., 1986; Brehme et al., 2006). However, results are not consistent in the literature and influenced by various factors, respectively.

1.4.2.2.3 Agonistic interactions

In the period of estrus, the cows are more motivated to involve in agonistic interactions than during diestrus. Aggressive interactions were exhibited more intensively – approximately doubled (Hurnik et al., 1975) – on the d of estrus than on all other d. The most frequent agonistic behavior was head-to-head butting which represented 64% of all fights (Hurnik et al., 1975). The number of butts was correlated positively with approach-walking (Phillips and Schofield, 1990; Kerbrat and Disenhaus, 2004) and pedometer readings, respectively, (Pennington et al., 1986; Van Vliet and Van Eerdenburg, 1996). Butting occurred at high incidence at the same time as that of mounting before standing estrus in the pre-ovulatory period (Esslemont et al., 1980). However, in studies conducted by Phillips and Schofield (1990) and Castellanos et al. (1992), the number of cows showing butting was observed at the same frequency during estrus as during diestrus. Push away-behavior, during which the initiating cow pushes the receiving cow with its head, was the only agonistic behavior displayed relatively infrequently in estrual cattle (Sveberg et al., 2011).

1.4.2.2.4 Social interactions

Chin-resting/chin-rubbing, sniffing/licking the anogenital region (vulva) of another cow and orientation are classified as social or sexual behaviors. Chin-resting and sniffing/licking represented 48.0% and 21.7%, respectively, of all sexual interactions on the d of estrus (Kerbrat and Disenhaus, 2004). Similar results were found by Lyimo et al. (2000). In order to determine important symptoms for detection of estrus, they analyzed correlations between estradiol concentration and some signs of estrus. Differences in correlation factors indicated that mounting, unrest, and chin-resting are more indicative of estrus than sniffing vulva. Increased frequencies of these signs were found during (Sveberg et al., 2011) and after standing estrus (Esslemont et al., 1980). However, chin-resting and sniffing were also observed in non-estrual cows and therefore not considered as a reliable tool to identify estrual animals (Phillips and Schofield, 1990). Chin-rubbing and anogenital licking were less relevant for detecting cows in estrus (Pennington et al., 1986, Negussie et al., 2002). Similarly, flehmen and circling (Esslemont et al., 1980) were only occasionally seen. Hurnik et al. (1975) revealed no significant differences in bellowing between cows in estrus and non-estrual cows.

Table 3. Characteristic behavioral symptoms of estrus.

Primary sign of estrus	Selection of further references
Standing to be mounted	Phillips and Schofield, 1990; Diskin and Sreenan, 2000; Lyimo et al., 2000; Lopez et al., 2004a
Secondary signs of estrus	
Mounting/Attempting to mount other cows	Pennington et al., 1986; Lyimo et al., 2000; Heres et al., 2000; Cuttulic et al., 2009
Activity/Restlessness	Kamphuis et al., 2012
Activity/Locomotion	Peter and Bosu, 1986; Maatje et al., 1997; Yániz et al., 2006
Approach-walking/	Pennington et al., 1986
Following/Circling	Kerbrat and Disenhaus, 2004
Bellowing/Vocalization	Hurnik et al., 1975; Negussie et al., 2002
Head butting	Glencross et al., 1981; Negussie et al., 2002
Chin-resting/-rubbing	Van Vliet and Van Eerdenburg, 1996
Sniffing/Licking	Van Vliet and Van Eerdenburg, 1996
Flehmen	Van Eerdenburg et al., 1996
Lying	Phillips and Schofield, 1990; Brehme et al., 2006
Standing	Phillips and Schofield, 1990; Kerbrat and Disenhaus, 2004
Inappetance/Feeding	Hurnik et al., 1975; Phillips and Schofield, 1990

1.5 Factors affecting estrous expression

Behavioral signs differ among individual cows in duration and intensity of estrus (Hurnik et al., 1975; Orihuela, 2000). Cow-related as well as environmental and management-related factors influence the expression of estrus (Gwazdauskas et al. 1983; Diskin and Sreenan, 2000; Roelofs et al., 2010) and are responsible for high inter-individual variations.

1.5.1 Cow-related factors

1.5.1.1 Parity

Duration of estrus is highly variable between heifers and cows, ranging from 2.6 h to 26.2 h in heifers (Stevenson et al., 1996) and from 3 h to 28 h in dairy cows (Allrich, 1994). Lower activity peaks were observed for multiparous than for primiparous cows (Yániz et al., 2006). López-Gatius et al. (2005b) calculated that each additional lactation number caused a 21.4% decrease in locomotion. Peralta et al. (2005) found a significant lower number of standing events for cows in the third lactation (5.6 ± 2.8) compared with those in the second (6.2 ± 3.5) and first lactation (9.2 ± 6.6) (Peralta et al., 2005). However, Walker et al. (1996) reported a 50% shorter duration of estrus in primiparous (7.4 ± 1.4 h) compared to older cows (13.6 ± 2.0 h). Similarly, Van Vliet and Van Eerdenburg (1996) noted significant differences between the mean duration of primi- and multiparous cows (10.4 ± 5.0 h versus 14.8 ± 7.2 h), while others found no effect of age on estrus-related characteristics (At-Taras and Spahr, 2001; Van Eerdenburg et al., 2002). Because mounting activity was lowest in heifers (5.5 mounts/h) and increased to 7.9 mounts/h for cows in the fourth lactation, Gwazdauskas et al. (1983) suggested an association with sexual experience. Van Eerdenburg et al. (1996) found, according to their scoring system, primiparous cows to have less pronounced intensity of estrus compared to multiparous cattle (361 ± 82 points versus 578 ± 331 points), although this result was not supported by Van Eerdenburg et al. (2002).

1.5.1.2 Breed

Behavior around estrus differs among species and breeds. The duration of estrus was shorter for *Bos indicus* cattle that are widespread in tropical regions than that reported for *Bos taurus* cows in the temperate zone (Plasse et al., 1970). Rae et al. (1998) revealed significant differences among Angus and Brahman heifers. Breeds varied in estrus duration (Angus heifers: 8.52 ± 1.2 h, Brahman: 6.65 ± 1.2 h, Angus/Brahman cross: 11.9 ± 1.2 h) and in the number of mounts (Angus heifers: 19 ± 3.6, Brahman: 25 ± 5.4, Angus/Brahman cross: 37 ± 5.5). Additionally, there is considerable variability in the inter-estrous intervals. Duration of the estrous cycle averaged 21 d for Holstein cows (Diskin and Sreenan, 2000). In Ethiopia, mean length was 29.2 ± 19.7 d for Fogera cows, and the duration of estrus averaged 10.6 ± 4.5 h (ranging from 2.2 h to 21.0 h) (Negussie et al., 2002) which was in close agreement with mean duration of estrus (10.3 ± 4.5 h) in Zebu cattle (Lamothe-Zavaleta et al., 1991).

As milk yield and expression of estrous signs were correlated (Lopez et al., 2004a), Holstein cows had – if compared with others (Normande cows) (Cutullic et al., 2009) – less intense estrous expression and poorer reproductive performance (Ranasinghe et al., 2010). There were significantly fewer services in Jersey x Holstein crossbreeds (2.2) than in pure Holstein cows (2.7) (Heins et al., 2012). The rate of occurrence of first estrus after parturition was greater in Danish Red cows than in Jersey and Holstein cows. Similar results were noted by others (Fonseca et al., 1983; Washburn et al., 2002; Løvendahl and Chagunda, 2010).

1.5.1.3 Health

It is well known that several diseases are associated with reduced reproductive performance in dairy cattle. The intensity of estrous behavior was 50% lower in cows suffering from lameness. These animals expressed a lower frequency as well as duration of standing estrus and secondary signs such as mounting other cows, chin resting (Collick et al., 1989; Sood and Nanda, 2006; Dobson et al., 2008), and activity behavior (number of steps) (Maatje et al., 1997). According to Walker et al. (2008), reduced intensity resulted from altered time budgets in lame cows which spent less time standing and walking and more time lying, thus having little chance of displaying estrous behaviors and being detected. The intervals from calving to first AI and from calving to conception were increased by four and 14 d (Collick et al., 1989), and the probability of delayed cyclicity was 3.5 times greater in lame cows compared with non-lame cows (Garbarino et al., 2004). Loss of body reserves during the early lactation period was associated with low fertility (Gillund et al., 2001), as NEB had negative impact on LH pulse frequency that lead to inhibition of estrogen synthesis by dominant follicles and failure of ovulation (Butler, 2000). López-Gatius et al. (2003) found a more than 10 d longer calving-to-conception interval in cows with low BCS. Similarly, development of ketosis, acidosis or displaced abomasum was correlated negatively with conception rate and time to become pregnant. Maatje et al. (1997), however, detected no influence of mastitis on activity behavior during estrus.

1.5.1.4 Milk yield

The effect of milk yield on estrous expression and duration has been the subject of numerous investigations. Authors noted increases in services per conception, d open (Lucy, 2001; Sakaguchi et al., 2004; Washburn et al., 2002), the incidence of inactive ovaries as well as decreases in cyclicity, pregnancy rate (López-Gatius, 2003) and estrous behaviors in high-producing Holstein Friesian herds (Lopez et al., 2004a, b; Yániz et al., 2006). Length of time during which high-yielding cows (≥ 39.5 kg/d) expressed estrous signs lasted 6.2 h compared to the duration of 10.9 h in cows with lower milk yields (< 39.5 kg/d) (Lopez et al., 2004a). To characterize the relationship between milk yield and duration of estrus, Wiltbank et al. (2006) noted a correlation coefficient of r = - 0.51. This may be the result of a lower serum estradiol concentration on the d of high-yielding cows' estrus (Lopez et al., 2004a; Sartori et al., 2004) due to increased metabolic clearance rate of steroid hormones (Sangsritavong et al., 2002; Wiltbank et al., 2006). Similarly, the frequency of standing events was lower for cows with milk production above than for cows with milk yields below the herd average (6.3 ± 0.5 versus 8.6 ± 0.5) (Lopez et al., 2004a). Negative effects of high milk production on activity behavior were reported by López-Gatius et al. (2005b) and Yániz et al. (2006). In contrast, no interaction between the level of milk production and the expression of estrus and conception rate was reported by Patton et al. (2007).

1.5.2 Environmental factors

1.5.2.1 Season

Although De Rensis and Scaramuzzi (2003) reported some contradictory effects of season on reproductive patterns – maybe affected by different definitions of heat stress provided in the literature (Roelofs et al., 2010), hot climatic conditions were major factors depressing reproductive efficiency due to reduced duration and intensity of estrus and a larger range in cycle length (Gangwar et al., 1965; Cartmill et al., 2001; Jordan, 2003) contributing to low detection and pregnancy rates (Hansen and Arechiga, 1999; Wolfenson et al., 2000; López-Gatius et al., 2005a). Lamothe-Zavaleta et al. (1991) noted an average duration of estrus of 12.4 h when the temperature was below 27 °C compared to 9.3 h when it was above 27 °C. Several authors studied the influence of heat stress in large commercial dairy Holstein herds and found higher conception rates in cows inseminated in the winter and spring in comparison to cows inseminated during summer and fall months (Santos et al., 2009) and during mild than during moderate heat stress (THI $\leq$ 76 versus THI > 76) (Peralta et al., 2005), respectively. Cows calving in the spring and winter had the greatest risk of delayed resumption of estrous cyclicity after calving and silent ovulation (Opsomer et al., 2000; Walsh et al., 2007), following alterations in photoperiodic stimulation (Dahl et al., 2000). Jordan (2003) reported some variations in follicular dynamics and endocrine profiles in heat stressed cows when compared with control cows. There was a reduction in LH secretion leading to suppressed synthesis of follicular steroids (Wilson et al., 1998; Wolfenson et al., 2000), thus, reduced plasma estradiol concentrations (Roth et al., 2000) contributing to impaired detection of estrus (De Rensis and Scaramuzzi, 2003). Further consequences of heat stress include higher incidence of ovulation failure (López-Gatius et al., 2005a), lowered progesterone secretion by luteal cells, impaired oocyte quality, and embryo development and survival (Wolfenson et al., 2000; Cartmill et al., 2001). A period of elevated temperature shortened the duration of standing activity – defined as the time between first and last mount recorded by heat mount detector – (2.97 h versus 6.76 h in cool weather), but had no impact on the number and duration of individual mounts (At-Taras and Spahr, 2001). Expression of mounting activity was not inhibited as long as the maximum environmental temperature on the estrous d remained within the cows' thermoneutral zone. Beyond 30 °C, as observed by Gwazdauskas et al. (1983), temperature impacted negatively the number of mounts. López-Gatius et al. (2005b) concentrated on the relationship between season and activity behavior during estrus and detected a significantly lower increase in walking activity during the summer season (May to September) than that measured during the period from October to April (369 ± 152% versus 384 ± 156%). Similarly, an increase in mean relative humidity higher than 95% was associated with a decrease in walking activity at estrus (Yániz et al., 2006).

Use of artificial cooling methods including installation of shaded areas, fans, sprinkler systems allowed overcoming the detrimental effects of hyperthermia on fertility in dairy cattle (Armstrong, 1994; Hansen and Arechiga, 1999), but the improvement of fertility did not correspond with normal winter fertility (De Rensis and Scaramuzzi, 2003). Indeed, hormonal treatments inducing timed AI and embryo transfer were insufficient to compensate for the weather-related decline in fertility (Wolfenson et al., 2000; Jordan, 2003) due to a higher percentage of cows suffering embryonic losses (Cartmill et al., 2001). Heat stress affected indirectly reproductive performance by reduced appetite and DMI which prolonged the period of NEB in early lactation (De Rensis and Scaramuzzi, 2003).

1.5.2.2 Circadian variation

Reproductive efficiency may be impaired due to cows displaying estrous behavior preferably during the nocturnal period (Hurnik et al., 1975; Van Vliet and Van Eerdenburg, 1996; Pinheiro et al., 1998). Several researchers found a diurnal distribution of the onset of estrous activity in two peaks: early in the morning and late in the afternoon. Cows primarily exhibited estrous behaviors between 0600 h and 1030 h, and 1400 h and 1830 h (Mattoni et al., 1988; Negussie et al., 2002). Others found no circadian variation in estrous activities (Xu et al., 1998).

1.5.3 Management-related factors

1.5.3.1 Housing

Duration and behavioral symptoms of cows' estrus reveal a dependency on housing system and floor surfaces. Most estrous behaviors were expressed more frequently in straw yards than in cubicle environments (Phillips and Schofield, 1990). Cows kept in cubicle housing exhibited more sub- und silent estrus than those kept at pasture. Similarly, the number of standing mounts was reduced under housed (52% of cows) than under pasture conditions (91% of cows) – irrespective of the detection method (Palmer et al., 2010). However, de Silva et al. (1981) and Gwazdauskas et al. (1983) found barn housed cattle displaying more standing events (11.2 mounts/h and 8.7 mounts/h) than pastured cattle (5.4 mounts/h and 5.5 mounts/h), possibly due to an increase in priority for feeding at pasture (Phillips and Schofield, 1990). There was an indirect effect of housing systems on reproductive efficiency as housed cows had a higher incidence of lameness and stress, which in turn reduced intensity of estrus (Dobson et al., 2008).

According to Britt et al. (1986), floor type was the most important factor affecting estrous behavior of dairy cows. Cows showed a clear preference for mounting – 3- to 15-fold greater – and further secondary signs (butting, sniffing, licking, chin resting) on soft than on concrete surface (Vailes and Britt, 1990). Equally, the time during which cows displayed standing and mounting behavior was longer on soft than on concrete surfaces (13.8 h versus 9.4 h) (Britt et al., 1986). Mounting activity was markedly inhibited by slippery floors, especially in cows that previously sustained a fall when attempting to mount another cow during estrus (Palmer et al., 2010).

1.5.3.2 Herd size

No consensus exists concerning stocking density. It was found that increasing stocking density enhanced the number of cows meeting and interacting sexually (Orihuela, 2000) as well as that overcrowding reduced the display of estrous signs because of no adequate space in housing systems (Diskin and Sreenan, 2000). Recent studies have demonstrated that the number of cows simultaneously in estrus affected both intensity of sexual activities (Britt et al., 1986; Diskin and Sreenan, 2000) and duration of behavioral signs (Pennington et al., 1986; Van Vliet and Van Eerdenburg, 1996; Roelofs et al., 2005a). The length of the estrous period varied between 11.6 ± 4.9 h and 16.1 ± 8.2 h with one or more cows becoming estrous (Van Vliet and Van Eerdenburg, 1996). Synchronization of estrus induced a high number of animals to be in estrus at the same time. Hurnik et al. (1975) who compared estrous behavior in synchronized and non-synchronized cows found significant changes in mounting activity: an increasing percentage of cows in estrus at the same time was associated positively with the average number and duration of mounts (11.2 mounts/h versus 52.6 mounts/h and 4.6 ± 1.12 s versus 7.4 ± 0.41 s for one cow or 3 cows simultaneously in estrus, respectively). Detection rates were significantly improved by the occurrence of another cow displaying estrous behavior (Cuttulic et al., 2009). Expression of cow's estrus that was based on measurement of walking activity increased by 6.1% for each additional estrual cow (Yániz et al., 2006).

In small herd sizes the likelihood of detecting more than one cow exhibiting estrus at a time becomes less (Diskin and Sreenan, 2000). Nevertheless, Van Eerdenburg et al. (2002) observed standing behavior in only 50% of the cows, although there was more than one cow in estrus at the same time, suggesting that the lack of standing behavior is not influenced by the presence of cows simultaneously in estrus and herd size, respectively. In addition, detection of estrus has become more difficult due to less interest of pregnant cows in mounting cows during estrus (Diskin and Sreenan, 2000).

The possibility of finding a partner with which to interact is particularly high in case of estrus synchronization. Cows receive some sexual stimulation by the estrual group, contributing to the manifestation of estrous behaviors. Thus, cows often participate in SAG during estrus (Kilgour et al., 1977), in which some cows are more attractive and sexually active than other animals in the herd (Orihuela, 2000). Sveberg et al. (2013) identified SAG as a novel sign of estrus of long duration: 1.45 h contrary to the total duration of mounts of 38 s. Social dominance has been reported to have a negative impact on expression of estrous symptoms. Subordinate cows may be less attractive to others and are suppressed to exhibit mounting behaviors by cows of high rank in the social hierarchy (Orihuela, 2000). They are described to be less fertile when they have experienced a decline in their social status within the herd (Dobson and Smith, 2000). Orihuela et al. (1988) observed that 60% of all exhibited mounts were initiated by high-ranking cows. Similar results were found by Hurnik et al. (1975).

However, other studies found no effect of estrous synchronization (López-Gatius et al., 2005b) and correlation between hierarchy order and sexual behaviors (Orihuela and Galina, 1997).

1.5.3.3 Nutrition

Fertility of modern dairy cows is affected by the process of postpartum metabolic adaptation (Peter et al., 2009) regulating the resumption of estrous activity (Butler, 2000, 2003). As milk yield of dairy cows is closely related to DMI – Harrison et al. (1990) found a correlation of $r = 0.88$, nutritional requirements increase rapidly in the early lactation (Butler, 2003). The most important factor to explain impaired reproductive performance is the cow’s energy balance, the difference between the available energy from feed intake and the amount of energy needed for maintenance and milk production (Sheldon et al., 2006). To meet the huge demands of lactation cows usually enter a period of NEB causing – dependent on the extent and duration of NEB – inhibited expression of estrous behaviors and further reproductive dysfunctions (Sheldon et al., 2006; Wathes et al., 2007). A status of NEB – occurring for a longer time in high-yielding than in less productive cows (Lucy, 2001) – decreases hypothalamic production of GnRH and, in turn, suppresses pulsatile LH secretion and circulating estrogen and progesterone concentrations (Sangsritavong et al., 2002; Washburn et al., 2002; Wathes et al., 2007), explaining the decrease in duration and intensity of estrus (Lyimo et al., 2000; Lopez et al., 2004a). Body reserves are mobilized to compensate for NEB and contribute to higher loss of body weight and BCS (Collard et al., 2000; Liefers et al., 2003) which in turn affects fertility by fewer cows showing initiated estrus (Santos et al., 2009). In addition, NEB has been related to delayed resumption of ovarian activity, prolonged postpartum anestrus (Liefers et al., 2003; Butler, 2003), delayed time of first detected estrus (De Vries et al., 1999), a greater incidence of irregular cycles (Wathes et al., 2007), decreased conceptions rate (Butler, 2003; Patton et al., 2007), increased pregnancy loss, (Wiltbank et al., 2006) and, thus, more d open (Reist et al., 2003). In contrast, cows in a positive energy balance were found to have 11.3 d lesser to first postpartum luteal activity (Liefers et al., 2003) reducing calving-to-conception interval (Patton et al., 2007).

Additionally, high dietary protein indicated by elevated plasma urea concentrations may be responsible for impaired reproductive performance (Sheldon et al., 2006). Nevertheless, López-Gatius et al. (2005b) expected no effect of NEB on the intensity of estrous expression and there have been, indeed, some high-yielding cows being able to maintain high fertility inspite of the described influence of milk production on reproductive function (Pryce et al., 2004).

1.5.3.4 AI and hormonal therapy

Detection of estrus is affected by the duration, frequency (Hurnik et al., 1975; Saumande, 2002), and timing of observation (Gwazdauskas et al., 1983; Van Vliet and Van

Eerdenburg, 1996). Synchronization of estrus by reproductive hormones has been used to stimulate fertility and to increase the efficiency of estrus detection in dairy cattle (De Rensis and Scaramuzzi, 2003). Duration and intensity of estrus were highly variable and were not different between estrous cycles induced by $PGF_{2\alpha}$ and those occurring spontaneously (Walker et al., 1996). However, others reported a longer duration of natural in comparison to induced estrus (21.7 h versus 19.8 h (Jaume et al., 1980) and 15.3 h versus 13.3 h (Vaca et al., 1985)). According to Valenza et al. (2012) using activity monitoring systems and heatmount detectors for identifying cows in estrus, increased activity and standing behavior was detected in only 71% and 66% of synchronized cows.

1.6 Methods of detecting estrus

1.6.1 Visual observation

Visual observation is of practical importance in exclusively herds where AI is performed. However, this method requires a substantive part of the herd manager's working time and is complicated by the short duration and poor expression of behavioral signs of estrus in modern dairy cows (Dransfield et al., 1998; Peralta et al., 2005) and is especially difficult in large herds (Lucy, 2001). Identification of estrus by visual observation varied considerably between 90% detected by Hall et al. (1959) to less than 50% (Peter and Bosu, 1986; Van Vliet and Van Eerdenburg, 1996; Heres et al., 2000; Van Eerdenburg et al., 2002; Peralta et al., 2005). Thereby, efficient detection depends on the length and frequency of observation periods and was reported to be most successful at a daily frequency of two or three observation periods of 30 min (Van Vliet and Van Eerdenburg, 1996) or four periods of 15 min (Saumande, 2002). However, undetected and falsely detected cycles lead to missed and untimely AI associated with consequent economic losses.

1.6.2 Fully automated systems

Over the past several years, there has been a clear trend toward the use of technological methods for accurate detection of estrus in dairy cattle (Fricke et al., 2014). Already in 1994, Senger postulated cost-effective methods replacing visual observation by permanent automatic monitoring of individual data. Further, the ideal system for detection of estrus provides minimal labor requirements and a high degree of accuracy at identifying physiological or behavioral signs. A number of diverse detection systems including temperature measurements, milk yield recordings, intravaginal resistance probes, hormone analyses, cameras, activity measurements, and heat mount detectors have been developed and refined to enhance detection of estrus and, thus, improve reproductive management in dairy farms (Firk et al., 2002). Several methods were eliminated from further investigations. Large daily fluctuations and the influence of too many non-estrus related factors minimized the potential of a trait for practical application. Similarly, methods considered to be too expensive and labor-intensive were eliminated (Firk et al., 2002). Recent research has concentrated on accurate analysis of routinely collected sensor-based data and constant surveillance of behavior (Ranasinghe et al., 2010; Burfeind et al., 2011; Valenza et al., 2012).

1.6.2.1 Pressure sensing system

Electronic pressure sensitive devices such as HeatWatch® (Walker et al., 1996; Xu et al., 1998; At-Taras and Spahr, 2001; Rorie et al., 2002) or DEC® (Saumande, 2002) are based on detection of onset and length of standing mounts accepted by estrual cows. The system consists of a pressure-sensitive transmitter which is embedded in a burlap pouch and glued to the sacral region anterior to the tail head (Walker et al., 1996; Xu et al., 1998; Saint-Dizier and Chastant-Maillard, 2012). This on-cow sensor is activated by the weight of a mounting animal for a minimum of 2 s to limit the number of false-positive results, although it has been found that up to 40% of mounts lasted less than 2 s (Walker et al., 1996). Via radio signal data (date, time, cow ID, number and duration of mounts, signal strength) are sent within a 1200-m radius to a receiver and recorded by the management software on a farm computer (At-Taras and Spahr, 2001; Rorie et al., 2002; Saint-Dizier and Chastant-Maillard, 2012). A defined algorithm analyses each cow's mounting profile with the software classifying a "standing" as three or more standing events in any 4-h period (Diskin and Sreenan, 2000; Peralta et al., 2005). Initiation of estrus is confirmed by the first activation of the sensor (Lopez et al., 2004a). The software provides various reports including lists and graphs of cows defined as standing or suspected of standing – depending on whether cows receiving or not receiving three or more mounts within the 4-h period (At-Taras and Spahr, 2001; Rorie et al., 2002). Use of that system resulted in detection of 82.1% of the ovulations (Lopez et al., 2004a) and improved detection of estrus compared with visual observation. In two different trials, At-Taras and Spahr (2001) found efficiencies of 86.8% and 71.1% for detection based on HeatWatch® in comparison to 54.4% and 54.7% provided by visual observation of cows. However, similar efficiencies – 48.0% identified by the system versus 49.3% by visual observation – were indicated in a study conducted by Peralta et al. (2005). The efficiency for the DEC® system was reported to be considerably lower, videlicet approximately only 50% of the efficiency obtained from visual observation (35.4% versus 68.8%) (Saumande, 2002). The potential of pressure-sensitive systems was affected significantly by housing conditions (Palmer et al., 2010), type of flooring (Britt et al., 1986; Vailes and Britt, 1990), weather (Peralta et al., 2005), and difficulties in maintaining the sensors in the proper position (Diskin and Sreenan, 2000). Displacements or losses of sensors up to 40% were described in some studies (Foote, 1975; Xu et al., 1998; Saumande, 2002).

1.6.2.2 Activity measurement

1.6.2.2.1 Pedometer

Pedometers attached to the leg of the cow record the number of steps taken per unit time as an indicator of walking activity being markedly increased during proestrus and estrus of dairy cows (Arney et al., 1994; Maatje et al., 1997; López-Gatius et al., 2005b; Roelofs et al., 2005a; Brehme et al., 2006; Yániz et al., 2006; Ranasinghe et al., 2010). Advancements in sensor technology have provided reading of activity in frequency from twice daily (Yániz et al., 2006) to 12 2-h periods (Liu and Spahr, 1993; Maatje et al., 1997; Roelofs et al., 2005a) or 24 1 h-periods (Ranasinghe et al., 2010) per d. Various researches evaluated these systems as a reliable method of identifying estrual animals (Lehrer et al., 1992; Senger, 1994) as well as useful for prediction of ovulation time (Roelofs et al., 2005a). Further, López-Gatius et al. (2005b) found a positive relationship between walking activity and pregnancy rate of dairy cows. Pedometer readings agreed with sexual activities including mounting, following, chin resting, rubbing, butting, sniffing expressed by estrual cows, and duration of estrus (Pennington et al., 1986; Liu and Spahr, 1993; Maatje et al., 1997).

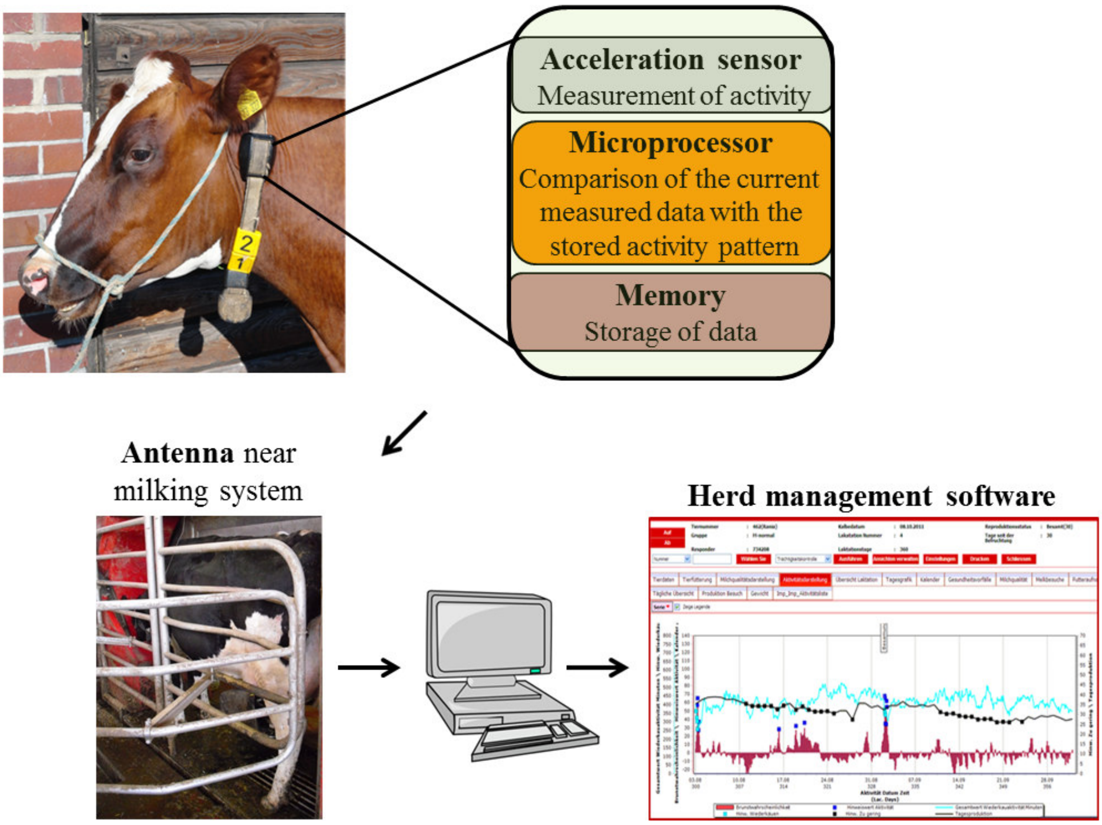
Cows coming into estrus are identified by an increase in locomotion above the mean activity value recorded – during the same time period – for preceding d (Roelofs et al., 2005a; Yániz et al., 2006). Pedometer recordings showed a diurnal rhythm in the number of steps (Roelofs et al., 2005b) which is important for the development of algorithms considering within-cow comparisons (Liu and Spahr, 1993). Alerts are generated using different algorithms and are set off if weighted activity has exceeded a user-defined threshold value (Liu and Spahr, 1993; Roelofs et al., 2005a). The detection rates and error rates for the different thresholds used to study the increase in the number of steps around estrus have been reported (Schofield et al., 1991; Liu and Spahr, 1993; Redden et al., 1993; Maatje et al., 1997; Roelofs et al., 2005a). Data stored in a memory are transferred to receivers usually placed near the milking system and sent to the management software (Maatje et al., 1997; Roelofs et al., 2005a; Ranasinghe et al., 2010) enabling herd managers to review the reproductive status of individual cows (Fricke et al., 2014). Thus, duration of estrous behaviors monitored by pedometers has been found to be shorter than duration of estrus visually observed (10.0 versus

11.8 h) (Roelofs et al., 2005a) but longer than the mean duration of estrus based on the interval between the first and the last standing event recorded by means of radiotelemetric monitoring of mounting activity (Dransfield et al., 1998; Xu et al., 1998). Use of pedometers is more efficient than visual observation of cows (Kiddy, 1976; Pennington, 1986; Peter and Bosu, 1986). Liu and Spahr (1993) detected 74% of estrous cycles by these systems compared to 58% achieved by herd managers. But, results depend markedly on the number of ovulations post partum. As estrus is usually silent in early lactation (Ranasighe et al., 2010), Peter and Bosu (1986) found 43% of the cows displaying no estrous signs at first ovulation, based on detection by means of pedometers, followed by expression of estrus at subsequent ovulations. However, incidences of silent ovulation based on visual observation seem much higher compared with technological systems (Shipka, 2000).

1.6.2.2.2 Accelerometer system

Activity meters using acceleration technology are attached to the neck collar of each cow (Müller and Schrader, 2003; Kamphuis et al., 2012; Valenza et al., 2012) and measure continuously horizontal accelerations related to upward movements of cow's head and neck during walking and mounting behavior (Elischer et al., 2013; Løvendahl and Chagunda, 2010). Data present average activity shown as a general activity index (Elischer et al., 2013; Silper et al., 2015) which can be stored in 1 h- (Løvendahl and Chagunda, 2010) or 2 h-intervals each d (Kamphuis et al., 2012; Valenza et al., 2012). Specially developed algorithms based on deviations of the current measured data from the stored activity pattern are used to separate cow's d to d activity from activities associated with estrous behavior. Herdsmen receive an alert after cows have exceeded a user-defined threshold (Løvendahl and Chagunda, 2010; Valenza et al., 2012). The effects of changing thresholds and time windows on detection performance was demonstrated by Kamphuis et al. (2012). Data are read by an antenna and automatically transferred via infrared communication to the herd management software providing lists and graphs to control reproductive (and health) status of individual cows (Kamphuis et al., 2012; Valenza et al., 2012).

Figure 2. Acceleration technology attached to cow’s neck collar.



Accelerometer readings showed a diurnal rhythm (Løvendahl and Chagunda, 2010) and corresponded highly with the behavioral patterns obtained from video recordings (Müller and Schrader, 2003). Duration of estrus based on acceleration technology between 10.33 h and 16.1 h (Løvendahl and Chagunda, 2010; Valenza et al., 2012; Silper et al., 2015) was comparable to the average duration reported for cows observed for estrus by pedometers (Roelofs et al., 2005a). Further, the percentage of cows detected in estrus did not differ between the accelerometer system and the Heatmount detectors (71% versus 66%, respectively) (Valenza et al., 2012). Thus, accelerometer systems are described as a useful tool to detect estrus (Müller and Schrader, 2003; Løvendahl and Chagunda, 2010) and to improve fertility in dairy cattle (Valenza et al., 2012). The technology is commercially available for measurement of activity only or combination with rumination characteristics (Kamphuis et al., 2012).

1.6.2.3 Video camera

Usability of video systems realizes identification of cow's standing mount position. Cameras fixed preferably in the upper corners at a height of 3 m are connected to the video management software providing visualization of stored video sequences. Detection is affected by camera resolution, as low resolution may result in difficulties in reading of the ear-tag number and, thus, identifying the cow (Saint-Dizier and Chastant-Maillard, 2012), disposition and the used threshold value. Although these systems are equipped with infrared technology, artificial lighting is necessary at nighttime (Bruyère et al., 2012). Compared with a duration of 40 min per d (four periods of 10 min) needed for visual observation, the time exposure to analyze the video sequences varied between 8 and 32 min, depending on the number of cows that were simultaneously in estrus (Bruyère et al., 2012). The efficiency for detection based on video recording was similar to that obtained from classical visual observation (81% versus 82%) (Saint-Dizier and Chastant-Maillard, 2012) whereas Bruyère et al. (2012) found a higher detection rate (80% versus 68.6%) and concluded that using video cameras for detection of estrus can replace visual observation. Nevertheless, as with visual observation, only cows with obvious behavioral estrous signs were detected. According to Foote (1975), this system did not save much time and may be mainly of research value.

1.6.2.4 Recording of vocalization

The vocal behavior of cattle gives information on the reproductive status of the vocalizing animal and may bear upon estrus advertisement (Watts and Stookey, 2000). Near the time of estrus vocalization rate was found to be increased (Negussie et al., 2002; Schön et al., 2007), with the extent of vocalizations depending on the status of the estrous cycle: di-estrus < pro- and postestrus < estrus (Dreschel, 2014). Vocalizations are recorded continuously by a clip-on microphone attached to a neck harness of the animal. Via a transmitter the recordings are transferred to a stationary receiver being connected to the sound card of the computer. By use of the available algorithm, serial signal windows are generated from the sound recording and only those with means exceeding a defined threshold are considered for detection of estrus. However, large individual variability of absolute vocalization rate might reduce the suitability of this trait for practical application (Schön et al., 2007).

1.6.2.5 Measurement of body temperature

Automated systems of monitoring body temperature around estrus are based on radiotelemetric transmission of information. The temperature rhythms have been recorded by rectal (Piccione et al., 2003) and vaginal thermometry (Mosher et al., 1990; Kyle et al., 1998). In a study conducted by Redden et al. (1993), transmitters enclosed by a support anchor with fingerlike projections were inserted into the vagina to a depth of 20 cm. Transmitter signals were picked up by specific receivers which were connected to a computer. Others used microprocessor-controlled temperature loggers (size = 92 mm × 20 mm; weight = 40.5 g) placed in the vaginal cavit (Suthar et al., 2011) or on-chip temperature sensors implanted in the cow's vulvar muscle – connected with receivers located in the collar (Morais et al., 2006). Peaks in vaginal temperature have been observed around the time of estrus (Kyle et al., 1998; Mosher et al., 1990). The detection rate of estrus based on vaginal temperature was 89.4%

when an increase of at least 0.4 °C for 3 or more consecutive hours above the average of the corresponding hours of the previous 3 d was considered as the peak in vaginal temperature (Kyle et al., 1998). Piccione et al. (2003) used a rectal probe inserted 15 cm into cow's rectum. With small seasonal variations, increases in body temperature occurred every 21 d on the d of estrus. Nevertheless, the records of the body temperature of four representative cows resulted in a detection rate of only 78% and a false positive rate of 12%. As the interval between the onset of increasing temperature and the time of ovulation was found to be consistent, the use of this predictor may be a reliable indicator of ovulation and the time of the LH surge (Mosher et al., 1990; Fisher et al., 2008). However, limitations may be due to variation in environmental temperature, disease-related hyperthermia, or some systemic or local inflammation, increasing the incidence of false positive results (Firk et al., 2002; Fisher et al., 2008).

An increase in milk temperature of 0.3 °C compared to the means of a 5-d baseline led to the detection of 50% of the cows (McArthur et al., 1992). Identification of estrus greatly depends on the frequency of measurements. Therefore, McArthur et al. (1992) concluded that twice daily measurements of milk temperature would not allow the detection of estrus.

1.6.2.6 Measurement of milk progesterone concentration

As the blood concentration of progesterone is closely associated with its concentration in milk (r = 0.81; Kamboj and Prakash, 1993), progesterone analysis of representative milk samples can be used to determine the reproductive status of the dairy cow. The samples taken during the milking session are collected in a sample intake unit and transferred automatically to the analyzing unit connected to a computer. The frequency of progesterone assays can be varied according to the stage of the estrous cycle (Saint-Dizier and Chastant-Maillard, 2012). Before being processed in a biological model developed by Friggens and Chagunda (2005) the milk progesterone values prepared over the last few d are smoothed using an extended

Kalman filter, with the algorithm distinguishing between different categories of cows: postpartum anestrus, estrus cycling, and potentially pregnant. Alerts are generated by the software in case of milk progesterone concentrations < 4 ng/ml (Friggens and Chagunda, 2005). Except comparatively major investment costs, in-line measurements of milk progesterone may have the potential to be a reliable tool in reproduction monitoring (Friggens and Chagunda, 2005; Saint-Dizier and Chastant-Maillard, 2012). But, due to the large inter-individual variation in timing of decreased levels, Roelofs et al. (2006), who noted values < 5 ng/ml 80 h (range: 54 h to 98 h) before ovulation, concluded that monitoring of progesterone alone is not adequate to predict ovulation.

1.7 Aims and structure of the thesis

The present thesis focuses on the analysis of activity, rumination, feed and water consumption as well as BW over the peri-estrous period. The publications on which thesis is based investigated whether these traits are related to estrus in dairy cattle. Further objectives were

- to determine the effects of parity and milk yield on rumination and activity during estrus,
- to analyze correlations between DMI, WI, and BW of the cows,
- to simultaneously investigate activity behavior and RT during estrus and over a 24-h period and
- to test the hypothesis that estrus-related variations in activity behavior correlate with variations in RT of estrual cows.

The aim of study 1 was to investigate whether RT of dairy cattle was affected by estrus. Therefore, 265 verified estrous cycles of Holstein-Friesian cows of four herds in Hesse were analyzed from d -3 to d 3 around estrus (d 0 = d of estrus). Rumination was registered for individual animals by a microphone-based sensor in 2-h time intervals.

The second publication focuses on the influence of estrus on feeding characteristics. The consumption of feed and water as well as BW of 34 estrual Holstein-Friesian cows were recorded on the research farm “Haus Riswick” of the Agricultural Chamber North Rhine-Westphalia. Daily DMI and WI were measured by troughs placed on an electronic floor scale.

Study 3 deals with the simultaneous analysis of RT and activity behavior of Holstein-Friesian and Simmental cows during estrous days as well as over a 24-h period. Moreover, the effects of parity and milk yield on activity (based on collar-mounted acceleration technology) and RT around estrus were evaluated.

1. Relationship between daily rumination time and estrus of dairy cows

S. Reith and S. Hoy

Department of Animal Breeding and Genetics, Justus Liebig University, Bismarckstrasse 16, D-35390 Giessen, Germany

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Relationship between daily rumination time and estrus of dairy cows

S. Reith[1] and S. Hoy
Department of Animal Breeding and Genetics, Justus Liebig University Giessen, Bismarckstrasse 16, D-35390 Giessen, Germany

ABSTRACT

The aim of the study was to investigate whether rumination time (RT) was related to estrus in dairy cattle. On 4 farms, cows were equipped with a microphone-based sensor system that allowed continuous recording (in blocks of 2 h) of RT. The analyzed data set consisted of 265 verified estrus cycles of 224 animals with artificial insemination leading to conception. The day of estrus (d 0) was defined as the day when estrus was identified either by measurement of physical activity or by visual observation. In estrous cows, RT was significantly reduced. With a duration of 355 min/d, the minimum RT was found on the day of estrus compared with the base level of 429 min/d during the reference period (the mean of 3 d before and 3 d after estrus). The average decrease in RT was 17% (74 min), ranging between −71 and +16% among animals. Herd and parity affected the RT decrease during estrus. Among the 4 analyzed herds, the RT decrease of cows in estrus ranged between 14% (60 min/d) and 24% (94 min/d). The decrease in RT was more pronounced in primiparous than in mature cows. In conclusion, RT is reduced on the day of estrus on average. The RT decrease during estrus was characterized by high variation among cows.
Key words: rumination time, estrus, dairy cow

INTRODUCTION

Rumination time (**RT**) is an appropriate parameter for early identification of metabolic disorders such as ruminal acidosis. Saliva secretion and rumen health are closely associated with daily RT (Maekawa et al., 2002). In addition, Murphy et al. (1983) noted that RT could be used for monitoring of ration composition and feeding practices.

Measurements of RT implemented by technical methods; for example, pressure transducers (Kaske et al., 2002) or piezo disks integrated within a cow's halter (Yang and Beauchemin, 2006), are mostly invented for research purposes. Recently, a microphone-based system (HR-Tag, SCR Engineers Ltd., Netanya, Israel) became commercially available for automatic recording of RT data. Rejection of feed boluses and mastication produce sounds that are registered by the acoustic sensor (Burfeind et al., 2011) and can be separated from sounds related to eating (Adin et al., 2009).

Schirmann et al. (2009) and Burfeind et al. (2011) found a high correlation (r = 0.88 and r = 0.93, respectively) between RT obtained from the HR-Tag and visual observation. Although RT can be used for monitoring the metabolic health of dairy cows, it is not known whether RT is influenced by fertility and reproductive management routines. A change that seems to be clearly associated with estrus behavior and that is pronounced in many cows is the increase in physical activity. Arney et al. (1994) and Schofield et al. (1991) observed a clear increase in the number of steps, 300% and 230%, respectively. Maltz et al. (1997) reported decreased milk yield during estrus as well as a decrease in daily BW accompanied by a significant reduction in food consumption. The aim of the study was to investigate whether RT was related to estrus in dairy cattle. A second objective was to determine the effect of lactation number on RT.

MATERIALS AND METHODS

Animals and Housing

The study was carried out on 4 farms, and herd size ranged from 50 to 70 lactating Holstein-Friesian dairy cows. The cows were housed in freestall barns with a free cow traffic routine that implied cows always had access to the cubicles, feeding areas, and automatic milking system (**AMS**). The animals were fed a TMR ad libitum throughout lactation. The ration consisted of grass silage and maize silage as roughage components, supplemented with concentrated feed to fulfill energy and protein requirements. Concentrates were supplied according to production level in the milking robot or in a separate feeding station. Cows were fed twice daily at approximately 0800 ± 1 h and 1700 ± 1 h. Cows were milked by a milking robot (Astronaut A3, Lely Ltd.,

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[1]Corresponding author: Stefanie.Reith@agrar.uni-giessen.de

Maassluis, the Netherlands). The cows could choose when and how often they were milked in the AMS, and their individual electronic tags were recorded (ID and individual cow data) in the AMS. The average 305-d milk production per cow was 9,800 kg (herd 1), 10,500 kg (herd 2), and 10,000 kg (herds 3 and 4).

After calving, reproductive management included a waiting period of 76 ± 30 d. The waiting period was determined based on daily milk yield and BCS of the cow. Calving to conception interval averaged 157 d (farm 1), 120 d (farm 2), 124 d (farm 3), and 123 d (farm 4). The herd managers identified estrus by visual observation and activity measurement. Physical activity was recorded by the HR-Tag monitoring system (SCR Engineers Ltd.). The cows calved year round and were artificially inseminated by the farm manager or veterinarian. In 126 cases (47.5%) of all analyzed estrus cycles, first AI led to pregnancy. Second AI was successful in 30.6%, and pregnancy was achieved after more than 2 inseminations in 21.9%. Diagnosis of pregnancy was carried out by ultrasonography 29 d after AI or by rectal palpation 42 d after AI.

Study Design

In total, 279 estrus cycles were available for study. Because of missing rumination data in 14 cases, the data set was reduced to 265 estrus cycles of 224 cows. All cycles analyzed led to conception. The day of estrus was defined as the day when estrus was detected either by measurement of physical activity (farms 2, 3, and 4) or by visual observation (farm 1). Insemination was performed by the herdsman in farms 2, 3, and 4; the day of estrus detection was identical to the day of AI. Cows of farm 1 were inseminated by a veterinarian; in 30% of estrus events monitored on farm 1, the day of estrus was the day before AI. Deviations in RT during estrus were detected by comparing the RT value on the day of estrus with RT values of the reference period (the mean of 3 d before and 3 d after estrus). Therefore, changes in RT were collected during each cow's peri-estrus period around the successful insemination. Despite a certain degree of inaccuracy concerning timing of estrus (estrus can be initiated at any time), the measurements of RT recorded in 2-h intervals were arithmetically averaged to one value per day for further analyses.

Cow data and reproductive data (calving, estrus and insemination data, pregnancy determination) were obtained directly from the herdsman or retrieved from the management software. The following data were recorded for each animal: herd, calving date, lactation number (**LN**), insemination date, number of inseminations, service period, calving to conception interval, duration of RT on d 0 and averaged over d −3 to −1 and +1 to +3. The LN of the cows ranged from 1 to 10. To analyze the possible effect of LN on RT, the cows were classified into 4 groups: LN 1, LN 2, LN 3, and LN >3.

Measurement of RT

For the automatic sensor-based detection of RT, the HR-Tag (SCR Engineers Ltd.) was used. The tag is attached to the left side of each cow's neck with a strap. A microphone enclosed within a plastic cover continuously records RT data in blocks of 2 h. The output data consist of cow RT, chewing rhythm, and the interval between feed boluses (SCR, 2011) and are analyzed by algorithms inside the tag. The current measured data are compared with the stored pattern in a microprocessor. In the memory of the HR-Tag, data of RT can be averaged and stored in 2-h intervals up to 24 h. All required data are transferred to receiver units installed in the AMS and sent to the management software on the farm computer. Based on validation trials (Schirmann et al., 2009; Burfeind et al., 2011), the HR-Tag works accurately for recording RT in dairy cows. The 2-h means of RT were used for further analyses in our study.

Statistical Analysis

Statistical analyses of RT data were performed using the program package SPSS 18.0 (SPSS Inc., Chicago, IL) and SAS 9.2 (SAS Institute Inc., Cary, NC). First, descriptive statistics were used for all variables. Rumination time and RT decrease during estrus were normally distributed as assessed by the Kolmogorov-Smirnov and Shapiro-Wilk tests. The RT data during estrus of cows were analyzed by using the MIXED procedure of SAS (SAS Institute Inc.). The following factors were included in the model:

$$Y_{ijklmn} = \mu + h_i + p_j + ai_k + day_l + cow_m(h_i) + e_{ijklmn},$$

where Y_{ijklmn} = the variable RT, μ = the intercept, h_i = the fixed effect of herd (i = 1 to 4), p_j = the fixed effect of parity group (j = 1, 2, 3, and >3), ai_k = the fixed effect of the number of AI (1, 2, and >2), day_l = the fixed effect of day (l = −3 to 3), $cow_m(h_i)$ = the random effect of cow within herd, and e_{ijklmn} = the random residual error. Further interactions between fixed effects were not included in the model because they were not found to be significant.

In a second analysis for each estrus, the decrease in RT was analyzed with the following fixed model (GLM):

$$Y_{ijkl} = \mu + h_i + p_j + ai_k + e_{ijkl},$$

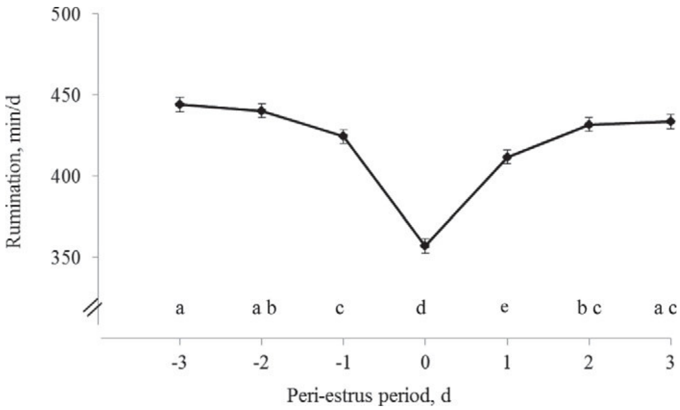

Figure 1. Dynamics of rumination time during the peri-estrus period (least squares means; bars indicate SEM) for 265 estrus events leading to pregnancy of the cow. Significance is represented by different letters ($P < 0.05$).

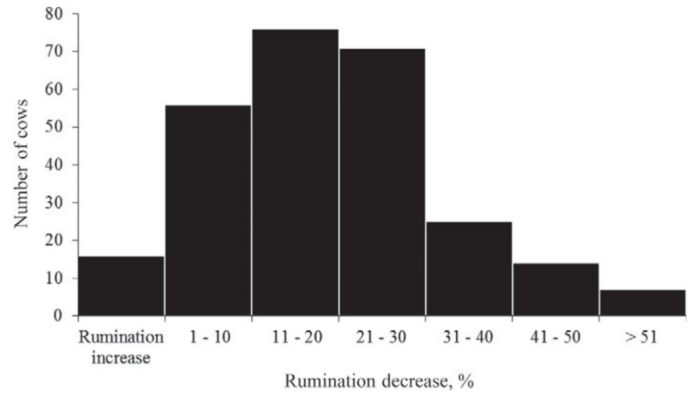

Figure 2. Distribution of the number of cows with different decreases (%) in rumination time during estrus.

where effects are explained as above and Y_{ijkl} = the variable RT decrease. Because very few cows had data in more than one parity, the random effect of cow was ignored.

RESULTS

Rumination time of dairy cows was considerably affected by day of estrus (Figure 1). In the reference period, cows spent 429 (±107) min/d ruminating. The duration of RT was significantly reduced on the day of estrus compared with all other days. The minimum level of daily RT was found on d 0 with 355 min/d, and RT decreased from 442 min/d (d −3) to 438 min/d (d −2) and 422 min/d (d −1). Cows reduced RT by about 16 min from d −2 to d −1. The decrease in RT from d −1 to the day of estrus was 67 min. After estrus, daily RT duration increased from 409 min/d (d 1) to 429 min/d (d 2) and 431 min/d (d 3). With an increase in RT of 55 min from the day of estrus to d 1, the pre-estrus decrease was not quite compensated. From d 1 to d 2, RT was enhanced further by about 22 min. On average, RT during estrus was shortened by 17% (74 min). The repeatability of RT for cow (81%) was calculated as the ratio between variance for cows within herd divided by the sum of cow variance and error variance.

Large individual differences in RT decrease during estrus were found among analyzed animals and estrus periods, respectively. The distribution of RT decrease is shown in Figure 2. Out of 265 analyzed estrus events, 94% were associated with a decline in RT. Decline in RT ranged from −0.9% (4 min/d) to −71% (247 min/d) relative to the base value. In 16 cases (6%), estrus was associated with increased RT. Cows in herd 1 and cows in herd 4, respectively, reduced RT from 474 and 480 min/d (reference period) to 396 and 389 min/d on the day of estrus. With 385 min/d in the reference period and 291 min/d during estrus, the lowest value of RT was found in dairy cows of herd 2. Compared with cows in herd 2, cows in the other 3 herds chewed the cud between 82 and 105 min longer per day (Table 1).

The extent of the decrease in RT during estrus was influenced by parity (Figure 3). The decrease in daily RT during estrus was less pronounced with increasing LN. Primiparous cows decreased RT, on average, by about 98 min/d, whereas cows with LN >3 showed a decline in RT, on average, of 69 min/d in estrus compared with the baseline level. The difference in RT decline between

Table 1. Means of rumination time during reference period and during estrus, and mean rumination decrease in estrous cows in 4 farms[1]

		Rumination time, min/d			
Herd	Cows, n	Reference period[2]	Day of estrus (d 0)	Rumination decrease, min/d	Rumination decrease, %
1	91	474	396	78	16
2	48	385	291	94	24
3	61	433	373	60	14
4	65	480	389	91	19

[1]Standard deviation of rumination decrease was 13%.

[2]Reference period: mean of d −3, −2, −1, 1, 2, and 3.

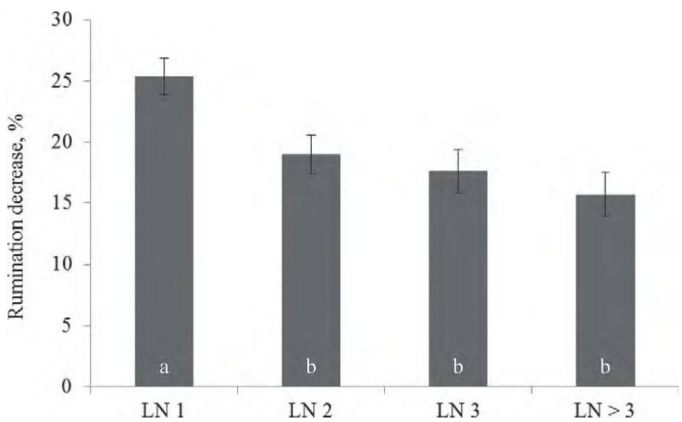

Figure 3. Decrease in daily rumination time on the day of estrus compared with the reference period (d −3 to −1 and +1 to +3) in dependence on lactation number (LN). Results are group least squares means (bars indicate SEM) for cows in parities 1 to >3 (LN 1 to LN >3). Bars with different letters (a, b) are significantly different ($P < 0.05$) from each other (LN 1: n = 78, LN 2: n = 68, LN 3: n = 55, LN >3: n = 64).

primiparous and older cows was significant. Compared with multiparous cows (cows with LN >1), the decrease in RT in primiparous cows was about 23 min more pronounced during estrus.

DISCUSSION

The current study showed that chewing the cud was influenced by the onset of estrus in cows. Daily RT quantifies behavioral changes and completes the picture of increased activity and restlessness (Arney et al., 1994; Roelofs et al., 2005), reduced lying time (Brehme et al., 2006), and decreased milk yield and feed intake associated with a decrease in BW (Maltz et al., 1997). During the reference period (the mean of 3 d before and 3 d after estrus), the basal RT averaged 429 min/d, which is consistent with results of other studies. By means of HR-Tag measurements, Adin et al. (2009) recorded a daily RT between 428 and 482 min. In all estrus cycles, the minimum value of RT was 357 min/d, registered on the day of estrus. The peri-estrus period was assigned into day categories. The day of estrus (d 0), mostly identical to the day of successful AI, was defined as the calendar day when estrus was detected by activity measurement or visual observation. Given the fact that estrus is initiated at any time of day, classification is difficult, as is described in other studies (Schofield et al., 1991; Arney et al., 1994). This may lead to inaccurate definitions of the exact day of estrus (d 0). The onset of estrus is usually gradual and occurs over several hours. This may also explain why d −1 and d 1 showed reduced RT, and that could have affected d −2 and 2 as well. During estrus, RT was reduced by 17% (74 min). To our knowledge, this effect has not been reported previously. A plausible explanation might be that the decline in RT is closely linked to the increase in physical activity recognized one of the first signs of a cow initiating estrus behavior. Compared with cows on nonestrus days, cows in estrus show a considerable increase in activity behavior, restlessness, mounting, standing to be mounted, chin rubbing, and sniffing (Phillips and Schofield, 1990; Van Vliet and van Eerdenburgh, 1996). An obvious increase in the number of steps was detected in several pedometer studies (Arney et al., 1994; Roelofs et al., 2005). Arney et al. (1994) observed activity gradually increasing 3 d before estrus and decreasing after reaching peak value. Rumination shows a reverse dynamic, with a gradual decrease starting 2 d before onset of estrus. The expression of estrus is regulated by estrogens, especially estradiol-17β (Allrich, 1994; Roelofs et al., 2010), reaching peak level 1 d before estrus (Lopez et al., 2004). Three days after the day of estrus, estradiol concentration returns to the basal level (Mondal et al., 2006). One of the main effects of these steroids is the enhancement of activity behavior, which is negatively correlated with RT. In addition, estrogens affect dietary behavior by reducing appetite and feed consumption (Uphouse and Maswood, 1998; Mondal et al., 2006). Rumination time is strongly associated with daily feed intake (Welch, 1982; Kaske et al., 2002). During estrus, cows spend less time feeding. Phillips and Schofield (1990) observed a reduction in feeding time of between 5 and 20%. Because of lowered feed intake, Maltz et al. (1997) reported a decrease in daily BW limited to 1 to 3 d during estrus. Restlessness caused by estrus reduces lying time. Brehme et al. (2006) noted that estrous cows do not lie down for 6 to 17 h. In view of the fact that cows spend more time on rumination while lying down in cubicles (Nørgaard et al., 2003), we assume that reduced lying time impedes RT of cows in estrus. Furthermore, Brehme et al. (2006) showed that lying time is a useful trait to identify cows with low estrus intensity. Weak estrus events are rarely observed by the herd manager.

Rumination behavior was not affected similarly in all estrous animals. Dairy cows of one herd in the current study exhibited marked variation in the degree of RT decline during estrus, even when fed and managed similarly to cows of the other herds. The variation in RT decrease, including the values of the cows on the 4 analyzed farms, indicate a high variability of −71 to +16% among animals. Not all bovine animals in estrus show equal intensity and duration of estrus (Van Vliet and van Eerdenburgh, 1996). Allrich (1994) noted a range from 3 to 28 h in duration of estrus.

According to Maekawa et al. (2002), multiparous cows spend more time ruminating than do primiparous cows. Compared with that in cows of high LN (LN >3) in the current study, the RT decline of young cows (LN 1) was increased by 23 min/d. The greater decrease in RT in primiparous cows is likely associated with activity behavior. Verifiable higher activity values occur in first-lactation cows during estrus (López-Gatius et al., 2005; Yániz et al., 2006). Roelofs et al. (2005) noted an increase in the number of steps taken by primiparous animals. Estrus behavior by young cows was about 3 h longer than that observed in older cows. López-Gatius et al. (2005) observed that cows' activity at estrus was reduced by 21.4% with each additional lactation.

CONCLUSIONS

On average, daily RT at estrus was significantly reduced compared with that on nonestrus days. However, our results indicated high variation in the RT decrease at estrus. Further research that includes factors such as accuracy, error rate, sensitivity, and specificity for different threshold values in RT decrease is necessary to determine whether RT is an indicator, alone or in combination with other parameters such as measurements of physical activity, of estrus in dairy cows.

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2. Influence of estrus on dry matter intake, water intake and BW of dairy cows

S. Reith[1], M. Pries[2], C. Verhülsdonk[2], H. Brandt[1] and S. Hoy[1]

[1] Department of Animal Breeding and Genetics, Justus Liebig University Giessen, Leihgesterner Weg 52, D-35392 Giessen, Germany

[2] Agriculture Chamber Northrhine-Westphalia, Nevinghoff 40, D-48147 Münster, Germany

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Influence of estrus on dry matter intake, water intake and BW of dairy cows

S. Reith[1†], M. Pries[2], C. Verhülsdonk[2], H. Brandt[1] and S. Hoy[1]

[1]*Department of Animal Breeding and Genetics, Justus Liebig University Giessen, Leihgesterner Weg 52, D-35392 Giessen, Germany;* [2]*Agriculture Chamber Northrhine-Westphalia, Nevinghoff 40, D-48147 Münster, Germany*

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The objectives of this study were to analyze whether dry matter intake (DMI), water intake (WI) and BW were influenced by estrus. A second objective was to determine whether correlations exist among these traits in non-estrous days. Data collection included 34 Holstein-Friesian cows from the research farm 'Haus Riswick' of the Agricultural Chamber North Rhine-Westphalia, Germany. On an individual basis, daily DMI and daily WI were measured automatically by a scale in the feeding trough and a WI monitoring system, respectively. BW was determined by a walk-through scale fitted with two gates – one in front and one behind the scale floor. Data were analyzed around cow's estrus with day 0 (the day of artificial insemination leading to conception). Means during the reference period, defined as days − 3 to − 1 and 1 to 3, were compared with the means during estrus (day 0). DMI, WI and BW were affected by estrus. Of all cows, 85.3% and 66.7% had reduced DMI and WI, respectively, on day 0 compared with the reference period. Lower BW was detected in 69.2% of all cows relative to the reference period. During the reference period, average DMI, WI and BW were 23.0, 86.6 and 654.8 kg. A minimum DMI of 20.4 kg and a minimum BW of 644.2 kg were detected on the day of estrus, whereas the minimum WI occurred on the day before estrus. After estrus, DMI, WI and BW returned to baseline values. Intake of concentrated feed did not seem to be influenced by estrus. Positive correlations existed between daily DMI and daily WI (r = 0.63) as well as between cows' daily BW and daily WI (r = 0.23). The results warrant further investigations to determine whether monitoring of DMI, WI and BW may assist in predicting estrus.

Keywords: estrus, dry matter intake, water intake, BW, dairy cows

Implications

Detection of estrus has a major impact on reproductive efficiency in dairy herds. Undetected estrous cycles leads to prolonged intercalving intervals and reduced milk production resulting in economic losses. However, detection rates are decreasing and visual observation of cows has become difficult. Dry matter intake, water intake and BW can be measured automatically and normally are indicators for early detection of cows with metabolic disorders. However, the possibility to use these traits as additional tools for identifying estrous cows – as shown in the current study – may help the herd manager to improve the detection of estrous cows and the profit of the dairy herd.

Introduction

Reproductive performance of dairy cows is largely dependent on accurate detection of estrus, especially on farms where timed artificial insemination is not applied. Because of increased milk production and less pronounced symptoms and duration of estrus, and incidence of reproductive disorders, detection of estrus is a major problem in dairy cattle (Wiltbank *et al.*, 2006). Lopez *et al.* (2004) reported a shorter duration of estrus (6.2 ± 0.5 h) in high-yielding cows than in cows with lower milk production (10.9 ± 0.7 h). In addition, milk yield was associated negatively with cyclicity and health status of the ovaries (López-Gatius, 2003). With increasing herd sizes, visual observation of individual cows for signs of estrus becomes impractical. To facilitate estrus detection, numerous estrus-detection aids have been developed in improved efficiency of detecting estrus.

Studies published previously revealed that standing to be mounted was most characteristic for estrus (Hurnik *et al.*, 1975; Phillips and Schofield, 1990). However, Lyimo *et al.* (2000) reported that standing to be mounted by another cow can no more be considered a sure indication of estrus because this behavior is not found in all cows (Diskin and Sreenan, 2000; Roelofs *et al.*, 2010). Van Eerdenburg *et al.* (2002) observed

† E-mail: Stefanie.Reith@agrar.uni-giessen.de

standing estrus in only 50% of the cows. Hereof, Kerbrat and Disenhaus (2004) updated knowledge about behaviors observed during estrus. Automatic observation of secondary signs becomes increasingly important to identify estrous cows. Data can be measured automatically on a daily basis for individual cows.

It is well known that restlessness was detected to be one of the most characteristic indicators of estrus. During estrus, behavior of cows is significantly more pronounced than in diestrus (Berka *et al.*, 2004; López-Gatius *et al.*, 2005; Yániz *et al.*, 2006). Arney *et al.* (1994) observed a 300% increase in the number of steps measured by pedometers during estrus. Activity – locomotion and mounting activity – was influenced by a multitude of different factors (Orihuela, 2000; Yániz *et al.*, 2006), and seemed to have a major impact on other traits (lying time, rumination duration) during estrus (Brehme *et al.*, 2006; Reith and Hoy, 2011 and 2012).

Besides these traits, dry matter intake (DMI), water intake (WI) and BW of cows can easily be recorded – daily and on an individual basis – by sensor-based technology. Actually, feed consumption and BW are used in some dairy farms to detect cows with metabolic disorders. Owing to a positive correlation, lower feed intake is mostly followed by a decrease in BW (Maltz *et al.*, 1997). This is particularly often the case in early lactation when cows with insufficient feed intake enter a period of negative energy balance, resulting in body tissue mobilization and BW loss, respectively (Tamminga *et al.*, 1997). In an investigation conducted by Van Straten *et al.* (2008), BW decline was up to 8.5% during the first 5 weeks after calving. Apart from roughage intake, Mol *et al.* (2001) confirmed that there is also a negative association between the voluntary intake of concentrates and cow diseases.

We previously reported that rumination time is reduced during estrus (Reith and Hoy, 2012). Our objectives of this study were to determine whether DMI, WI or BW decrease in estrous cows. A second objective was to determine whether correlations existed among those measures and estrous behavior, as well as between DMI, WI and BW in non-estrous days.

Material and methods

Cows and study design

A total of 34 Holstein-Friesian dairy cows were included in the study. Data were recorded on the research farm 'Haus Riswick' of the Agricultural Chamber North Rhine-Westphalia, Germany. The animals were housed year-round in a free stall barn with cubicles equipped with rubber mats and a slatted floor with free access to the feeding area. The floor of the holding pen was covered with rubber flooring. An *ad libitum* partial total mixed ration (pTMR) was fed. The ration consisted of grass silage, maize silage, chopped straw and concentrated feed (rapeseed meal, maize, wheat, sugar beet molasses and urea) to fulfill the requirements for energy, protein and minerals, and was calculated to achieve daily milk yield of 25 kg (energy corrected milk) per cow. Depending on the production level, concentrates were additionally offered in a feeding station. Water was available continuously and *ad libitum* via a trough placed in the feeding area. The cows were milked twice daily at 0530 and 1630 h in a milking carousel with 32 milking stalls. Average daily milk yield of the analyzed cows was 30 kg/day. The cows were housed in different groups with a group size of 24 animals. Per group, 12 feeding and 2 water troughs were available. The cows were at various stages of lactation, ranging from 59 to 213 days in milk. Mean lactation number of cows was 2.5.

Activity measurement via pedometers (GEA Farm Technologies, Düsseldorf, Germany) was used for identifying cows in estrus. In the present study, the cow was considered to be in estrus (day 0) when detected by activity measurement or/and visual observation. In response to estrus, cows were inseminated artificially by an experienced insemination technician between 0900 and 1100 h. To ensure that cows showed true estrus, only cycles of cows with insemination leading to conception were included. Means of DMI, WI and BW of 3 days before and 3 days after day 0 were defined as reference period. Deviations in DMI, WI and BW during estrus were calculated by comparing the value on the day of estrus with values of the reference period (days −3, −2, −1, 1, 2, 3). The following data were recorded for each cow: date of estrus, daily DMI, daily WI and daily BW at the day of estrus, and at the days −3 to −1 and 1 to 3.

Measurements of individual DMI, WI and BW

Each cow was equipped with a tag fitted on the neck collar for identification and to save its individual data. Daily DMI of the cows was measured by a feeding trough placed on an electronic floor scale (Waagen Döhrn, Wesel, Germany). Total DMI was separated into DMI of the pTMR and DMI of concentrated feed. For analysis, DMI was defined as DMI of the pTMR in the feeding trough (excluding intake of concentrated feed from the separate feeding station). Concentrate intake (CI) was separately analyzed. Each cow had access to one feeding trough when the identification tag passed the antenna of the DMI monitoring system. The difference between the weight of the feed before entering and after leaving the trough was recorded at each individual feeding event. A grid around the feeding trough mostly prevented feed losses by throwing feed out. Via data line, data arrived at the farm computer and were retrievable from the management software.

On the same technical basis, cow's individual WI was daily registered by a scale (Waagen Döhrn) on which a trough was installed (Figure 1). Thus, the difference between the weight of water before and after WI was individually determined. On the basis of validations, WI accessed at the trough had a deviation of 0.05 kg.

Measurement of BW was accessed by a walk-through scale (GEA Farm Technologies). Weights were measured twice daily after milking.

The single measurements of DMI, WI and BW were summarized to one value per day by the management software. In terms of data retrieved from the computerized data storage system, a 'day' was defined as the calendar day (from midnight

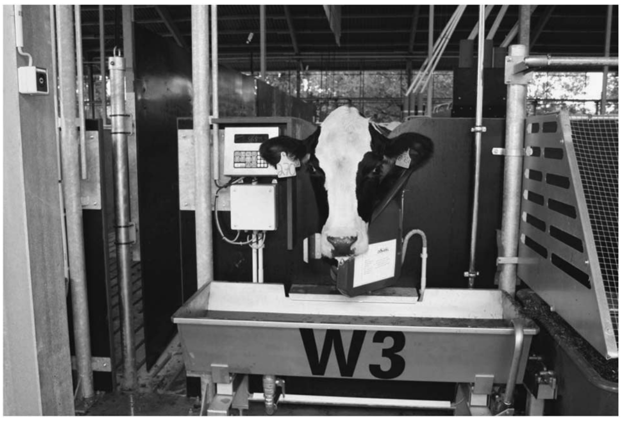

Figure 1 Water trough for the automatic measurement of cow's daily water intake (Source: C. Verhülsdonk).

to midnight). Values for DMI and CI were indicated in kilograms of fresh matter and dry matter (DM). During the data collection, the average DM content of the ration was about 46%. Because of missing data of WI, CI and BW in the periestrous period, the sample size was different for each trait.

Statistical analysis

Data of DMI, CI, WI and BW during estrus of cows were analyzed using the program package SPSS 20.0. For all traits, descriptive statistics and correlations between traits were calculated. For the analysis of the daily measurements, the following factors were included in the model of generalized linear mixed model:

$$Y_{ijklm} = \mu + \text{day}_i + p_j + \text{BW}_k + \text{cow}_l(p_j) + e_{ijklm}$$

where Y_{ijklm} is the variable 'DMI', μ the overall mean, day_i the fixed effect of day ($i = -3$ to 3), p_j the fixed effect of parity group ($j = 1$ = first parity and >1 = cows with greater parity), BW_k the fixed effect of BW ($k \leqslant 645$ and >645 kg), $\text{cow}_l(p_j)$ the random effect of cow-within parity group and e_{ijklm} the random residual error. Similarly, the variables CI, WI and BW were analyzed with the effects explained as above. For calculation of 'BW,' class of BW was excluded. Correlations were calculated between the average DMI, CI, WI and BW during the reference period (days − 3, − 2, − 1, 1, 2, 3), and the change of all traits from the reference period to day of estrus calculated as percentage from the average value in the reference period.

Results

Changes in DMI, WI and BW during estrus

DMI, WI and BW of dairy cows were significantly influenced by the occurrence of estrus with a great variability among cows. Means, standard deviations and ranges of the traits are in Table 1. On average, cows consumed 23 kg of DM in the pTMR per day during the reference period with a maximum DMI of 28.4 kg/day. During the reference period, the amount of DMI was constant. Individual feeding behavior was altered during estrus relative to the reference period. On the day of estrus, mean DMI was 20.4 kg. The day of estrus

Table 1 *Number of cows[1], means, standard deviations, minimums and maximums of dry matter intake (DMI), concentrate intake (CI), water intake (WI) and BW during the reference period, and variations on the day of estrus compared with the reference period[2]*

	Cows (*n*)	Mean	s.d.	Minimum	Maximum
DMI (kg/day)	34	23.0	2.4	16.1	28.4
DMI variation (%)	29	− 14.6	11.6	− 1.0	− 45.4
CI (kg/day)	26	5.2	1.6	1.5	7.3
CI variation (%)	11	− 12.7	16.6	− 1.1	− 55.4
WI (kg/day)	33	87.6	22.3	43.4	132.5
WI variation (%)	22	− 15.3	14.1	− 0.1	− 58.5
BW (kg)	26	654.8	61.2	519.2	784.5
BW variation (%)	18	− 3.0	3.3	− 0.1	− 12.4

[1]Number of cows (*n*) was different for the different traits because of missing values (in full or in part) resulting in a reduced data set for CI, WI and BW.
[2]Reference period: means of days − 3, − 2, − 1, 1, 2 and 3.

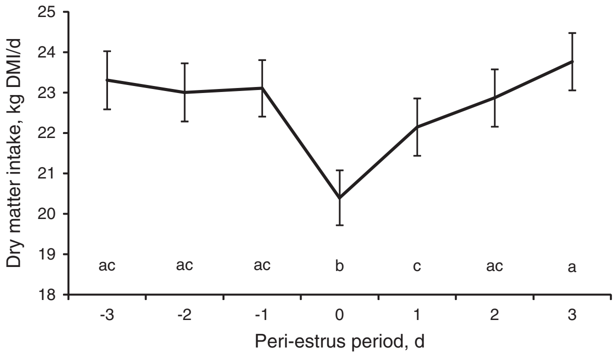

Figure 2 Changes in dry matter intake (DMI) before and after estrus ($n = 34$). Results are given as LSQ means ± s.e. [a,b,c]Means with different superscripts differ significantly at $P < 0.01$.

was different ($P < 0.001$) from all other days. From day − 1 to the day of estrus, DMI was decreased about 2.9 kg. Of all 34 animals, 29 cows (85.3%) had reduced DMI by 14.6% on average. In 15 of these 29 cows, decrease in DMI was >10% (ranging from 12.8% to 45.4%). In five cases in which DMI increased during estrus, DMI ranged from 2.9% to 18.7% relative to reference values. After estrus, DMI increased from 22.2 (day 1) to 22.8 (day 2) and 23.8 kg (day 3) (Figure 2).

CI was not affected by the occurence of estrus. No clear tendency could be found around estrus. Of 26 cows, CI was reduced in 11 cows and increased in 15 cows, respectively. During the reference period, the consumption of concentrates averaged 5.2 kg/day and differed between 1.5 and 7.3 kg/day depending on the stage of lactation of the analyzed cows.

Dynamics of daily WI followed the same pattern during estrus compared with DMI. Mean daily WI during the reference period was 87.6 kg and ranged from 43.4 to 132.5 kg/day. On the day of estrus, cows drank 81.5 kg water. With 80.5 kg, the minimum level was detected on the day before estrus, yet. The decrease from day − 2 to day − 1 was nearly significant ($P = 0.07$). After estrus, WI increased from 84.4 (day 1) to 86.7 (day 2) and 92 kg (day 3) (Figure 3). Of the total 33 cows, 22 (66.7%) consumed less water during estrus with a mean decrease of 15.3%.

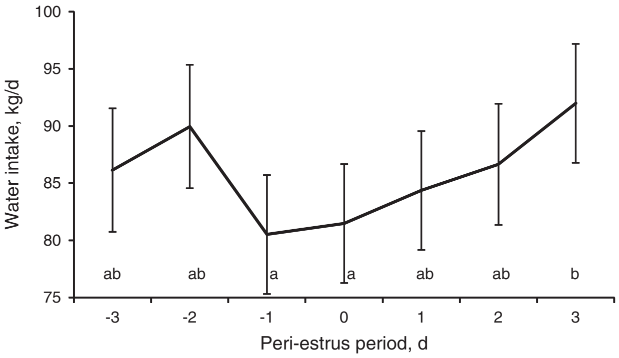

Figure 3 Changes in water intake (WI) before and after estrus (n = 33). Results are given as LSQ means ± s.e. [a,b]Means with different superscripts differ significantly at $P < 0.05$.

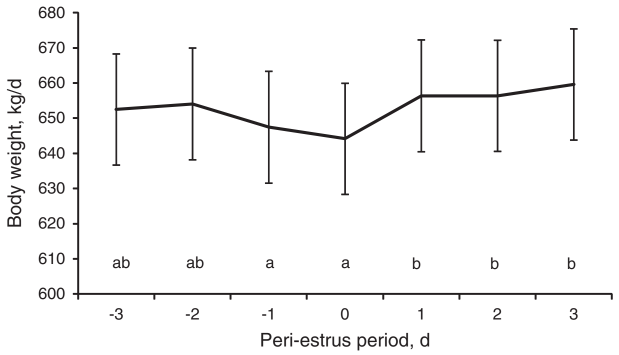

Figure 4 Changes in BW before and after estrus (n = 26). Results are given as LSQ means ± s.e. [a,b]Means with different superscripts differ significantly at $P < 0.05$.

Cow's daily BW was markedly influenced by estrus. The day of estrus was significantly ($P < 0.05$) different from the days after estrus. Before estrus, the decrease from day −2 to the day of estrus was nearly significant (P = 0.07), whereas the decrease from day −1 to the day of estrus was not found to be significant. During the reference period, BW averaged 654.8 kg and varied between 519.2 and 784.5 kg among cows. The minimum value was measured on the day of estrus with a mean BW of 644.2 kg (Figure 4). Of the total of 26 analyzed cows, BW was reduced in 18 cows (69.2%), with an average decline of 3% and a range from 0.1% to 12.4%.

Correlations between DMI, WI, and BW and estrus

Correlations among traits are presented in Table 2. During the reference period, daily DMI and daily WI were closely related. In addition, a positive correlation existed between daily BW and daily WI. No clear relationship was found between daily BW and cow's DMI.

Table 2 *Coefficients of correlation (r) between dry matter intake (DMI), water intake (WI) and BW during the reference period*[1]

	DMI (kg/day)	WI (kg/day)
WI (kg/day)	0.63**	–
BW (kg)	0.09	0.23

A significant (**$P < 0.01$) positive relationship was found between WI and DMI of dairy cows.
[1]Reference period: means of days −3, −2, −1, 1, 2 and 3.

Discussion

Changes in DMI, WI and BW during estrus

It is undisputed that accurate detection of estrus is essential for reproductive performance of dairy cows. An analysis performed by Plaizier *et al.* (1998) verified considerable economic benefits associated with improved detection rates. With the help of sensor-based monitoring systems, traits can be measured daily and on an individual basis. In the present study, DMI, WI and BW had been identified as three traits that were influenced by estrus – defined as the day of artificial insemination leading to conception – of Holstein cows.

Physiologically, estrus of cows is divided into four different stages accompanied by the display of specific behaviors. In proestrus – in the present study most probably consistent with day −1 – onset of estrous behavior is induced by the release of steroid hormones, primarily estrogens (Allrich, 1994). Lyimo *et al.* (2000) found estrus being strongly correlated with estradiol concentration reaching peak level on the day before estrus (Lopez *et al.*, 2004). A reduction in feed intake could mostly be caused by estrogens (Uphouse and Maswood, 1998). Mondal *et al.* (2006) reported increasing quantities of estradiol-17β 6 days before estrus that reached peak level on the day of estrus and declined to basal level on day 3 of the estrous cycle. In the current study, day 1 may be characterized by the time of ovulation. Roelofs *et al.* (2005) detected an average interval between increased activity behavior measured by pedometers and ovulation of 29.3 h. As a result, they defined optimal time for artificial insemination to be between 11 and 16 h after onset of pedometer estrus. In postestrus, activity of progesterone resulted in inhibition of estrous behavior (Allrich, 1994). In most cases, the day of estrus was identical with the day of artificial insemination. Our definition of 'estrus' referred to the calendar day when estrus was detected primary by activity measurement. However, onset of cows' estrus occurs round the clock. Therefore, more exact classification was not possible. This fact may be one of the reasons why day −1 and day 1 also showed decreases in the analyzed traits.

During estrus, 85% of cows had less DMI with the minimum level recognized on the day of estrus (day 0) compared with the reference period. A decline in feed intake during estrus is also described by Diskin and Sreenan (2000). In addition, estrous cows spent less time at the feeder (Hurnik *et al.*, 1975). Although seemingly obvious, this was not found to be the case in other studies. On the contrary, De Silva *et al.* (1981) found no change in feed consumption during estrus, and Lukas *et al.* (2008), however, observed an increase in feed intake by 0.61 kg in estrous cows. Mean daily DMI obtained for the reference period in this study was similar to the result found in the studies by Dado and Allen (1994) and Yang and Beauchemin (2006), in which cows had an average DMI of 22.8 kg DM/day and 23.8 kg DM/day, respectively. Using the same technique – feeding troughs as described in the current investigation – Kaufmann *et al.* (2007) reported a daily DMI of 21.6 kg.

Daily CI was not clearly affected by estrus. It seemed that cows – independent of the reproductive cycle – were motivated to visit the concentrate feeding station to obtain their individual ration of the tasty concentrate mixture.

Among the analyzed cows, great variation has been found in daily water consumption. The results of the present study showed a decrease in daily WI, which is similar to that found by Meyer *et al.* (2004) and Lukas *et al.* (2008). In the own investigation, the lowest value was found 1 day before the day of estrus. Compared with DMI (and BW), the low level of WI during estrus lasted for about 2 days (day − 1 and day 0) after which water consumption then returned – relatively fast – to base level, and rose higher. It might be that cows have to compensate loss in WI during estrus.

Thus, the observed declines in food consumption and WI were found to be further behavioral signs of estrus. Comprehensive knowledge of traits indicate that estrus may be important for improving detection rates on dairy farms.

Decreased DMI and WI probably were consequences of increased activity behavior in estrous cows including mainly restlessness and mounting (Van Eerdenburg *et al.*, 2002). Several pedometer studies confirmed an obvious rise in the number of steps. In comparison with non-estrous days, activity behavior of cows in estrus was increased by a factor of 2.3 (Redden *et al.*, 1993) to 4 (Berka *et al.*, 2004). Increased levels of general activity were also found in studies by Reith and Hoy (2011) and Kamphuis *et al.* (2012), in which neck transponders attached to the neck collar of each cow were used for the automatic identification of estrus. Arney *et al.* (1994) observed a stepwise increase in activity starting already 3 days before estrus. The current results indicated that gradual deviations in data were measured 1 and 2 days, respectively, before the day of estrus. Brehme *et al.* (2006) found similar dynamics for lying time of estrous cows and reported that these cows are recumbent for 6 to 17 h. They investigated lying time in parallel to daily activity behavior and concluded that the detection of estrus can be improved by analyzing data of more than one single trait. In a previous study, we showed that time spent ruminating also correlated with cows' estrus. Very similar to the dynamics of feed intake, the dynamics of rumination time followed nearly the same pattern in the estrous period. In contrast with the reference period in which cows ruminated on average 429 min/day, rumination time was reduced to a duration of 355 min on the day of estrus (Reith and Hoy, 2012). Obviously, reduced rumination time can be interpreted as the consequence of decreased DMI during estrus.

The values of DMI and WI were enormously scattered among cows. One of the greatest effects of between individual variation is age. Compared with primiparous animals, multiparous cows consumed more feed (19.2 *v.* 17.1 kg), and spent more time eating (260 *v.* 213 min/day; Maekawa *et al.*, 2002). Dado and Allen (1994) studied cows' WI and added that older cows showed increased WI by about 30%. When investigating changes during estrus, age was correlated with a decrease in rumination time (Reith and Hoy, 2012), and the increase in activity was less pronounced in older cows (Yániz *et al.*, 2006). In a detailed review, Orihuela (2000) listed several further reasons for inter-individual variations such as dominance order, milk yield, nutrition and a number of environmental factors. It can be assumed that ration composition as well as feeding practice additionally had a major impact on DMI and WI in a dairy herd.

The average BW (655 kg) of the cows in the current investigation was in agreement with results of other studies using Holstein cows (Maekawa *et al.*, 2002; Meyer *et al.*, 2004; Kume *et al.*, 2010). In the majority of cows, BW was reduced with a weak decline recorded on the day of estrus. In another investigation, we analyzed each cow's BW data obtained by a scale installed in the automatic milking system and found the same pattern (Reith and Hoy, 2011). Maltz *et al.* (1997) reported that the decrease in BW near estrus lasted between 1 and 3 days. In addition, the researchers detected lower BW associated with a significant reduction in DMI and a decrease in rumen content, respectively. They indicated that in the dairy industry BW and its changes can, indeed, contribute to predict estrus. In some cases, BW responded clearly and better than milk yield in the period of estrus.

Correlations between DMI, WI, and BW and estrus

DMI is one of the most important factors affecting WI of dairy cows (Kume *et al.*, 2010). In the present study, a close relationship between DMI and WI existed during the reference period, which is similar to that detected by Holter and Urban (1992) ($r = 0.69$), higher to that reported by Meyer *et al.* (2004) ($r = 0.107$) and lower to the correlation indicated by Kume *et al.* (2010) ($r = 0.83$). Incidentally, further research on this could be interesting to clarify whether the causal relationship would come from both traits. On the basis of their correlation calculation, Lukas *et al.* (2008) and Kramer *et al.* (2009) concluded that daily water consumption can be an indirect trait to predict individual changes in feed intake as well as to identify sick cows.

Not only DMI but also BW affected daily WI. It was detected that cows with higher BW seemed to consume more water. This would be in accordance with the results of Meyer *et al.* (2004) and Kume *et al.* (2010) who also obtained with $r = 0.417$ and $r = 0.75$ positive correlations between BW and WI. Investigating the relationship between DMI and BW, Maltz *et al.* (1997) observed a continuous increase in feed intake of cows paralleled by an increase in BW. Such relationships suggest that DMI, WI and BW (as well as milk yield) follow a specific pattern being characteristic for the different physiological states – be it the *postpartum* period or estrus – during cows' lactation. Herd managers can take advantage of this knowledge for improved monitoring of individual cows. As it is described by Firk *et al.* (2002), detection rates for physiological disorders or estrus would be higher when different traits are considered simultaneously. More investigations are necessary to determine whether the analyzed traits are useful for combined analysis.

In the present study, it was shown that each trait was significantly (DMI: $P < 0.001$ and BW: $P < 0.01$) or nearly significant (WI: $P = 0.07$) reduced during estrus. Despite the fact that the given sample size is relatively small, we are confident that DMI and WI were truly influenced by the estrous cycle of dairy cows.

Final remarks

Worldwide, numerous studies were carried out on cows' reproductive performance and have revealed severe problems in detecting estrous cows. The results of the current study indicate that DMI, WI and BW are influenced by estrus. Actually, monitoring feed intake and WI on a daily individual basis is only practical in research farms. If the declines differ significantly from daily fluctuations, it may be possible to define an algorithm used as an additional management aid. Alerts placed on lists could then inform the herd manager about changes in DMI, WI and BW, indicating not only illness but also estrus of modern dairy cows. The results give rise to further research with the aim to analyze whether monitoring of DMI, WI and BW may help to improve the detection of estrus in practise.

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3. Simultaneous analysis of activity and rumination time, based on collar-mounted sensor technology, of dairy cows over the peri-estrus period

S. Reith*, H. Brandt, S. Hoy

Department of Animal Breeding and Genetics, Justus Liebig University Giessen,

Leihgesterner Weg 52, D-35392 Giessen, Germany

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S. Reith *, H. Brandt, S. Hoy

Department of Animal Breeding and Genetics, Justus Liebig University Giessen, Leihgesterner Weg 52, D-35392 Giessen, Germany

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ABSTRACT

The aim of this study was to investigate activity and rumination time (RT) measured by collar-mounted acceleration and microphone-based technology (HR-Tag monitoring system, SCR Engineers Ltd., Netanya, Israel) of dairy cows over the peri-estrus period. The data base consisted of 453 estrous cycles and cows, respectively. To ensure true estrus, only cows with AI leading to conception were included in the study. The reference period was defined as the mean of 3 d prior and 3 d post the day of estrus. With large intra- and inter-individual variation, activity and RT were significantly influenced by cows' estrus. On the day of estrus, activity behavior was on average increased by 38.7%, whereas data of daily RT were on average reduced by 19.6% (83 min/d). The percentage of estrual cows with increased activity was 76.5%. In contrast, 86.2% of all cows showed decreased RT during estrus. Circadian rhythms of activity and RT were bimodal. Cows displaying estrus showed highest activity and lowest RT during the nocturnal and early morning hours between 0200 and 0800 h and 0400 and 1000 h on the day of estrus. Clear estrus-related deviations from base level were measured much earlier for RT than for activity. Activity behavior tended to be more pronounced in primiparous cows and high-yielding cows (>40 kg/d) on the day of estrus compared with multiparous herd mates and cows with lower milk production (≤ 40 kg/d). Rumination time was associated positively with parity and negatively with cows' milk production on the day of estrus. During the reference period, RT was 384 min/d and 443 min/d for primiparous and multiparous (>3 lactations) cows and 445 min/d and 407 min/d for low- and high-yielding cows. Comprehensive knowledge of characteristics indicating estrus is important for improving estrus detection. Further research is recommended to investigate the potential benefit of combining data of activity and RT for practical application in daily herd management.

1. Introduction

Numerous studies conducted worldwide have shown severe problems in detection of estrus in dairy cows. This is particularly true in cows that have been selected for higher milk yields (Pryce et al., 2004; Yániz et al., 2006), which results in the changes to the normal reproductive physiology. These changes include large individual animal variation in estrus regularity (Friggens and Labouriau, 2010), reduced estrus duration (Friggens and Labouriau, 2010; Lopez et al., 2004), and less pronounced intensity of estrus symptoms (Kerbrat and Disenhaus, 2004; López-Gatius et al., 2005). Additional reasons for deficiencies in fertility were described in detailed review papers by Lucy (2001) and Wiltbank et al. (2006). In addition, detection of estrus is complicated by the fact that cows show estrus

* Corresponding author. Tel.: +49 641 9937642; fax: +49 641 9937639.
E-mail address: Stefanie.Reith@agrar.uni-giessen.de (S. Reith).

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activity mostly at night (Peralta et al., 2005; Van Vliet and Van Eerdenburg, 1996) and environmental factors such as housing conditions (Palmer et al., 2010; Pennington et al., 1985) and herd size influence management of the individual cow. Nevertheless, a study by Cummins et al. (2012) indicated that genetic merit for fertility traits also has pronounced effects on estrous cycle characteristics including expression of estrous behavior. Bearing this in mind, researchers increasingly turned away from studies focused on using visual observation of cows for signs of estrus. Already in 1994, Senger postulated cost-effective methods to replace visual observation with permanent automatic monitoring of individual data (Senger, 1994).

The cows' activity behavior and time spent walking were found to be the most specific and reliable indicators of estrus (Firk et al., 2002; Roelofs et al., 2010) and were positively correlated with activity data recorded by pedometers measuring locomotion (Roelofs et al., 2005; Yániz et al., 2006) and neck transponders recording activity in total (Elischer et al., 2013; Kamphuis et al., 2012; Valenza et al., 2012). Nevertheless, some cows expressed little or no changes in activity resulting in missed estrous cycles (Brehme et al., 2006; Kerbrat and Disenhaus, 2004). Besides activity monitoring systems, technological progress has led to the development of additional devices to measure behavioral changes due to estrus including lying time (Brehme et al., 2006), feed consumption and water intake (Reith et al., 2014), and standing estrus (Rorie et al., 2002; Saumande, 2002). To date, no work has been published on the relationship between activity and rumination time (RT) during estrus of each cow.

Rumination time is mainly used in dairy farms to predict impending metabolic disorders and calving (Schirmann et al., 2013; Soriani et al., 2012, 2013), and provides useful information to control feeding strategies and rations concerning its structure and components (Welch, 1982). Today, there are commercial systems available to record both RT and activity behavior every 2 h for the individual cow in the herd (Kamphuis et al., 2012; Soriani et al., 2013). In validation studies, researchers who compared data for RT generated by these systems with those obtained by visual observation confirmed the suitability of these systems to accurately monitor daily RT in a practical setting on farms (Burfeind et al., 2011: $r=0.88$; Schirmann et al., 2009: $r=0.93$).

The aims of the present study were to simultaneously investigate cows' activity behavior measured by collar-mounted acceleration technology and daily RT in the peri-estrus period as well as over a 24 h period, and to test the hypothesis that estrus-related variations in activity behavior correlate with variations in RT of estrual cows. In a second study, the effects of parity and milk yield on activity and RT around estrus were evaluated.

2. Material and methods

2.1. Animals, housing and routines

The study was conducted on five commercial farms located in Hesse, Germany with an average herd size between 55 and 70 cows. Four herds (herds 1 to 4) consisted of high producing Holstein-Friesian cows (total $n=239$), while herd 5 was a Simmental herd (70 cows). Parities (lactation number (LN)) of the cows ranged from one to 11. Throughout the year, all cows were housed in free stall barns with cubicles and were milked by an automatic milking system (AMS) with a changing milking frequency. The cows were free to decide on their time spent lying/resting, standing, feeding/drinking, and visiting the AMS. Overall, management routines and feeding practices were similar among the analyzed herds. Mean annual milk production of the Holstein and the Simmental cows was 10,000 kg or greater and 6,800 kg, respectively. Average milk yield ($n=162$, see second analysis) on the day of successful AI was 37.7 kg with a range from 12.4 to 59.3 kg. The animals received a partial total mixed ration with the following components: grass silage, maize silage, barley straw, dried molasses sugar beet pulp and/or malted barley, rapeseed meal and/or soy extraction grist and minerals. Diets were offered twice a day at 0800 ± 1 h and 1700 ± 1 h for ad libitum intake. The mixed ration was calculated for a daily milk yield of 20 kg (Simmental cows) and 28 to 30 kg (Holstein cows). For cows achieving higher levels of production concentrates were provided at 1 kg/2 kg milk produced in restricted amounts in the milking robot and in concentrate-feeding stations. The forage: concentrate ratio (on the basis of DM) was on average between 54:46 and 60:40 for Holstein cows and 69:31 for the Simmental herd. Water was available continuously and ad libitum.

For breeding, estrous cycles were noticed at the end of the voluntary waiting period of 45 d (herd 5), 50 d (herd 2), and 60 d (herds 1, 3, 4) after calving. The cows were inseminated artificially on the basis of increased activity recorded by the HR-Tag monitoring system (SCR Engineers Ltd., Netanya, Israel) and/or visual observation with a mean number of services per cow of 1.9. The interval between calving and first AI averaged 74 ± 39.6 d (mean ± S.D.), and calving-to-conception interval was on average 107 ± 60.7 d (mean ± S.D.). Hormonal treatment was used if estrus had not been detected by 120 d after calving. Among all cows, 47.2% ($n=226$) and 30.5% ($n=146$) conceived to first and second AI, respectively. Pregnancy diagnosis was performed by ultrasonography (herd 2 (55 cows)) 29 d or by transrectal palpation (herds 1, 3, 4, 5, (148 cows)) at approximately 42 d post insemination.

2.2. Study design

The day when the cow was inseminated artificially was defined as estrus (d of estrus = d 0), where the period from midnight to midnight was considered as 'a day'. To ensure that cows showed true estrus, only cycles of cows considered to be pregnant after AI were included. After having found no significant differences in data at d −10 to −3 and 3 to 10, results are presented from d −3 to −1 and 1 to 3. The means of 3 d prior and 3 d after d 0 were determined as reference period and compared with the value at d 0.

In order to identify circadian rhythms, a second reference period (reference period 'circadian rhythm') was defined with d −1 was considered separately and therefore excluded from the first reference period. Mean activity and RT of the reference period 'circadian rhythm' (d −3, −2, 1, 2, 3) were compared with mean activity and RT on the proestrous (d −1) and estrous days (d 0). Comparisons were performed per 2-h time interval.

According to LN and milk yield, the cows (n=162) were assigned to the following categories: 1, 2, 3, >3 (LN) and <35 kg, 35 to 40 kg and >40 kg (milk yield) (Table 2). Milk yield was calculated as an average of each daily milk yield during the reference period.

In order to determine a correlation between activity and RT, the cows were divided into four groups: cows with increased activity and decreased RT during estrus (A+/RT−), cows with increased activity and increased RT during estrus (A+/RT+), cows with decreased activity and decreased RT during estrus (A−/RT−), and cows with decreased activity and increased RT during estrus (A−/RT+).

2.3. Data acquisition

In total, 453 estrous cycles were available for the study. The database per cow (estrous cycle) consisted of 84 records (twelve 2-h values/d × 7 d during the peri-estrous period) for activity and RT each. Data were collected between January 2011 and July 2013 and analyzed in each cow over the peri-estrus period in the cow's complete calving-to-conception interval. Activity values of <20 μ/2 h and >100 μ/2 h and RT values of <180 min/d and >660 min/d as well as a day-to-day variation in RT >120 min during the reference period were considered as implausible. After the exclusion of missing values (in full or in part) or implausible values for activity and RT, the data set was reduced to 441 and 360 estrous cycles (Table 1). For 348 cows, data for both traits were available. The following data were collected for each cow: calving date, estrus date(s), number of AI per conception, calving-to-conception interval, herd, LN, activity, RT, and milk yield.

For measurement of activity, all cows were equipped with the HR-Tag monitoring system (SCR Engineers Ltd., Netanya, Israel) attached to the neck collar of each cow. After integration of a cow into the herd, the tags stayed permanently on their collar throughout their lifetime at each farm. An acceleration sensor continuously records individual cow activity, and calculates a general activity index in 'activity units'. According to Elischer et al. (2013) and the manufacturer, respectively, the tag collected only horizontal accelerations related to upward movements of cow's head and neck during walking and mounting other cows. Downward movements during eating were not considered. Raw activity data were analyzed in a microprocessor by complex algorithms which separated cow's day to day activity from activities associated with estrous behavior. At each visit to the AMS, data stored in 2-h intervals were read by an antenna and automatically transferred on a real time basis to the herd management software on a farm computer. Lists presenting data and graphs were generated by the software program and always retrievable for individual cow. The 2-h values (raw data) were arithmetically averaged to one value per day for further analysis.

Daily RT of the cows was measured by a microphone-based method in the HR-Tag monitoring system (SCR Engineers Ltd., Netanya, Israel) and has been described previously (Reith and Hoy, 2012a).

In a second analysis – this study was conducted between December 2012 and July 2013 –, data for individual milk yield was available for 162 cows. Individual milk yield was automatically registered at each milking. Depending on production level and the cow's milking frequency, recordings were spread over the day. For the analysis, data were summarized to one value per day.

Table 1
Number of activity records[a], rumination time (RT) records[b], and estrous cycles in each lactation number for Holstein Friesian cows (4 herds) and Simmental cows (1 herd).

	Holstein Friesian cows		Simmental cows	
Lactation number	Activity records/ estrous cycles	RT records/ estrous cycles	Activity records/ estrous cycles	RT records/ estrous cycles
1	6468/77	6216/74	4956/59	2604/31
2	5628/67	5544/66	3948/47	2772/33
3	4452/53	4452/53	2604/31	1848/22
>3	5376/64	5040/60	3612/43	1764/21

[a] Activity records: The data base per cow (estrous cycle) consisted of 84 activity records, resulting in 37,044 activity records for 441 estrous cycles.

[b] RT records: The data base per cow (estrous cycle) consisted of 84 RT records, resulting in 30,240 RT records for 360 estrous cycles.

Table 2
Number of estrous cycles (n=162) in each milk yield category and lactation number for Holstein Friesian cows (4 herds) and Simmental cows (1 herd).

	Milk yield			Lactation number			
Breed	<35 kg	35 to 40 kg	>40 kg	1	2	3	>3
Holstein Friesian cows	31	40	52	25	39	27	32
Simmental cows	18	12	9	8	12	9	10

2.4. Statistical analysis

Statistics were carried out using the program package SPSS 19.0 (SPSS Inc., Chicago, IL) and SAS 9.2 (SAS Institute Inc., Cary, NC). Descriptive statistics were calculated for all variables. Activity and RT were normally distributed as determined by the Kolmogorov–Smirnov and Shapiro–Wilk tests. The activity data during estrus of cows were analyzed by applying the MIXED procedure of SAS (SAS Institute Inc.). The following factors were included in the model:

$$Y_{ijklm} = \mu + h_i + p_j + \text{day}_k + \text{cow}_l(h_i) + (p*\text{day})_{jk} + e_{ijklm},$$

where Y_{ijklm}=the variable activity, μ=the intercept, h_i=the fixed effect of herd (1 to 5), p_j=the fixed effect of parity (1, 2, 3, and >3), day_k=the fixed effect of day (−3 to 3), $\text{cow}_l(h_i)$=the random effect of cow within herd, $(p^*\text{day})_{jk}$=the interaction between parity and day, and

e_{ijklm} = the random residual error. Similarly, the variable 'RT' was analyzed with the effects explained as above.

To identify a circadian rhythm of activity and RT, the reference period 'circadian rhythm' (d −1 was excluded) was compared with the day of estrus (d 0) and the day before the day of estrus (d −1) over a 24 h period. Each 2 h-value on d 0 and d −1 was compared with the respective 2 h-value of the reference period.

The following mixed model was used:

$$Y_{ijklmn} = \mu + h_i + \text{hour}_j + p_k + \text{period}_l + (\text{hour*period})_{jl} + \text{cow}_m + e_{ijklmn}$$

where Y_{ijklmn} = the variable activity, μ = the intercept, h_i = the fixed effect of herd, hour_j = the fixed effect of hour (2 to 24), p_k = the fixed effect of parity (1, 2, 3, and > 3), period_l = the fixed effect of period (reference period 'circadian rhythm', d −1, d 0), $(\text{hour*period})_{jl}$ = the interaction between hour and period, cow_m = the random effect of cow, and e_{ijklmn} = the random residual error. The variable 'RT' was analyzed similarly.

In a second investigation, the variable 'milk yield*day' was included resulting in the following mixed model:

$$Y_{ijklmn} = \mu + h_i + p_j + \text{my}_k + \text{day}_l + \text{cow}_m(h_i) + (p*\text{day})_{jl} + (\text{my*day})_{kl} + e_{ijklmn}$$

where Y_{ijklmn} = the variable activity, μ = the intercept, h_i = the fixed effect of herd (1 to 5), p_j = the fixed effect of parity (1, 2, 3, and > 3), my_k = the fixed effect of milk yield (< 35 kg, 35 to 40 kg, and > 40 kg), day_l = the fixed effect of day (−3 to 3), $\text{cow}_m(h_i)$ = the random effect of cow within herd, $(p\text{*day})_{jl}$ = the interaction between parity and day, $(\text{my*day})_{kl}$ = the interaction between milk yield and day, and e_{ijklmn} = the random residual error. Similarly, the variable RT decrease was analyzed.

Differences between estimated least square means were tested using Student–Newman–Keuls test procedure.

3. Results

All factors included in the statistical models were significant. In the mixed model to identify circadian rhythms, all effects except parity were significant.

3.1. Activity and rumination time during estrus

During estrus, activity behavior measured by neck transponders was on average increased (***$P < 0.001$) in each herd. There was no difference between the Holstein and the Simmental cows. Fig. 1 provides the results obtained from the calculation of means. Days −1 and 0 were highly increased compared with all other days in the reference period. This finding was significant at the ***$P < 0.001$ level. A total of 335 cows (76%) with increased activity during estrus – 106 cows (24%) showed reduced activity – was used to determine percentiles values describing the variability of increased activity among all cows (Table 3). The median increase was 34.6%. The arithmetic increase (± SD) calculated for all cows was 38.7% (± 27.0) with a range from 28.4% to 45.0% between the herds. The repeatability for activity calculated from the variance components between and within cows was 76%.

During diestrus, cow activity remained at a nearly constant level, with activity values between 31.1 and 32.7 μ/2 h. On d −1, a clear increase in activity was observed (average 38.7 μ/2 h). From d −2 to the day before estrus (d −1), activity was increased by 5.9 μ/2 h and further by 3.5 μ/2 h from d −1 to the day of estrus with a maximum of 42.2 μ/2 h. After the day of estrus, cows' activity behavior decreased by 11 μ/2 h from d 0 to d 1 to the base value in the reference period.

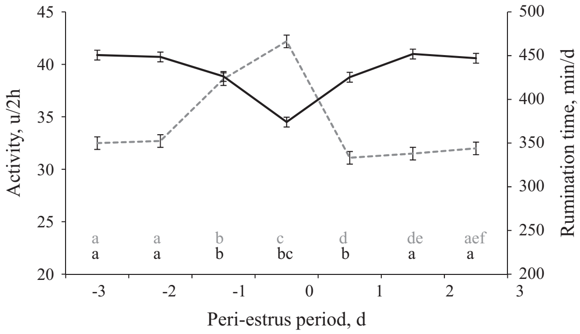

Fig. 1. Dynamics of activity (dashed gray line) and rumination time (RT) (solid black line) in the peri-estrus period of 441 and 360 estrous cycles, respectively, leading to conception of the cows. The bars indicate standard error. Significance is shown by different letters ($P < 0.05$).

Values of RT before and after estrus were different from those recorded on the day of estrus (***$P < 0.001$) (Fig. 1). On average, RT of cows was reduced during estrus: 374 min/d compared with 442 min/d during the reference period (d −3, −2, −1, 1, 2, 3). With values between 447 min/d and 452 min/d, RT of the d −3, −2, 2, and 3 differed only slightly from each other. The decrease in RT before estrus and the increase after estrus were approximately equal. From d −1 to the day of estrus, RT was reduced by 52 min. After estrus, RT increased by 51 min to 425 min detected on d 1 and reached pre-estrus level. Of 360 cows, 311 cows (86%) spent less time ruminating during estrus. The arithmetic decrease (± SD) in RT was 19.6% (± 13.1) and 83 min (± 55), respectively. The calculated percentile values are given in Table 3. The repeatability of RT was 78%.

Simultaneous analysis of activity and RT of the individual cows in estrus showed that 69% of all cows (n = 348) increased activity and decreased RT on the day of estrus compared to all non-estrous days (Fig. 2). However, in 13.8% and 23.5% of the cows, RT was increased and/or activity was reduced during estrus. Estrus remained undetected by both activity and RT in 6.3% of cows.

3.2. Circadian rhythm of activity and rumination time

Each 2-h interval of the reference period 'circadian rhythm' was different (***$P < 0.001$) from the corresponding 2-h interval of the proestrous (d −1) and estrous day (d 0) (Fig. 3). On d −1 and 0, activity values were at a much higher level. The circadian rhythm of activity behavior was bimodal with two peak phases occurring between 0800 and 1000 h and between 1600 and 1800 h. This pattern was not different among all days of the reference period 'circadian rhythm'. Minimum activity was found between 0200 and 0600 h and between 1200 and 1400 h. With an increase of more than 50%, the highest incidence of estrous activity was detected at nighttime and in the

Table 3
Percentile values describing the variability of the number of cows with different increases in activity and decreases in rumination time during estrus[a].

	Percentile							
	Cows, *n*	5%	10%	25%	50%	75%	90%	95%
Activity increase[b] (%)	335	2.1	5.6	18.0	34.6	55.5	73.6	87.0
Rumination decrease[b] (%)	311	2.1	3.6	9.0	17.4	27.5	38.0	45.2
Rumination decrease (min)	311	9.1	16.1	40.6	76.0	114.9	160.0	186.3

[a] Estrus (d 0)=the day of AI leading to cow's conception.
[b] Increase in activity/decrease in rumination time on the day of estrus compared with the reference period (d −3, −2, −1, 1, 2, 3).

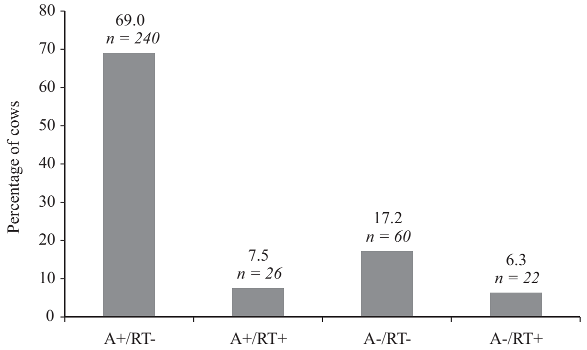

Fig. 2. Percentage of cows with increased activity during estrus (A+), decreased activity during estrus (A−), increased rumination time (RT) during estrus (RT+), and decreased RT during estrus (RT−). All cows were considered to be pregnant after AI.

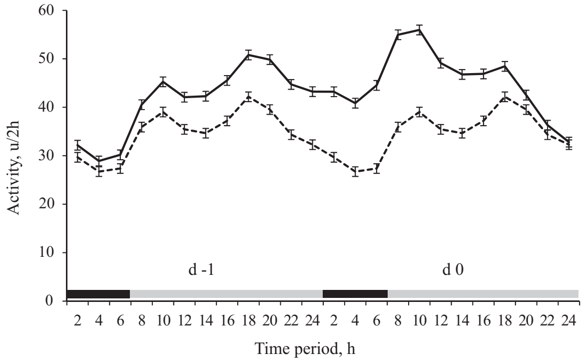

Fig. 3. Cows' activity (n=417) during the reference period 'circadian rhythm' (dashed line) (the mean of d −3, −2, 1, 2, 3) and d −1 (proestrous day) and 0 (estrous day) (solid line) over 24 h (nocturnal hours: black bars along the *x* axis, daytime hours: gray bars along the *x* axis). The acceleration system measured activity in 2-h time intervals where 0200 h indicated mean activity between midnight and 0200 h, 0400 h indicated mean activity between 0200 and 0400 h, …, and 2400 h indicated mean activity between 2200 h and midnight. The bars show the standard error.

early morning hours between 0200 and 0800 h on d 0. Between 2000 h on d −1 and 1400 h on d 0 activity increased more than 30% compared to the reference values.

A bimodal circadian rhythm, inverse to that of activity behavior, was also detected for cows' RT (Fig. 4). During the reference period 'circadian rhythm' and during estrus, minimum levels were measured between 0800 and 1000 h and between 1600 and 1800 h. Maximum RT occurred at night between 0200 and 0400 h and around noon between 1200 and 1400 h. During the estrous period, RT was on average lower than during non-estrous days (***$P<0.001$). The highest difference in RT between the reference period 'circadian rhythm' and estrus was found between 0400 and 1000 h on d 0. From 1800 h on d 0 RT values were identical to those of the reference period 'circadian rhythm'.

3.3. Activity and RT of primiparous and multiparous cows during estrus

Activity behavior during estrus of cows was associated with parity, as higher parity number resulted in lower activity (Table 4). During the reference period, values for multiparous cows ranged from 31.4 μ/2 h to 35.2 μ/2 h compared with primiparous cows (37.5 μ/2 h). On the day of estrus, means for activity varied between 36.3 μ/2 h (parity group >3) and 45.7 μ/2 h (parity group 1). Before estrus, activity increased from the reference period to d −1 and reached peak values on d 0. The largest difference between d 0, d −1, and the reference period was found for cows in parity 1.

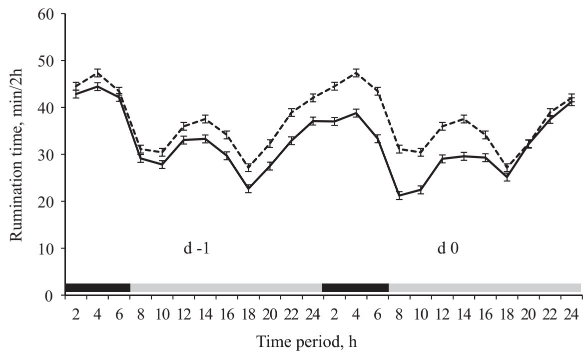

Fig. 4. Cows' rumination time (RT) (n=361) during the reference period 'circadian rhythm' (dashed line) (the mean of d −3, −2, 1, 2, 3) and d −1 (proestrous day) and 0 (estrous day) (solid line) over 24 h (nocturnal hours: black bars along the *x* axis, daytime hours: gray bars along the *x* axis). The microphone recorded RT in 2-h time intervals where 0200 h indicated mean RT between midnight and 0200 h, 0400 h indicated mean RT between 0200 and 0400 h, …, and 2400 h indicated mean RT between 2200 h and midnight. The bars show the standard error.

Similarly, RT differed by parity and was prolonged with increasing age (Table 4). During the reference period, cows in parity 1 ruminated on average 384 min/d whereas mean

RT was 444 min/d for cows in parity > 3. The same trend was observed on d −1 and on d 0. In each parity group, the lowest values were found on d 0 compared with non-estrus days. The decrease in RT during estrus was more pronounced in primiparous cows than in older ones.

3.4. Activity and RT of low- and high-yielding cows during estrus

For cows in different milk yield categories, mean activity was between 39.7 μ/2 h and 40.9 μ/2 h during estrus, which was considerably higher compared with the diestrous period (33.4 μ/2 h and 35.7 μ/2 h, respectively) (Table 5). Moreover, activity behavior tended to be higher with increasing daily milk yield. The estrus-related increase in activity was less pronounced in high-yielding cows (> 40 kg) than in cows with lower milk production.

In contrast to high-yielding cows, low-yielding cows spent more time ruminating (446 min/d versus 407 min/d during the reference period and 396 min/d versus 337 min/d on d 0). In each group, lowest values were detected on the day of estrus. Cows with a daily milk yield > 40 kg showed the greatest decrease in RT during estrus in comparison to cows with a daily milk production ≤ 40 kg.

4. Discussion

Detection of estrus has been identified as one of the primary causes of reduced reproductive performance in dairy cows. Technological progress has led to the development and application of sensor-based monitoring systems that continuously monitor and record detailed information, and use these data to diagnose the status of the cow (sick or healthy, in estrus, etc.). Although measuring activity by pedometers is thoroughly investigated as an indicator of estrus (Brehme et al., 2006; Ranasinghe et al., 2010; Yániz et al., 2006), only few data on activity measurement by means of acceleration systems attached to the neck collar are available in the literature. The present study revealed an increase of 38.7% in activity behavior recorded by collar-mounted technology on the day of estrus (d 0) compared with the reference period (d −3, −2, −1, 1, 2, 3). Similarly, Firk et al. (2003) and Kerbrat and Disenhaus (2004) found a higher number of cases of restlessness, mounting, walking, and agonistic behavior around estrus. Locomotive activity was between 2.3 and 4 times higher on the day of estrus compared to non-estrous days (Schofield et al., 1991; Arney et al., 1994).

The most probable reason for increased activity during estrus might be the release of estrogens (estradiol-17β) starting in the pre-estrus period. With $r=0.57$ and $r=0.7$, Lopez et al. (2004) and Lyimo et al. (2000) found a significant correlation between estradiol concentration, duration of estrus, and estrous behavior. Maximum circulating hormone concentrations were recorded one day before estrus (Lopez et al., 2004) or between 16 h before and 9 h after estrus (Mondal et al., 2006). Unlike Arney et al. (1994), who reported an increase in activity starting on d −3, the increase in activity in this study lasted about 2 d and did not start until d −2. On d 1 activity declined to base level again, coincident with a decline in estradiol concentration.

The opposite temporal pattern was found for RT. Relating to our earlier observations (Reith and Hoy, 2012a; Reith et al., 2012), we were able to confirm that estrual cows spent significantly less time ruminating. The characteristic pattern of RT during estrus was also found in estrous cycles with AI

Table 4
Effect of parity on activity and rumination time during the peri-estrous period (Least squares means ± standard error).

	Activity (μ/2 h)				Rumination time (min/d)			
Parity	1	2	3	> 3	1	2	3	> 3
Cows, *n*	32	52	34	44	28	48	30	40
Reference period	37.5 ± 1.8	35.2 ± 1.3	33.2 ± 1.7	31.4 ± 1.4	384.4 ± 21.8	436.7 ± 16.0	440.6 ± 20.7	443.9 ± 17.6
Day −1[a]	40.1 ± 1.8	39.2 ± 1.3	36.8 ± 1.7	35.6 ± 1.4	374.7 ± 21.7	434.4 ± 15.9	444.2 ± 20.7	437.6 ± 17.5
Day 0[b]	45.7 ± 1.8	39.3 ± 1.3	40.0 ± 1.7	36.3 ± 1.4	316.6 ± 21.7	370.7 ± 15.9	389.4 ± 20.7	385.3 ± 17.5

[a] Day −1 = the day before estrus.
[b] Day 0 = the day of AI leading to cow's conception.

Table 5
Effect of milk yield on activity and rumination time during the peri-estrous period (Least squares means ± standard error).

	Activity (μ/2 h)			Rumination time (min/d)		
Milk yield (kg/d)	< 35	35 to 40	> 40	< 35	35 to 40	> 40
Cows, *n*	53	46	63	56	45	56
Reference period[a]	33.4 ± 1.5	33.9 ± 1.4	35.7 ± 1.3	445.8 ± 17.8	426.7 ± 16.6	407.3 ± 16.5
Day −1[b]	37.0 ± 1.5	36.5 ± 1.4	40.3 ± 1.3	445.6 ± 17.8	435.2 ± 16.6	387.1 ± 16.4
Day 0[c]	39.7 ± 1.5	40.3 ± 1.4	40.9 ± 1.3	395.5 ± 17.8	364.1 ± 16.6	337.1 ± 16.4

[a] Reference period = mean of the d −3, −2, −1, 1, 2, 3.
[b] Day −1 = the day before estrus.
[c] Day 0 = the day of AI leading to cow's conception.

not resulting in conception even if the decrease in RT was less pronounced in those estrous cycles in comparison to estrous cycles with subsequent successful AI (Reith and Hoy, 2012b). Another factor that might contribute to decreased RT is feed intake. In general, it is known that feed intake is closely related to RT (Adin et al., 2009). Dry matter intake (and water consumption) of cows approaching the day of estrus was significantly lower compared with the reference period (Reith et al., 2014). Decreased values were also obtained by Schirmann et al. (2013) who investigated RT, feeding time, and DMI before calving. Unlike activity, base level of RT was reached on d 2. Rumination time increased gradually after estrus showing that cow's estrus had a marked influence on RT in the post-estrus period just as during pre-estrus and on the day of estrus. It could be possible that the increase in RT might be postponed because of the decrease in activity after estrus. After the day of estrus, activity decreased abruptly to values slightly below average. It seemed as if cows required time to recover from estrous activity.

Measurement of data in 2-h time intervals allowed illustration of circadian rhythms. During non-estrous days, various researchers have described bimodality in the circadian activity behavior of cows. High values were found in the daytime with two (Roelofs et al., 2005) or three peaks (Løvendahl and Chagunda, 2010), which were consistent with feeding and milking times of cows. This also accords with our observations except that an influence of milking time on activity can be excluded since the cows were milked using AMS. Data from Fig. 3 can be compared directly with the data shown in Fig. 4. Hence, activity was found to be decreased during nocturnal hours and in the afternoon when cows spent most time ruminating. The findings of the current study are in agreement with those of Van Vliet and Van Eerdenburg (1996) and Peralta et al. (2005), who measured activity by neck transponders and found increased values between 0100 and 0600 h. In practice, cows displaying estrus at nighttime and in the early morning hours could remain undetected by herd managers when only visual observation of estrus is used.

Similarly, the results of the present study revealed a circadian rhythm for RT, as described previously (Adin et al., 2009; Krause et al., 2002), and showed that RT was highly influenced by feeding time and activity behavior. The opposite patterns for activity and RT indicated that cows were not able to ruminate, feed, and be active simultaneously. In agreement with Schirmann et al. (2012), maximum values at night, in the early morning hours, and in the afternoon indicated that RT is likely to coincide with lying times. The proportion of nighttime RT was found to be 63.2% of total RT per d (Soriani et al., 2013). Adin et al. (2009) detected rumination peaks between 2400 h and 0600 h as well as at one hour after feed intake. Similarly, Krause et al. (2002) reported highest RT between two feedings. However, they noted that RT could be distributed throughout the day for cows fed different diets. To our knowledge, no further studies exist on circadian pattern of RT during estrus.

Of all cows, 76.5% (69% (A+/RT−)+7.5% (A+/RT+)) showed increased activity behavior. The finding seems to be consistent with the 71% found by Valenza et al. (2012) and the 62% to 77% announced by Kamphuis et al. (2012), who also used accelerometer systems for activity measurement. In general, it seems that nearly a quarter of all cows remained undetected by activity. When RT was additionally examined, simultaneous analysis of both traits showed that 86.2% (69% (A+/RT−)+17.2% (A−/RT−)) of all cows had detectable changes during estrus, indicating that combining data from different sources may be useful to herd and reproductive management. Surprisingly, the percentage of cows with increased activity behavior during estrus was lower than the number of estrual cows detected by RT in the current study. There may be an advantage of using RT for detection of estrus because some cows failed to display estrous activity or failed to be detected when activity was too low in intensity and/or duration to be classified as in estrus by the activity monitoring system. Possibly, social effects – individual cows may be more motivated to increase activity behavior in the presence of other estrual cows – and cow-related factors such as lameness may prevent cows from showing this specific sign of estrus. Although the automatic measurement of activity is one of the most prevalent methods (Roelofs et al., 2005; Schofield et al., 1991), Brehme et al. (2006) and Redden et al. (1993) noted that it is useless to detect cows with weak or silent estrus signs. Therefore, it is possible to hypothesize that some cows showed silent estrus in our study and might be identified by RT rather than by activity. Because of the fact that cows prefer ruminating while lying down (Östermann and Redbo, 2001; Schirmann et al., 2012), this hypothesis may be supported by Brehme et al. (2006), who found a decrease in lying time of 2.5 h in cows exhibiting silent estrus compared to those with normal estrus. Palmer et al. (2010) compared estrus characteristics in cubicle-housed and pastured cows and noted that cows kept in cubicle housing expressed more silent and sub-estrus events than cows at pasture.

Both activity and RT during estrus were associated with average daily milk yield and parity of dairy cows. There appeared to be a weak trend for a less pronounced activity increase in cows with higher daily milk production. An antagonistic relationship between activity and milk yield was also observed in a number of recent studies (Lopez et al., 2004; López-Gatius et al., 2005; Yániz et al., 2006). Lopez et al. (2004) further found that milk yield was significantly correlated with duration of estrus and estradiol concentration. Probably, this may reflect fertility problems including, among others, lower expression of estrous activity in high-yielding cows. The impact of negative energy balance on the reproductive physiology of the dairy cow and the incidence of silent ovulations were discussed as major causes of impaired fertility performance of modern high-yielding dairy cows (Lucy, 2001; Ranasinghe et al., 2010).

A difference existed between the activity behavior of primiparous and multiparous cows, as primiparous cows tended to be more active during estrus than older cows. This is in agreement with most results found in the literature. Researchers noted that duration of estrus as well as intensity of estrus signs were longer and less pronounced in multiparous cows compared with primiparous cows and heifers (Lopez et al., 2004; Roelofs et al., 2005; Yániz et al., 2006). Arney et al. (1994), on the other hand, found greater locomotion in multiparous cows and discussed their results in the context of selection which may have led to earlier culling of cows with low estrous activity.

Daily RT was highest for low-yielding cows and lowest for high-yielding cows during the reference period. Possibly, this could be explained by a low forage:concentrate ratio in diets created for more productive cows to supply the energy demand for high milk yields. Cows fed rations with a relatively large proportion of concentrates (based on the actual milk production) associated with decreased particle size and fiber digestion reduced forage intake resulting in reduced RT (Krause et al., 2002; Maekawa et al., 2002). Maekawa et al. (2002) reported lower daily RT (498 min) for cows consuming diets with a forage:concentrate ratio of 40:60 than for cows fed 60:40 rations (584 min), indicating that cows spent less time ruminating with decreasing proportion of forage and decreasing forage:concentrate ratio of the feed ration, respectively. In reviewing the literature, variations of RT during estrus in cows with different levels of milk production had not been investigated in prior studies. Rumination time of high-yielding cows was reduced to a greater extent on the day of estrus than that of herd mates with low milk production. This raises the questions of whether silent estruses – occurring more often in high-yielding cows – may be better detected by monitoring of RT than by activity measurement.

During the reference period, an increase in RT was associated with higher parity, agreeing with the results of Maekawa et al. (2002) who further observed DMI and eating time to be greater in multiparous cows. With due regard to cow's age, a causal relationship between feed consumption and RT is obvious. The decrease in RT from the reference period to the day of estrus was more pronounced in primiparous estrual cows than in multiparous estrual cows. Probably, this is due to the negative correlation between parity and activity increase during estrus, as described above.

Conclusions

Progressive use of sensor techniques in dairy management leads to provision of detailed information about the individual cow regardless of herd size. The present study showed that measurement of (neck) activity and RT can be used to identify estrual cows. During estrus, daily activity measured by collar-mounted acceleration technology was increased, whereas daily RT was reduced. One of the most important results was that some cows were only detected in estrus by observing RT data. The above-mentioned observations provide the idea of combining activity and RT for detection of estrus. Therefore, research should concentrate on the development and evaluation of algorithms. It is plausible to hypothesize that RT may be used to indicate cows with silent estrus. This may be another important issue for future studies.

Conflict of interest statement

I wish to confirm that there are no conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all of us.

I confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

As the Corresponding Author I am the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). I am responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. I provide a current, correct email address (Stefanie.Reith@agrar.uni-giessen.de).

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5 General discussion

Worldwide, there exist studies in which researchers reported decreased fertility in dairy cows associated with problems in detection of estrus having serious consequences for herd management. According to Washburn et al. (2002), strategies have to be developed to reduce reproductive declines, and Gröhn and Rajala-Schultz (2000) added that optimized reproductive management leading to improved estrus detection may reduce the number of cows removed from the herd and culled for reproductive reasons (Gröhn and Rajala-Schultz, 2000). Since in most dairy farms herd managers use AI, Galina and Orihuela (2007) emphasized that the success of insemination (and embryo transfer) depends greatly on the identification of estrual cows. With increasing number of animals per herd as well as manpower costs, more importance is attached to technical methods (Diskin and Sreenan, 2000), and behavior plays a key role in each system.

It is well known, that the behavior of cows in estrus is significantly different compared to behavior on non-estrous d. Researchers concentrated on secondary symptoms of estrus which seemed more indicative than standing behaviour, which represented only 1% of the whole estrous period (Senger, 1994) and thus is difficult to detect. Behaviors most frequently observed were mounting/attempted mounting, standing to be mounted, sniffing genitalia, chin-resting, bellowing, head butting, and inappetence (Pennington et al., 1986; Negussie et al., 2002). Due to data published by Heres et al. (2000), the ranking of characteristic signs was as follows: attempting to mount followed by restlessness, standing to be mounted, and sniffing vulva. Expression of secondary behaviors lasted longer compared with the period during which cows stand to be mounted as differences in behavior occurred on average 9 h before the onset of standing estrus and persisted until 18.4 h after the end of this primary symptom (Esslemont et al., 1980; Yoshida and Nakao, 2005). A significant increase in the frequency of secondary signs received and initiated was detectable in the period between 6 to 1 h before and 3 h after standing estrus (Sveberg et al., 2013). Despite contradictory results pointed out in a variety of recent studies which analyzed the number of cows showing standing behavior (Lyimo et al., 2000; Negussie et al., 2002), Kerbrat and Disenhaus (2004) and Palmer et al. (2010) updated knowledge and denied the suitability of "standing estrus" for accurately identifying estrual cows, especially cows kept in cubicle housing. A stronger focus on other behavioral signs related to estrus has been suggested to assist humans in detecting cattle starting estrus (Kerbrat and Disenhaus, 2004).

The major aim of the studies was to assess the hypothesis that cows' activity, rumination, consumption of DM and water as well as BW are changed over the peri-estrous period so with allowing the automatic detection of estrus.

Generally, rumination can be measured visually (Krause et al., 2002; Maekawa et al., 2002) or by technical methods, e. g. pressure transducers (Kaske et al., 2002) or piezo disks integrated within cow's halter (Yang and Beauchemin, 2006) which were mostly created for research purposes. Recently, sensor-based systems are used for automatic recording of individual RT and – contrary to measurement of feeding behavior – are commercially available. Regurgitation of feed boluses and re-mastication produce sounds that are registered by the acoustic sensor (Burfeind et al., 2011), excluding the sounds related to eating (Adin et al., 2009). Because cows are not able to show eating and ruminating behavior simultaneously, jaw movements can be separated in eating and rumination by the rate of chews, by the duration of pauses (Beauchemin et al., 1989; Matsui and Okubo, 1991) and by the amplitude (Kononoff et al., 2002). As rumination is primary used for detection of metabolic diseases (Maekawa et al., 2002; DeVries et al., 2009), a considerable amount of research has been published on RT in relation to diets and feed characteristics (Krause et al., 2002; Adin et al., 2009) as well as feed change (Hoy, 2014), and, in addition, to lameness (Almeida et al., 2008) and acute stress (Herskin et al., 2004).

In study 1, it was described for the first time that individual RT of Holstein cows declined during estrus in comparison to non-estrous d. Lack of significant differences in data during diestrus indicated that the cow's individual time spent ruminating remains at a nearly constant level. This is essentially even true – as observed by Beauchemin et al. (1990) – for the number of chews as well as the chewing speed during re-masticating a bolus a number of times. Consequently, data set was reduced to 7 d near estrus for statistical analysis.

The minimum RT was registered on the d of estrus (355 min) compared to the average of 429 min/d during the reference period. As the dynamics of RT followed the same pattern, this finding could be confirmed in study 3 which included additionally a herd of 70 Simmental cows. With an average decrease of 19.6% (83 min), RT of dairy cows was significantly affected by estrus. Daily RT during the reference period in our investigations (Studies 1 and 3) was in agreement with the results of previous reports showing that cows spend about one-third of the d ruminating (Beauchemin et al., 1989; Dado and Allen, 1994; Adin et al., 2009). During this period, the bolus was re-chewed for about a minute in 9 to 11 periods (Odyuo et al., 1991; Albright, 1993) of 18 - 25 min (Odyuo et al., 1991). Matsui and Okubo (1991) observed rumination periods of 30 - 60 min. A chewing cycle consisted of 50 - 80 chews (Law and Sudweeks, 1975; Melin et al., 2007) interrupted by pauses of 5 - 7 sec in which the animal swallowed and regurgitated (Law and Sudweeks, 1975; Matsui and Okubo, 1991; Kaske et al., 2002).

There is common consensus, that RT is closely linked to feed intake and eating time (Welch, 1982; Kaske et al., 2002; Adin et al., 2009). Recent investigations confirmed a correlation near calving of dairy cows as rumination and feeding time as well as DMI were significantly restricted in the last 6 h before the onset of calving (27%, 57% and 56% relative to the 72- to 7-h time frame) (Büchel and Sundrum, 2014). Hoy (2015b) found a significant decrease in time spent ruminating within 4 h prior to calving. Similar results are reported in other studies (Adin et al., 2009; Soriani et al., 2012; Schirmann et al., 2013; Hoy, 2015a). Thus, it is obvious that a reduction in RT is the consequence of a drop in cow's feed consumption during estrus, reflecting an inhibited demand for ruminating when forage intake decreases independent of the reproductive cycle (Welch and Smith, 1970). Study 2 focused on cow's DMI and WI which can nowadays be recorded continuously and with high accuracy as validated by Chapinal et al. (2007). Hypothesizing an effect of estrus on feed intake, we, indeed, found that most cows (85.3%) consumed less DM of the forage ration – CI was not inhibited – during estrus (20.4 kg) compared with 23.0 kg outside of estrus, which is within the range described in the literature (Dado and Allen, 1994; Yang and Beauchemin, 2006; Kaufmann et al., 2007) in the reference period. This is consistent with results of Diskin and Sreenan (2000) and Halli et al. (2015). In comparison to the remaining 20 d of the 21 d - estrous cycle, DMI was reduced by 10.5% on the d of estrus accompanied by decreases of 9.1% and 20.8% in the number of visits to the feeding trough and in feeding time, respectively (Halli et al., 2015). The latter has even been described in earlier work by Hurnik et al., (1975). However, these results differ from some published studies indicating no alterations (De Silva et al., 1981) or even an increase in feed consumption at estrus (Lukas et al., 2008). Supported by others (Meyer et al., 2004; Lukas et al., 2008), we detected estrus having a reducing effect on WI as well as on BW, which also accords with our previous observations (Reith and Hoy, 2011). Maltz et al. (1997) pointed out a 1- to 3-d drop in BW around estrus, confirming depressed DMI and WI. Nevertheless, Kerbrat and Disenhaus (2004) did not find any difference between the time spent eating and drinking during a cow's receptive phase and the period of sexual rest.

Study 2 was unable to draw a direct comparison between RT and feed consumption. For financial reasons, monitoring feed intake on a daily individual basis is not practical in commercial farms, which meant that it had been necessary to analyze RT and feed intake separately. Thus, study 2 was carried out on a research farm which possessed troughs installed on a scale to measure daily intake of DM and water. On a later occasion, Pahl et al. (2015) who were able to investigate RT and feed intake simultaneously in a research herd confirmed our assumption that the decrease in RT was directly related to the drop in feed consumption during estrus. Nevertheless, due to only a slight correlation of r = - 0.19 between RT and DMI reported by Büchel and Sundrum (2014), a decline in cow's DMI (WI) may not explain adequately the estrus-associated reduction in RT.

The decreases in the values of the feeding characteristics including RT are obviously caused by increased restlessness, as estrus has an enhancing influence on activity behavior of dairy cows (Yániz et al., 2006; Valenza et al., 2012). Since it was first published by Farris (1954) numerous studies have concentrated on the investigation of cows' activity for characterization of duration and intensity of estrus. Technology has improved greatly; its measurement promises to be an effective practical tool to improve not only reproductive efficiency but also herd management in terms of observation of cows' integration into the herd during the early lactation (Reith and Hoy, 2012) as well as automated detection of lameness (Van Hertem et al., 2013) and fresh cow disorders (Edwards and Tozer, 2004). While most studies focused on monitoring locomotion (Arney et al., 1994; Yániz et al., 2006; Ranasinghe et al., 2010), only few publications on activity measurement by acceleration technology are available in the literature. In study 3, collar-mounted systems are utilized to continuously measure estrus-related accelerations caused by upward movements of cow's head and neck during walking and mounting (Løvendahl and Chagunda, 2010; Elischer et al., 2013). Most cows initiating onset of estrus displayed increased restlessness (Kamphuis et al., 2012; Valenza et al., 2012), and activity correlates positively with most of the other behaviors including standing estrus, mounting, chin-resting, sniffing, and butting (Pennington et al., 1986; Van Vliet and Van Eerdenburg, 1996; Lyimo et al., 2000). During diestrus – in agreement with other studies (Arney et al., 1994; Koelsch et al., 1994; Brehme et al., 2006) – activity behavior of cows is characterized by similar, constant patterns, whereas analysis of data around estrus revealed a rise of 38.7% above the mean activity value recorded for preceding d. Kerbrat and Disenhaus (2004), who utilized pedometers for measurement, noted an increase in time spent walking of 342% with a range from 21 to 913% on the estrous d relative to the d before estrus. Although the increase in leg activity was described to be greater than this in neck activity, both systems offered in dependence on the chosen algorithm the potential to monitor deviations in cow's behavior (Liu and Spahr, 1993; Koelsch et al., 1994; Sakaguchi et al., 2007). Activity behavior enhanced stepwise starting in pro-estrus 2 d before estrus. Return to base level on the d after estrus indicated termination of sexual restlessness. However, Arney et al. (1994) described a gradual increase from 80 h to 16 h prior to estrus before locomotion increased more rapidly to a peak on d 0 followed by an exponential reduction to baseline values.

The pro-estrous period is distinctly marked by a hormone transition resulting in a significant rise in estrogen production caused by the pre-ovulatory follicle (Lyimo et al., 2000). The onset of the expression of visible behavioral symptoms by estrogens, especially estradiol 17β (Vailes et al., 1992; Allrich, 1994; Roelofs et al., 2010) and their inhibitory influence on dietary behavior (Uphouse and Maswood, 1998; Mondal et al., 2006) is well documented in the literature. Despite the lack of blood samples, it is highly probable that increased restlessness as well as reduced RT found in study 3 were caused by the release of these steroids. Maximum estradiol concentration (7.76 pg/ml) was reached at the same time as the highest behavior score (Lyimo et al., 2000) and was correlated with duration of estrus (Lopez et al., 2004a). High activity levels on d 0 were followed by a rapid decline to basal levels on d 1, which is usually referred to as the d of ovulation (Peters and Lamming, 1983), occurring on average 30 h after onset of estrus and 27.6 h after onset of mounting activity (Walker et al., 1996), respectively, and 18.8 h after cessation of estrus (Roelofs et al., 2005b). Following ovulation, the di-estrous period is characterized by maximum luteal function and progesterone dominance by which the secretion of GnRH from the hypothalamus is suppressed (Morris and Diskin, 2007; Lonergan, 2011). During this period, cow activity as well as RT remained at a constant level. No significant differences in data were measured between d -10 to -3 and 3 to 10 - probably due to the absence of estrogens. Progesterone concentrations decrease 2 d before initiation of the next estrus (Gartland et al., 1975), and this is the time marked by alterations in cow data.

Various studies have demonstrated that estrous behaviors start throughout each 24-h period and many of them are of short duration (Xu et al., 1998). In study 3, the highest incidence of estrous activity was found during nocturnal hours and in the early morning. In farms, cows expressing estrus at nighttime and in the morning hours could remain undetected by herd managers when only visual observation of estrus is used. Night-time observations (Hurnik et al., 1975) as well as observation outside feeding and milking (Van Vliet and Van Eerdenburg, 1996) promise increased detection rates. For further studies, the bimodality in circadian rhythms of activity and RT during estrus needs to be considered for development of algorithms. Decidedly, our results confirmed that a continuous (twelve 2-h values per d) observation of cows is one of the main advantages of automatic detection of estrus.

Duration as well as intensity of typical signs of estrus varied considerably between individuals (At-Taras and Spahr, 2001), depending on a number of environmental, cow- and management-related factors (Orihuela, 2000; Roelofs et al., 2010). Similarly, great variability in activity and RT existed among cows as we found cows showing greater restlessness or ruminating much more than others subjected to the same treatment. Findings of Vailes et al. (1992) revealed that some animals usually displayed mounting behavior and others were not active mounters. Prior mounting behavior of very active estrual cows was significantly associated with mounting behavior (successful mounts and mounting attempts) at subsequent observations (Vailes et al., 1992). In study 3, although all cycles led to conception, 24% of the cows were not detected by increased activity. Yet, we could show that simultaneous analysis of RT and activity may improve the detection rate of cows starting estrus. These data greatly underscore the relevance of considering more than only one trait for identification of cows that would otherwise not be inseminated. Unexpectedly, the number of cows with enhanced activity behavior at estrus was lower than that identified by RT, suggesting that measurement of rumination may detect more cows approaching estrus compared with measurement of activity. Although the latter is one of the most reliable sign of estrus (Schofield et al., 1991), this behavior is useless to detect cows with silent and weak estrus (Redden et al., 1993; Brehme et al., 2006) as well as in case of lameness (Kiddy, 1976). Some cows show no or only less pronounced visual signs (Kerbrat and Disenhaus, 2004) resulting in missed estrous cycles (Brehme et al., 2006). In order to improve estrus detection, multivariate data analysis was advanced in several investigations presenting an attempt to support the herd manager in detecting cows' estrus. Simultaneously analyzed traits incorporated into a multivariate detection system were activity as the primary trait combined with milk yield, milk temperature (Maatje et al., 1997), milk progesterone concentrations (Ranasinghe et al., 2010), vaginal temperature (Redden et al., 1993), lying time (Brehme et al., 2006), and data about previous estruses (Firk et al., 2003). Sensitivity and specificity varied depending on methods of calculation and definitions of algorithms (Firk et al., 2002). For modern practical application it is important to use automated and cost-effective approaches, as postulated by Senger (1994), which can be easily integrated into daily herd management.

Based on the analysis of the effect of parity and milk yield on alterations in behaviors during estrus data showed that RT was significantly more affected by the sexually active phase in primiparous cows, when compared to older cows (Studies 1 and 3). As proven in study 3, these cows with less time spent ruminating at estrus were characterized by tendential higher activity behavior, and this corroborates the close relationship between activity and RT during estrus. In the literature, there is large discrepancy about the influence of parity on reproductive traits. Several studies have shown a shorter duration of estrus for multiparous than for primiparous cows (Lopez et al., 2004a). The time during which estrual animals show increased activity behavior lasted 8.12 h in cows and 9.24 h in heifers (Løvendahl and Chagunda, 2010). Additionally, most researchers (López-Gatius et al., 2005b; Roelofs et al., 2005a; Yániz et al., 2006) noted reduced locomotion observed for multiparous in comparison to primiparous cows, and the frequency of mounting behavior as well as total number of behavioral activities (according to the scoring system developed by Van Eerdenburg et al., 1996) are inhibited significantly in older cows (Law et al., 2009). For detection of estrus, it seemed as if cows with higher parity require careful observation. In non-estrous d, older cows spent more time ruminating compared with primiparous cows (studies 1 and 3) which is supported by Maekawa et al. (2002). They further noted – according to earlier results of Dado and Allen (1994) – a higher feed consumption accompanied by longer time spent feeding in multiparous cows.

A number of recent publications have documented an antagonistic relationship between reproduction and the level of milk production (Harrison et al., 1990; Hansen, 2000; Lucy, 2001; Pryce et al., 2004; VanRaden et al., 2004) – especially in the Holstein breed being well known for its high milk yield (Cutullic et al., 2009; Norman et al., 2009). Our research revealed a weak trend for less pronounced estrous activity in cows with higher milk production, probably due to lower circulating concentrations of estradiol released in pro-estrus (Lopez et al., 2004a, Wiltbank et al., 2006). Similar results have been suggested in further studies based on pedometer measurements (López-Gatius et al., 2005b; Yániz et al., 2006). López-Gatius et al. (2005b) observed a decrease of 1.6% in walking activity, when a cow's milk production increased by 1 kg. In practice, occurrence of silent estrus in high-productive cows impedes their identification dramatically. Regarding RT, we observed this group showing the greatest decrease in time spent ruminating. If RT may be worthwhile for identifying (high-yielding) cows with silent estrus may be an issue for further research.

6 Conclusion

Detection of bovine estrus significantly affects reproductive efficiency and profitability of dairy herds. Prior to the publication of the presented studies there is little to no information on the effects of estrus on rumination, DMI, WI, and BW of dairy cattle. Detailed knowledge of all signs of estrus is required for good management, and the development of improved methods of identifying estrual animals depends on the knowledge of behavioral alterations at the onset of estrus. The studies of this thesis present several new findings with respect to the reproductive status of dairy cows. Feeding characteristics including RT – usually used for early identification of diseases – were identified to be useful for obtaining information on the onset of estrus. There were significant differences between estrous and non-estrous d with regard to rumination, consumption of feed and water as well as BW, which declined in most cows.

Additional conclusions arising from study 3 are that activity behavior measured by collar-mounted acceleration technology was increased concomitant with reduced RT in the majority (69.0%) of cows. The fact that some cows (17.2%) were only detected by RT provides the idea of combining data of activity and RT for detection of a high proportion of cows. Therefore, further studies will need to be undertaken on the development and evaluation of algorithms with the objective of reducing false positive detections. Large differences in the expression of behaviors which can, inter alia, be attributed to parity and milk yield as well as circadian rhythms have to be considered for development of thresholds.

The findings presented in this thesis suggest that the alterations in these traits revealed the potential for early prediction of cow's estrus. Thus, the herd manager will receive reliable and practical information about the onset of estrus in dairy cattle resulting in improved reproductive performances in dairy cattle.

7 Summary

The detection of estrus is one of the major factors affecting the reproductive performance of dairy cows, especially in farms using AI. Failure to detect estrous behaviors and false positive results leads to missed inseminations and, thus, economic losses. Technical methods providing detailed information and continuous monitoring of the individual cow have been developed to support herd managers in determining the onset of estrus. Whereas increased activity behavior is regarded as indicative of estrus, little to nothing is known about the effect of estrus on daily rumination or feed and water consumption.

The overall objective of the studies presented in this thesis was to investigate whether these traits were associated with estrus in dairy cattle.

To ensure true estrus, only estruses leading to conception after AI were included in the investigations. Values of the estrous day (d 0) were compared with the reference period defined as the mean of 3 days prior and 3 days post d 0. Recording of data in 2-h time intervals provided information on circadian rhythms. For measurement of individual RT, cows of five commercial farms were equipped with the HR-Tag monitoring system (SCR Engineers Ltd., Netanya, Israel) containing a microphone attached to the neck collar of each cow.

In study 1, it was found for the first time that RT was – with large inter-individual variation – significantly influenced by cow's estrus as animals spent less time ruminating on d 0 relative to the base level during the reference period (374 versus 442 min). Rumination time decreased gradually starting two days before the onset of estrus. The minimum level was identified on d 0, after which RT returned to base level again. As RT is closely linked to feeding characteristics, these results predicted that further feeding characteristics may also be reduced at estrus. Hence, study 2 carried out on a research farm focused on cow's feed and water consumption which were automatically measured by troughs placed on an electronic floor scale. Indeed, with a decline of on average 14.6%, most cows (85%) consumed

significantly less dry matter of the forage ration – CI was not affected by estrus – during estrus in comparison to non-estrous days (20.4 versus 23.0 kg). Similarly, estrual cows drank less water. WI was reduced by 15.3% in 67% of all cows, with the lowest value determined on the day before estrus relative to the reference period. Consequently, BW recorded by a walk-through scale showed lower values in 69% of the animals at estrus, reflecting the decrease in rumen content caused by reduced DMI and WI.

Simultaneous analysis of RT and activity behavior in study 3 verified our hypothesis of a direct relationship between the alterations in these traits. Monitoring of activity was performed by a collar-mounted system that continuously measures estrus-related accelerations resulting from upward movements of the head and neck during walking and mounting. With no difference between Holstein and Simmental cows activity enhanced on average by 38.7%. On d 1 activity declined to base level again, concomitant with the increase in RT. The percentage of estrual cows with increased activity was lower than of those with shorter RT (76.5 versus 86.2%).

Both RT and activity behavior during estrus were correlated with parity as well as average daily milk yield. Changes during estrus were more pronounced in primiparous cows than in multiparous herd mates. Additionally, study 3 revealed that cows with a daily milk yield > 40 kg exhibited the greatest decline in time spent ruminating compared to those with a production ≤ 40 kg per day. A weak trend for a less pronounced activity increase was detected in high-yielding cows.

The circadian rhythms of RT and activity behavior were bimodal. Activity behavior was found to be decreased in the afternoon and during nocturnal hours in the reference period when cows spent most time ruminating. Cows expressed highest activity and lowest RT between 0200 and 0800 and 0400 and 1000 h on the day of estrus.

In conclusion, previously unknown as being indicative for estrus, rumination was found to be significantly reduced at the onset of cow's estrus. Furthermore, feed and water consumption as well as BW were markedly decreased by estrus, whereas activity behavior measured by collar-mounted acceleration technology was significantly increased. Comprehensive knowledge of the behavioral characteristics of cow's estrus is essential to recognize cows in estrus. The results of the presented studies give rise to further research including the development of algorithms to determine how the analyzed traits may support herd managers in detecting estrual animals and, thus, to improve daily reproductive management.

8 Zusammenfassung

Die Brunsterkennung ist eine der Haupteinflussfaktoren für die Fruchtbarkeitsleistung von Milchkühen. Dies gilt insbesondere für solche Betriebe, in denen die künstliche/instrumentelle Besamung praktiziert wird. Ein Übersehen der Brunst sowie falsch-positive Ergebnisse bei der Trächtigkeitsuntersuchung resultieren meist in Fehlbesamungen und somit in ökonomischen Verlusten. Technische Hilfsmittel, die zur Unterstützung des Herdenmanagers bei der Bestimmung des Brunstbeginns entwickelt wurden, liefern detaillierte Informationen und eine kontinuierliche Kontrolle des Einzeltieres. Während ein gesteigertes Aktivitätsverhalten charakteristisch für das Vorliegen einer Brunst ist, war bisher wenig bis nichts über die Effekte der Brunst auf die tägliche Wiederkauzeit sowie Futter- und Wasseraufnahme bekannt.

Das übergeordnete Ziel der in dieser Dissertation vorgestellten Studien war es zu untersuchen, ob es einen Zusammenhang zwischen der Brunst und diesen Zielgrößen bei Milchkühen gibt.

Um sicherzugehen, dass es sich um eine echte Brunst handelte, wurden ausschließlich Zyklen, die zur Konzeption nach KB geführt haben, in die Untersuchungen einbezogen. Die Werte am Tag der Brunst (Tag 0) wurden mit der Referenzperiode, dem Mittel der 3 Tage vor und 3 Tage nach dem Tag 0, verglichen. Die Erfassung der Daten in 2-Stunden-Intervallen ermöglichte die Darstellung von Tagesrhythmen.

Für die Messung der Wiederkauaktivität waren die Kühe von 5 Betrieben mit dem HR-Tag-System (SCR Engineers Ltd., Netanya, Israel) ausgestattet, dessen integriertes Mikrofon am Halsband der Kuh befestigt war.

In Studie 1 wurde erstmals nachgewiesen, dass die tägliche Wiederkaudauer – mit einer großen inter-individuellen Variabilität – signifikant durch die Brunst der Kühe beeinflusst wird; die Tiere ruminierten am Tag 0 wesentlich weniger im Vergleich zum

Referenzzeitraum (374 versus 442 min). Während der graduelle Rückgang der Wiederkauzeit bereits zwei Tage vor Brunstbeginn startete, wurde das Minimum am Tag 0 identifiziert.

Da die tägliche Wiederkauaktivität eng im Zusammenhang mit dem Futteraufnahmeverhalten steht, führten diese Ergebnisse zu der Vermutung, dass auch die Futteraufnahme während der Brunst verändert ist. Demzufolge wurde in Studie 2 der Fokus auf die Futter- und Wasseraufnahme, die automatisch per Wiegetrog gemessen wurden, gelegt. Tatsächlich konsumierten die meisten Kühe (85 %) mit einer Abnahme von im Mittel 14,6 % signifikant weniger Trockenmasse der Grobfutterration am Tag des Östrus – die Kraftfutteraufnahme wurde nicht durch die Brunst beeinflusst – im Vergleich zu den di-östrischen Tagen (20,4 versus 23,0 kg). Gleichermaßen tranken die Tiere weniger. Bei 67 % aller Kühe war die Wasseraufnahme um 15,3 % reduziert mit dem niedrigsten Wert am Tag vor der Brunst. Die Lebendmasse, die mittels einer Durchtreibewaage erfasst wurde, war bei 69 % aller brünstigen Tiere am Tag 0 verringert, was auf den geringeren Panseninhalt bzw. die Depression des Futter- und Wasserkonsums zurückzuführen ist.

Die simultane Auswertung des Ruminations- und Aktivitätsverhaltens in Studie 3 verifizierte die Hypothese, dass es eine direkte Beziehung zwischen den Veränderungen dieser beiden Faktoren gibt. Das Aktivitätsmonitoring erfolgte über ein Halsband-System (HR-Tag), das kontinuierlich brunstbedingte Kopf- und Halsbewegungen während des Gehens und Aufspringens aufzeichnete. Die Aktivitätssteigerung betrug im Mittel 38,7 %, wobei es keine Unterschiede zwischen Holstein- und Fleckviehkühen gab. Am Tag nach dem Östrus fiel die Aktivität, einhergehend mit einer Zunahme der Wiederkaudauer, wieder auf den Basalwert ab. Der Anteil an brünstigen Kühen mit verkürzter Wiederkauaktivität war höher als jener mit einer erhöhten Aktivität (86, 2 versus 76,5 %).

Sowohl die Wiederkaudauer als auch das Aktivitätsverhalten standen im Zusammenhang mit der Parität und mit der mittleren täglichen Milchleistung. Die brunstbedingten Veränderungen waren bei primiparen Kühen stärker ausgeprägt als bei multiparen Herdenmitgliedern. Zudem zeigte Studie 3, dass die Kühe mit einer Tagesproduktion > 40 kg die stärkste Abnahme in der Wiederkauzeit aufzeigten, verglichen mit den Kühen mit einer Leistung von ≤ 40 kg pro Tag. Hingegen war der Aktivitätsanstieg bei Hochleistungskühen tendenziell weniger stark ausgeprägt im Gegensatz zu den niederleistenden Kühen.

Für die Rumination und das Aktivitätsverhalten konnte jeweils ein bimodaler täglicher Rhythmus festgestellt werden. Während in der Referenzperiode die Aktivität nachmittags und in den Nachtstunden herabgesetzt und die Wiederkauzeit erhöht war, zeigten die Kühe am Tag der Brunst die höchste Aktivität und die kürzeste Wiederkauzeit zwischen 02:00 und 08:00 Uhr bzw. 04:00 und 10:00 Uhr.

Schlussfolgernd ist festzuhalten, dass die Wiederkauzeit, die bisher als Indikator für die Brunst unbekannt war, zum Brunstbeginn der Kühe signifikant verringert ist. Zudem sind die Futter- und Wasseraufnahme sowie die Lebendmasse deutlich reduziert, wohingegen das Aktivitätsverhalten signifikant erhöht ist. Umfangreiche Kenntnisse über die verhaltensbezogenen Merkmale der Brunst sind für die Erkennung brünstiger Kühe essentiell. Die Ergebnisse der vorgestellten Studien geben Anlass zu weiterer Forschung, in der mit Hilfe von Algorithmen bestimmt werden kann, wie die analysierten Faktoren den Herdenmanager bei der Detektion brünstiger Kühe unterstützen und somit das tägliche Reproduktionsmanagement verbessern können.

9 References of the general part

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Eidesstattliche Erklärung

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