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Evaluation of stress caused by drying-off dairy cows and its relation to milk yield and udder pressure

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Meiner Oma

„Ein Text ist nicht dann vollkommen, wenn man nichts mehr hinzufügen, sondern nichts mehr weglassen kann!“

(Antoine de Saint-Exupéry)

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1. INTRODUCTION

The dry period and dry cow management were demonstrated to be important for health (Kim and Suh, 2003), milk production (Annen et al., 2004; Andersen et al., 2005) and fertility (Beever, 2006) of dairy cows. The optimal dry period length (Rastani et al., 2005; Watters et al., 2008; Santschi et al., 2011) and advantages and disadvantage of continuous milking and drying-off (Rémond et al., 1992; Madsen et al., 2008) have been researched in several studies.

Considering the dry-off management in most European countries and in large parts of North America, farmers follow common recommendations featuring an abrupt cessation of milking at the end of lactation (Newman et al., 2010). This method was recommended in the 1930s and has been applied for decades (Steyn, 1940) irrespective that milk yield has been increasing considerably during the last semi century (German Cattle Breeders' Federation, 2013). In 1975, 88.2% of the cows produced less than 9 kg milk per day at the time of dry-off (Natzke et al., 1975). Only 25 years later, Dingwell et al. (2001) evaluated an average milk yield at the time of dry-off of 16.6 kg per day based on Ontario dairy herd improvement records. Furthermore, about 20% of the cows had a daily milk yield exceeding 22 kg. Given such an increase of milk yield, a re-evaluation of traditional dry-off procedures might be indicated.

While animal welfare became a major public concern (von Keyserlingk et al., 2009), animal welfare aspects of drying-off especially high yielding dairy cows have not been considered or evaluated. Tucker et al. (2007) hypothesized that a sudden dry-off causes discomfort by the accumulation of milk over several days after dry-off. Discomfort was also described by Leitner et al. (2007), who associated noticeable agony with udder engorgement and milk leakage after dry-off. It was hypothesized that extensive udder engorgement after dry-off reflects the high pressure within the udder, caused by a slowly decreasing milk secretion and cessation of milking. Agony and discomfort might originate from these pressures (Leitner et al., 2007). This assumption is substantiated by findings in women that suffer regularly from breast soreness and pain after a short weaning duration (Neighbors et al., 2003). Scientific evidence of a relationship between a sudden dry-off, udder pressure after dry-off and discomfort or pain, however, has not been provided yet.

While an objective quantification of pain in animals is currently not possible (Anil et al., 2002; Rutherford, 2002), it is well established that pain and discomfort are strong causes of stress (Smith et al., 1999; Martini et al., 2000). In contrast to pain, a quantification of stress is feasible (Anil et al., 2002; Rutherford, 2002). Considering these contexts, it was hypothesized that there is a relationship between sudden dry-off, udder pressure, discomfort and elevated stress levels after dry-off.

The overall objective of this thesis was, to assess stress caused by drying-off dairy cows and to evaluate an association between milk yield at dry-off, udder pressure and stress after dry-off. It is meant to provide basic information necessary to re-evaluate dry cow management in high yielding dairy cows considering animal welfare aspects.

In order to relate udder pressure and stress after dry-off, a validated method to measure udder pressure objectively was essential. In recent studies different manual scoring systems were applied in order to define udder firmness (Gleeson et al., 2006; Leitner et al., 2007; O'Driscoll et al., 2011). Manual palpation, however, might be subjective, the techniques have never been validated and information about repeatability is not available. In addition to the scoring systems, manometers and spring pressures measuring devices were used in former studies to determine udder firmness or udder pressure objectively (Mayer et al., 1991; Tucker et al., 2007, 2009). Though, these devices were either uniquely designed for an experiment and are not commercially available (Phillips, 1954; Tucker et al., 2007, 2009) or they were cumbersome and had technical limitations (Witzel and McDonald, 1964; Mayer et al., 1991). For example, several authors used manometers connected to cannulas either inserted into the teat canal (Mayer et al., 1991) or surgically implanted into the udder tissue (Witzel and McDonald, 1964). In field studies, especially in studies concerning animal welfare an application of these devices is unsuitable.

Dynamometers have been validated and are widely used to control crisp and firmness of fruits in plant and food research (Feng et al., 2011). One of these commercially available hand held dynamometer (Penefel DFT 14 Agro Technologies, Forges les Eaux, France) is designed for free hand usage, works on batteries and allows a non-invasive measurement (Gamrasni et al., 2010; Sabban-Amin et al., 2011). There is a dearth of information, however, on the applicability of such a dynamometer for udder pressure measurement.

Therefore, the objective of the first study was to validate a dynamometer (Penefel DFT 14) for udder pressure measurement in dairy cows. Specifically I set out 1) to evaluate the inter-investigator repeatability, 2) to study the effect of location of measurement on udder pressure, and 3) to examine the relationship between pressure changes before and after milking and milk yield.

The results of this study have been published in the Journal of Dairy Science (Impact Factor 2013: 2.566):

S. Bertulat, C. Fischer-Tenhagen, A. Werner, W. Heuwieser. 2012. Technical note: Validating a dynamometer for non-invasive measuring of udder firmness in dairy cows. Journal of Dairy Science 95:6550-6556.

In conclusion of this first paper mentioned above, a valid method to measure udder pressure was found. Considering the aim of this thesis, however, in addition to a validated device to measure udder pressure, a reliable method to quantify stress was required.

The measurement of cortisol and cortisol metabolites is an established method to estimate stress (Anil et al., 2002; Rutherford, 2002). While blood is the most common sample material to measure cortisol (Echternkamp, 1984), it has certain limitations. Mere handling, restraining and blood sampling cause an increase in blood cortisol (Echternkamp, 1984; Hopster et al., 1999) and confound the resulting cortisol concentrations. A different approach for the evaluation of chronic stress levels in animals is the measurement of fecal cortisol metabolites (Möstl et al., 2002; Morrow et al., 2002). Fecal samples can be obtained without stressful restraining and manipulating the cow. A direct relationship between fecal glucocorticoid metabolites, blood cortisol and the adrenal activity has been demonstrated by Morrow et al. (2002). The most important glucocorticoid metabolites measured in cow feces are 11,17-dioxoandrostanes (Palme and Möstl, 1997; Palme et al., 1999; Möstl et al., 2002).

Therefore, the objectives of the second study were 1) to quantify the changes of udder pressure and of the fecal 11,17-dioxoandrostane concentration after sudden dry-off, 2) to determine the effect of milk yield prior to dry-off on udder pressure and the fecal 11,17-dioxoandrostane concentration, and 3) to evaluate the relationship between udder pressure and fecal 11,17-dioxoandrostane concentration in the early dry period.

The results of this study were recently published in the Journal of Dairy Science (Impact Factor 2013: 2.566):

S. Bertulat, C. Fischer-Tenhagen, V. Suthar, E. Möstl, N. Isaka, W. Heuwieser. 2013. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yield. Journal of Dairy Science 96:3774-3787

While this study was able to show that a sudden dry-off had a distinct effect on udder pressure and stress levels, the relevance of these data for dairy husbandry and animal welfare remained unclear. Dry-off procedures applied in studies vary to a great extend (Odensten et al., 2005; Tucker et al., 2009; Zobel et al., 2013), but valide data on dry-off strategies applied on commercial dairy farms are rare and out-dated (Dingwell et al., 2001). In order to prove the relevance of these results, especially for German dairy farms and to substantiate the relevance of this thesis a third study was conducted.

The objectives of this third study were 1) to evaluate current dry-off strategies on German dairy farms using a questionnaire, 2) to quantify behavior indicative of stress after dry-off on commercial dairy farms and, 3) to compare dry-off strategies used on commercial dairy farms to recommendations given in the current literature.

The results of this study were not published and are presented in this thesis as an additional contribution.

2. RESEARCH PAPERS

a. Technical note: Validating a dynamometer for non-invasive measuring of udder firmness in dairy cows

b. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yield

The manuscripts of the papers are formatted according to the guidelines for authors of the Journal of Dairy Science.

a. Technical note: Validating a dynamometer for non-invasive measuring of udder firmness in dairy cows

***Technical note*: Validating a dynamometer for non-invasive measuring of udder firmness in dairy cows**

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ABSTRACT

Most measurements of udder pressure are based on devices connected to the gland cistern via cannulas. These devices are either inserted in the teat canal or surgically implanted into the udder tissue. In this study, instead of invasively measuring intramammary udder pressure, we measured the udder firmness noninvasively on the udder surface via a dynamometer. These are commonly used in food research to determine crispiness and firmness of fruits. The objective of this study was to validate a hand-held dynamometer for measuring udder firmness in dairy cows. Specifically we set out to determine inter-investigator repeatability considering potential confounders such as investigator, location, and cow. Through modifications in the standard operating procedure for the measurements, inter-investigator repeatability increased from correlation coefficient = 0.80 (n = 275) to correlation coefficient = 0.94 (n = 634). Measurements in different locations within the left hind quarter revealed a firmness gradient from the upper to the lower measuring point. Measurements between the 4 quarters within a cow displayed differences, except between both hind quarters. In 94.8% of the udders, firmness decreased due to milking. The correlation coefficient, however, between firmness changes and milk yield was low (r = 0.42, n = 153). Our data provide evidence that the dynamometer, although imperfect, does provide a reasonable measure of udder firmness and can be a useful tool in research related to animal health and welfare. However, a standardized operating protocol should be followed to minimize confounding by investigator, location, and quarter.

Keywords: udder firmness, dynamometer, validation, dairy cow

Udder pressure in dairy cows has been measured for multiple reasons such as investigating aspects of milk secretion and ejection (Tgetgel, 1926), determining effects of different milking routines (Tucker et al., 2007) or dry-off strategies (Tucker et al., 2009) on udder pressure. Most approaches have been based on measurement of the intramammary pressure using manometers connected via fluid-filled tubes to cannulas either inserted into the teat canal (Mayer et al., 1991) or surgically implanted into the udder tissue (Witzel and McDonald, 1964). These methods provided useful information, but the equipment is cumbersome and has several technical limitations that precluded use in the field. A different approach was adopted in 3 studies (Phillips, 1954; Tucker et al., 2007, 2009) that used spring pressure to measure udder firmness noninvasively on the udder surface instead of an invasive intramammary udder pressure measurement. These previously used devices, however, are not commercially available.

Possible applications of a noninvasive udder pressure measurement are manifold and the determination of udder firmness could be interesting in various areas of research. Palpations scores (Gleeson et al., 2007; O'Driscoll et al., 2011) are an established tool in daily veterinary practice. One application is the diagnosis of mastitis (Nielsen et al., 2004; Wilson et al., 2004).

Manual palpation, however, might be subjective; the technique has never been validated and information about repeatability is not available. A tool potentially facilitating an increased accuracy of mastitis diagnosis and better prediction of treatment outcome might be a valuable improvement.

Most recently, the influence of drying-off dairy cows on their animal well-being was investigated (Odensten et al., 2007; Valizaheh et al., 2008). To evaluate relationships between udder firmness and animal welfare on commercial dairy farms, a noninvasive measuring method would be advantageous.

In plant and food research, pressure measurement devices (i.e., dynamometers) are widely used to control crispiness and firmness of fruits (Feng et al., 2011). The force needed to insert the measuring tip into the fruit flesh is measured with such a device. One of these commercially available hand-held dynamometers (Penefel DFT 14; Agro Technologies, Forges-les-Eaux, France) is designed for free-hand usage, works on batteries, and allows a noninvasive measurement (Gamrasni et al., 2010; Sabban-Amin et al., 2011).

The overall objective of this study was to validate the Penefel DFT 14 dynamometer for udder firmness measurement in dairy cows. Specifically, we set out (1) to evaluate the inter-investigator repeatability, (2) to study the effect of location of measurement on firmness, and (3) to examine the relationship between firmness changes before and after milking and milk yield.

The study was conducted between April and August 2011 on a commercial dairy farm in Brandenburg, Germany. Cows were managed according to the guidelines set by the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (Hellmann and Radeloff, 2000). Eighty Holstein-Friesian dairy cows (31 primiparous and 49 multiparous) were included in the study 7 d before drying-off (343 ± 39 DIM; mean ± SD) and followed up for 9 d after drying-off. Cows were housed in a straw-bedded freestall barn and fed a roughage mix delivered twice per day at 0830 and 1700 h. Late-lactating cows received (on a DM basis) 54.3% corn silage, 25.4% haylage, 16.3% distillers grains, 0.9% corn, 0.8% soy, 2.0% rapeseed, and 0.3% basic mineral mix. Dry cows received, on a DM basis, 64.7% haylage, 32.8% corn silage, 1.7% hay, 0.3% corn, and 0.5% mineral mix. Concentrate was available for each cow individually via an automatic feeder (35% wheat, 35% rye, 24% rapeseed extract, 5% soy, and 1% oil, on a DM basis). Lactating cows were milked twice daily in a 2 × 8 Herringbone milking parlor (Alpro System; DeLaval, Tumba, Sweden) from 0600 to 0900 h and 1600 to 1900 h.

Before enrollment, udders were palpated and milk was visually examined on a dark surface. One day before dry-off, examinations were repeated and a California mastitis test was performed. Cows with signs indicative of mastitis, udder or teat lesions, alterations of the udder tissue, or less than 4 functional quarters were excluded. Udder examinations were repeated once per week until the cow completed the study. After drying-off, milk was not examined. Cows were retrospectively withdrawn if any off the signs mentioned above were observed.

The udder firmness was measured using a Penefel DFT 14 dynamometer. The device (size = 250 × 93 × 30 mm; weight = 415 g) consists of a pressure sensor with a measuring tip connected to a processing unit and a digital display (Figure 1). The dynamometer measures the maximum weight bearing on the tip in kilograms. The measuring range is 0.05 to 14 kg, with a precision of ±0.04 kg according to the manufacturer. The output value is the arithmetic mean of a certain number of measurements and their coefficient of variation. According to the manufacturer, the device can be used with a specific support or free hand.

The dynamometer used for this study was equipped with a 15-mm diameter tip. A plastic plate (70 × 100 mm) 20 mm behind and parallel to the surface of the measuring tip was added to standardize the penetration depth of the measuring tip into the udder tissue. The unit was programmed to a threshold of 0.3 kg and to display mean and coefficient of variation of 5 consecutive measurements. Values with a coefficient of variation exceeding 10% were discarded and the measurement repeated. All measures were carried out free hand.

Before the actual measurements, 7 investigators were trained to use the dynamometer in the following manner. Based on information gathered from the manufacturer and scientific literature pertaining to measurements of fruits, a draft standard operating procedure (SOP) was written. The 7 investigators conducted the measurements using the draft SOP and when disagreement occurred, they reviewed the definition until agreement was reached. Four experiments were conducted to determine inter-investigator repeatability (experiments 1 and 2), to quantify effects of location within a given quarter and between quarters (experiment 3), and to compare measurements of udder firmness and teat distances before and after milking (experiment 4).

In experiment 1, the following criteria were added to the SOP. The cow had to stand with all 4 legs on a level surface during the whole measurement. The measuring tip had to be pushed against the surface of the udder at a right angle. Firmness measurements were conducted in the middle of the left hind quarter. In all cases, both investigators used the same dynamometer and recorded the firmness measurements independently within 2 ± 1 min. Because inter-investigator repeatability was low, the SOP was modified and a second experiment was conducted.

In experiment 2, the measuring point was marked with an animal marker pen (Raidex GmbH, Dettingen, Germany) in the middle of the left hind quarter to ensure that both investigators conducted the measurement at a consistent location. It was also added to the SOP that all 4 edges of the supporting plate had to touch the udder surface to standardize penetration depth. Furthermore, if the cow had shifted one of her legs before both investigators concluded their measurement, both measurements had to be repeated. In all cases, both investigators used the same dynamometer and recorded the measurements independently within 2 ± 1 min.

In experiment 3, the criteria established in experiment 2 were used. To estimate the effect of the measuring location within a given quarter and between different quarters within 1 udder, udder firmness was measured in 6 different locations. To determine firmness differences within 1 quarter, 198 measurements each were carried out in the middle, lower, and upper third of the left hind quarter (Figure 1). To study differences in firmness between different quarters, the firmness of each quarter was measured independently, but at the same level in 56 cows. Measuring points were marked with an animal marker pen before measuring started. Firmness measurements were carried out within 2 ± 1 min by the same investigator using the same dynamometer.

To compare udder firmness and teat distances before and after milking in relationship to milk yield, 80 cows were enrolled in experiment 4. Both udder firmness and teat distances were measured on 2 d before and after the evening milking. Measurements of teat distances and udder firmness were conducted by the same 2 independent investigators. The first measurements were conducted in the barn 1 h ± 30 min before milking, whereas the second measurements were conducted directly after milking in the milking parlor. As in experiment 2, the measuring point was marked in the middle of the left hind quarter to ensure a consistent location. Teat distances between the front, hind, left, and right teats were determined with a 300-mm sliding caliper (Conrad Electronic SE, Hirschau, Germany). The measuring point was the opening of the teat canal. Values were recorded with an accuracy of ± 0.5 cm. The sum of the 4 measured teat distances within an udder was defined as the total teat distance (TTD). All measurements were carried out within 2 ± 1 min by the same investigator using the same dynamometer and sliding caliper. Milk yield per cow and milking were recorded in the parlor.

Data were entered into Excel (version 2010; Microsoft Corp., Redmond, WA) and statistical analyses were performed with SPSS for Windows (version 20.0; SPSS Inc., Munich, Germany). The correlation and the difference between 2 investigators before and after marking a measuring point were investigated using Pearson correlation and paired t-test, respectively. In experiment 1 and 2, inter-investigator repeatability for an individual pair of investigators was only calculated if a minimum of 30 paired observations were documented. The inter-investigator variation was calculated by dividing the difference between the udder firmness measurements of both investigators (|F2 – F1|) by the firmness measured by investigator 2 (F2; Schirmann et al., 2009). The results were read in percent. The interoperator agreement index (IAI) was calculated as follows: IAI = 1 – {(|XA – XB|)/[(XA + XB)/2]} (van der Vlugt-Meijer et al., 2006; White et al., 2008). Comparison of clinical measurements with a correlation coefficient can be inappropriate. Therefore, the agreement between observers was tested and analyzed with a Bland-Altman plot (Bland and Altman, 1986). The effect of the different investigators on udder firmness values was evaluated in a linear mixed-model ANOVA with repeated measurements. The random effect of cows was included in this model. Moreover, the diagonal covariance structure was used, because it resulted in the model with lowest Akaike information criterion value. Post hoc comparison was performed with the least significant difference test. Comparing the firmness values measured in different locations, the correlation coefficient was determined for each possible combination and a repeated-measures ANOVA was performed. The least significant difference test was used for post hoc comparison. The correlation and differences of TTD between 2 investigators were investigated using Pearson correlation and a paired t-test, respectively. The TTD and udder firmness measurements of both investigators were averaged to compare values before milking and after milking. The association between udder firmness, TTD, and milk yield were determined using Pearson correlation. All values reported are least squares means ± standard error. The significance level was set at $P < 0.05$.

Eighty cows were enrolled in the experiments. Nine cows and 1 cow were excluded within the first week because of a positive California mastitis test and 1 case of clinical mastitis, respectively. In total, 2,838 udder firmness measurements were documented.

Three pairs of investigators had more than 30 paired observations in the first and second experiment and were analyzed separately (i.e., pairs A, B, and C). In experiment 1 (275 paired observations), the correlation coefficient was 0.80 ($P < 0.001$) and the mean deviation (0.02 ± 0.21 kg) was not significant ($P = 0.15$). For investigator pairs A and B, however, the firmness measurements differed (Table 1). The Bland-Altman plot (Figure 2) illustrated a mean disagreement between the investigators close to 0, indicating good accuracy of the measurements. Greater disagreement was observed for higher means of firmness measurements. The average inter-investigator variation was 20.0%. The IAI for all measurements within experiment 1 averaged 0.81 ± 0.16 (Table 1). These results demonstrate that repeatability of the udder firmness measurement as implemented according to the SOP was not sufficient in experiment 1. The linear mixed-model ANOVA proved that the individual pair of investigators had an effect on the level of disagreement between measurements (P = 0.001). Additionally, an effect of the number of measurements within 1 cow could be shown (P = 0.008), whereas the influence of the individual cow on the disagreement was almost negligible (P = 0.043).

A modified SOP was established in experiment 2 to improve the inter-investigator repeatability. The penetration depth and the measuring point were defined and measurements disturbed by movements of the cow were excluded. Because of this last exclusion criterion, 9.3% of the measurements had to be repeated. As in the first experiment, pairs A, B, and C were analyzed separately. All correlation coefficients increased considerably (Table 1). The t-test did not demonstrate disagreements for any pair of investigators. The mean deviation within pair A, B, and C decreased in comparison to experiment 1 (Table 1). Considering all paired observations (n = 634), the correlation improved (r = 0.94, P < 0.001) and the mean deviation decreased (0.005 ± 0.12, P = 0.323) compared with experiment 1. Again, the Bland-Altman plot (Figure 2) illustrated a mean disagreement between the investigators close to 0. In contrast to experiment 1, disagreement did not increase with higher firmness and residuals were equally distributed between different firmness values. Overall, the average inter-investigator variation decreased to 11.3%. The mean IAI was 0.89 ± 0.10 for all measurements and higher in comparison to experiment 1. Differences between individual pairs of investigators were negligible (Table 1). Compared with experiment 1, the effects of the individual cow (P = 0.77) and the investigators (P = 0.047) on the disagreement between both investigators were reduced and can be considered as barely existent. The effect of the number of measurements performed on a given cow did not change (P = 0.008) after modifying the SOP. The decreased effect of the investigator can be explained with the standardization of the penetration depth. We assume that the effect of the number of measurements was caused by a familiarization of the cow with the measuring procedure and, therefore, cannot be influenced by procedural changes as implemented between experiment 1 and 2. In conclusion, udder firmness measurement using the Penefel DFT 14 can be conducted with high inter-investigator repeatability following the experimental design of experiment 2. Previous studies also using an extramammary udder firmness measurement (Phillips, 1954; Tucker et al., 2007, 2009) did not validate the measuring device or controlled the repeatability of measurements. An SOP was not provided in those previous studies;

however, Tucker et al. (2007, 2009) described a marking of the measuring point and defined the penetration depth of their device.

Experiment 3 was carried out to determine firmness gradients within the left hind quarter. Firmness differed between the 3 locations (n = 196, $P < 0.001$). Average firmness of the upper and lower measuring point was 15.5% lower (0.69 ± 0.29 kg) and 21.1% higher (1.01 ± 0.52 kg) compared with values measured at the middle measuring point (0.86 ± 0.39 kg). The correlation coefficients varied from 0.52 (lower to upper measuring point, $P < 0.001$) to 0.69 (middle to lower measuring point, $P < 0.001$). One explanation for the pressure distribution within a quarter is Pascal's law, which proves that, in fluids, firmness is highest at the lowest level. We also assume that factors such as distension of the udder tissue, the shape of ligaments, or alterations of the tissue influence the firmness of the udder. Regardless of the reasons, our data emphasize the importance of a defined measuring point for a repeatable measurement as described by Tucker et al. (2007). In the second part of experiment 3, all 4 quarters were measured at the same level. Firmness values differed significantly ($P < 0.05$), except between the hind quarters ($P = 0.234$). Firmness values within both front quarters were lower than in the 2 hind quarters ($P < 0.001$). The correlation coefficients varied between 0.632 (left hind to right front quarter, $P < 0.001$) and 0.81 (right to left front quarter, $P < 0.001$). This is in agreement with older findings on milk yield and intramammary udder pressure (Kitts et al., 1963; Graf and Lawson, 1968). Both studies found that intramammary pressures measured in the hind quarters were higher compared with the front quarters. According to Lawson and Graf (1968), udder pressure was 18% higher in the hind quarters, which is similar to our firmness data (16%). It is well known that milk yield of the hind quarters is also higher compared with the front quarters (Tančin et al., 2006). Therefore, a relationship between pressure (firmness) and difference in milk yield between front and hind quarters is plausible. According to hydrostatic principles, a higher volume of fluid causes a higher pressure on the wall of an elastic body. To achieve comparable firmness values, measurements should be carried out at the same quarter and at the same level. Implementation of an exact SOP is recommended.

In experiment 4, udder firmness and distances between teats were measured before and after milking by 2 independent investigators and related to milk yield. The inter-investigator repeatability for the TTD measurements was assessed first. The correlation coefficient was 0.98 (n = 307, $P < 0.001$) and the mean difference between the TTD measured by 2 investigators was 0.06 ± 2.12 cm ($P = 0.62$). In 96.1% of all measurements, differences of TTD were 4 cm or less. Overall, inter-investigator repeatability for the TTD was excellent (IAI = 0.96 ± 0.04) and an independence of TTD and investigator was demonstrated. Comparing values before and after milking, both, firmness and TTD decreased in 91.5% of the udders. Mean TTD before milking was 42.6 ± 9.4 cm and after milking 35.2 ± 8.7 cm (i.e., a 17.6% decrease). In comparison the firmness decreased from 0.89 ± 0.32 kg before to 0.52 ± 0.15 kg after milking (i.e., a 36.5% decrease). This observation differs considerably from previous studies (Graf and Lawson, 1968), demonstrating a decrease in pressure from 34.3 ± 0.86 mm of Hg before to 3.1 ± 0.27 mm of Hg after milking (i.e., a 91% decrease). This disagreement can be explained by the different methods used. Whereas we used a dynamometer and measured from the outside, Graf and Lawson (1968) cannulated the udder and measured an intramammary pressure. It is plausible that after milking intramammary pressure decreases to almost zero, as no fluid is in the mammary gland. With a dynamometer, however, udder firmness as a function of the tissue firmness and the amount of milk within the udder is being measured. A direct comparison of absolute values with previous studies (Tucker et al., 2007, 2009) also measuring the force required to indent the udder tissue is not meaningful because of different diameters of the tips.

The relationship between firmness change and milk yield ($r = 0.42$, $P < 0.001$) demonstrated in our experiment is consistent with the results described in the study mentioned above ($r = 0.52$, $P < 0.01$). Furthermore, relationships existed between milk yield and TTD changes from before to after milking ($r = 0.54$, $P < 0.001$) and between firmness and TTD changes ($r = 0.39$, $n = 153$, $P < 0.001$) from before to after milking, respectively. Despite these significant relationships the predictive value of udder firmness or TTD for milk yield is limited. Comparing firmness values of the same udder at different times, higher firmness indicates a larger filling of the udder and a greater time span to the last milking. This substantiates the importance of measuring udder firmness every day at the same time.

Our data provide evidence that the dynamometer, although imperfect, is a valuable device to measure udder firmness. Inter-investigator repeatability is high when an SOP is implemented to minimize the influence of potential confounders such as investigator, location, and cow. It is recommended to carry out measurements at the same quarter and the same level with a marked measuring point and defined penetration depth. Measurements should be taken at the same time after milking to compare firmness values of different days.

The relationship between extramammary udder firmness and intramammary udder pressure remains unclear and warrants further studies to understand how udder firmness reflects pressure conditions inside the udder. Nevertheless, a dynamometer can be useful in research and commercial applications. Relevant research questions that could benefit from such a tool include relationships between udder firmness and pain, prevalence of edema, inflammation-mediated alterations in the tissue, or prevalence of milk leakage after dry-off.

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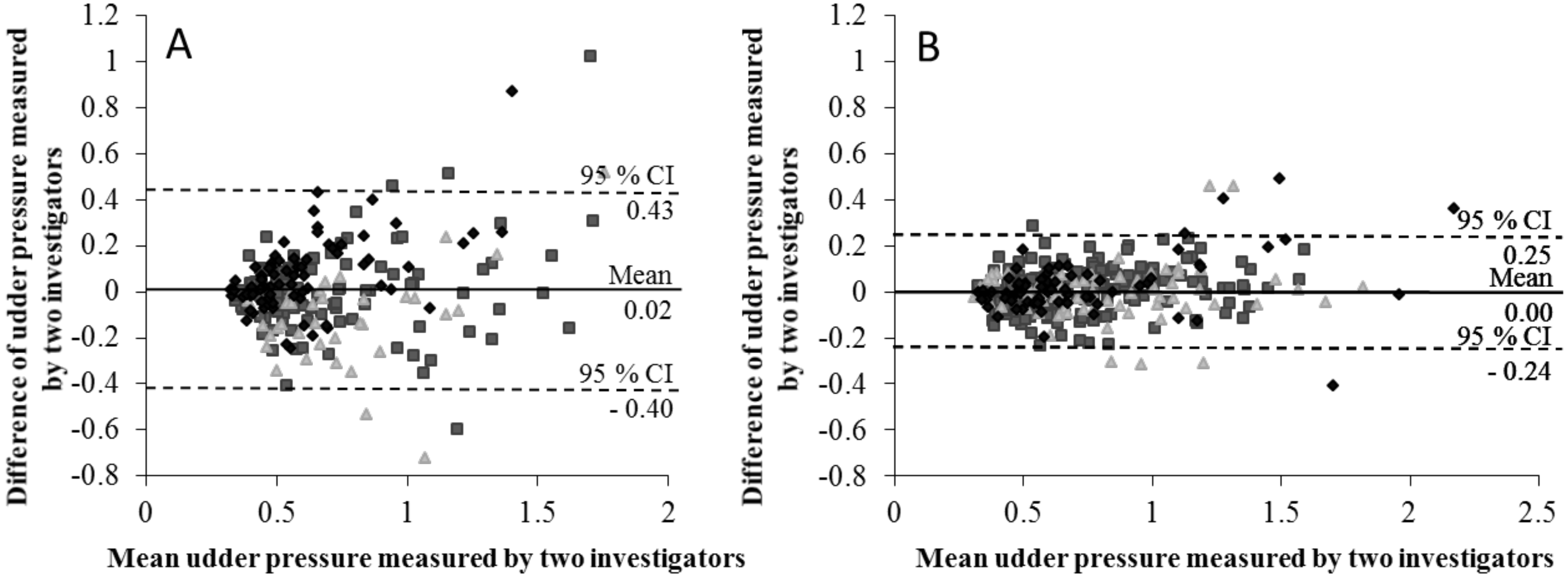
Figure 1. Dynamometer (Penefel DFT 14) and udder with marked measuring points (experiment 3).

Table 1. Inter-investigator repeatability in experiment 1 and 2 for three pairs of investigators with at least 30 paired observations.

Investigator	Paired observations	Coefficient of correlation		Difference of paired measurements		Agreement index (AI)	Inter-investigator variation (%)
		r	*P* - value	Mean ± SD (kg)	*P*-value	Mean ± SD (kg)	
Experiment 1							
Pair A	74	0.83	< 0.001	0.07 ± 0.17	0.001	0.82 ± 0.15	20.4
Pair B	36	0.80	< 0.001	-0.12 ± 0.21	0.002	0.76 ± 0.19	20.5
Pair C	102	0.85	< 0.001	0.00 ± 0.20	0.961	0.83 ± 0.15	19.7
Experiment 2							
Pair A	68	0.95	< 0.001	0.02 ± 0.13	0.133	0.90 ± 0.09	10.5
Pair B	76	0.94	< 0.001	0.00 ± 0.12	0.792	0.91 ± 0.09	9.3
Pair C	190	0.95	< 0.001	0.00 ± 0.09	0.771	0.88 ± 0.09	11.6

Figure 2. Differences between udder firmness' measured by two investigators versus the mean of both measurements (in kg). Data are shown for experiment 1 (A) and experiment 2 (B) and are divided according to the pair of investigators (pair A ■; pair B ◆; pair C ▲).



b. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yield

Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yield

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ABSTRACT

Sudden dry-off is an established management practice in the dairy industry. But milk yield has been increasing continuously during the last decades. There is no information whether the dry-off procedure, which often results in swollen and firm udders, causes stress, particularly in high-producing dairy cows. Therefore, we evaluated the effect of a sudden dry-off on extramammary udder pressure and the concentration of fecal glucocorticoid metabolites (i.e., 11,17-dioxoandrostane, 11,17-DOA) as an indirect stress parameter. Measurements were carried out within the last week before dry-off and until 9 d after dry-off considering 3 groups of milk yield (i.e., low: < 15 kg/d, medium: 15–20 kg/d, and high: > 20 kg/d). Udder pressure increased in all yield groups after dry-off, peaked at d 2 after dry-off and decreased afterwards. Pressures were highest in high-yielding cows and lowest in low-yielding cows. But only in high-yielding cows was udder pressure after dry-off higher than before dry-off. Baseline 11,17-DOA concentrations depended on milk yield. They were highest in low-yielding (121.7 ± 33.3 ng/g) and lowest in high-yielding cows (71.1 ± 30.0 ng/g). After dry-off, 11,17-DOA increased in all yield groups and peaked at d 3. Whereas in medium- and high-yielding cows 11,17-DOA levels differed significantly from their respective baseline during the whole 9-d measuring period, low-yielding cows showed elevated 11,17-DOA levels only on d 3 after dry-off. However, especially the increase in 11,17-DOA after dry-off between the 3 yield groups was considerably different. Mean 11,17-DOA increase from baseline to d 3 was highest in high-yielding cows (129.1%) and considerably lower in low-yielding cows (40.1%). The highest fecal 11,17-DOA concentrations were measured on d 3 after dry-off, indicating that the stress was most intense on d 2, which is due to an 18-h time lag; at about the same time, udder pressure peaked. Our results showed a negligible effect of a sudden dry-off on low-yielding cows. High-yielding cows, however, faced high extramammary pressures and increased glucocorticoid production. Considering animal welfare aspects, a review of the current dry-off strategies might be warranted.

Keywords: dry-off, fecal glucocorticoids, stress, udder pressure

INTRODUCTION

Animal welfare in farm animals has become a major public concern (von Keyserlingk et al., 2009). Recently, numerous studies were conducted to assess potential stressors in cows, such as the transition period and its effects on postpartum health (Huzzey et al., 2011), different lactation stages (Fukasawa et al., 2008), weaning and separation of calf and dam (Loberg et al., 2008), and vaginal examinations (Pilz et al., 2012).

Although a sudden dry-off is a common management practice on commercial dairy farms (Dingwell et al., 2001), there is a dearth of science-based information on the question whether the dry-off procedure causes stress, particularly in high-producing dairy cows. Recently, studies analyzed the effect of different feeding strategies during dry-off on metabolic parameters (Odensten et al., 2005) and the influence of herd management strategies during the dry period on the prevalence of IMI (Green et al., 2007). Only one study, however, addressed animal welfare during dry-off (Tucker et al., 2009). The authors investigated the effect of drying-off dairy cows on their lying behavior, time budget, and udder characteristics (e.g., udder firmness). That study focused on the comparison of 2 different dry-off strategies (feed restriction vs. reduced milking frequency) in late-lactating cows. Consequently, milk yield prior to dry-off was low (9.6 ± 2.9 kg of milk/d). In many areas throughout North America and in most European countries, however, farmers follow common recommendations featuring an abrupt cessation of milking at the end of lactation (Newman et al., 2010). Without feed restriction (Tucker et al., 2009) or intermittent cessation of milking (Odensten et al., 2005), milk yield at the last day of milking is quite a bit higher. A decrease in milk production during the last week before dry-off by 22 to 47% and 3.7 to 10.4% was demonstrated in cows with intermittent and abrupt cessation, respectively (Dingwell et al., 2001). Dingwell et al. (2001) examined Ontario DHI records and discovered an average milk yield at the time of dry-off of 16.6 kg per day. Furthermore, it was demonstrated that about 20% of the cows had a daily milk yield exceeding 22 kg at the time of dry-off. In the 1990s, even peak lactation rarely exceeded 25 kg per day (Schutz et al., 1990). This increase in milk yield during the last 20 yr warrants recognition, especially because management procedures hardly changed.

Frequently, dairy farmers have reported increased vocalization, reduced feed intake, and prolonged standing times after dry-off in addition to apparent udder swelling (S. Bertulat, unpublished data). Such behavioral changes indicate elevated stress levels and can be signs of discomfort and pain (Anil et al., 2002; Rutherford, 2002). Similar behavior during the dry-off period in cows with restricted feed rations was described by Valizaheh et al. (2008). Those authors associated increased vocalization with the experience of distress during the dry-off procedure. Due to their study design, however, hunger was the suspected reason.

Nevertheless, considering this evidence, we hypothesized that a relationship exists between dry-off and elevated stress. The pressure within the udder after dry-off, caused by slowly decreasing milk secretion and cessation of milking, might cause discomfort and consequently create stress accompanied by behavioral changes. This assumption is substantiated by findings in women that suffer regularly from breast soreness and pain after a short weaning duration (Neighbors et al., 2003).

Unfortunately, an objective quantification of pain in animals is currently not possible (Anil et al., 2002; Rutherford, 2002). But estimating stress as a result of pain or discomfort by measuring cortisol and cortisol metabolites is an established method (Anil et al., 2002; Rutherford, 2002).

Blood is the most common sample material to measure cortisol (Echternkamp, 1984) and the analytical method is well proven (Neher, 1958). For the determination of low and chronic stress levels, the use of blood samples, however, has certain limits (Cook et al., 2000). Cortisol concentration increases in the blood immediately after stress exposure. Therefore, mere handling, restraining cows, and puncturing the blood vessel cause an increase in blood cortisol (Echternkamp, 1984; Hopster et al., 1999) and confound the resulting cortisol concentrations.

Because limitations of blood cortisol measurements have become evident for several research topics, various alternatives have been investigated. Analytical methods using feces (Morrow et al., 2002), saliva (Negrão et al., 2004), milk (Fukasawa et al., 2008), hair (Comin et al., 2011), and urine (Pompa et al., 2011) have been shown to be advantageous for effectively measuring glucocorticoid metabolites as equivalents of stress. For our study, the determination of cortisol in the feces seemed to be most promising. Fecal samples can be obtained without stressful restraining and manipulating the cow and measuring fecal cortisol metabolites offers a feedback-free method that has proven to be useful for the evaluation of chronic stress (Möstl and Palme, 2002). A direct relationship between fecal glucocorticoid metabolites, blood cortisol, and adrenal activity has been demonstrated (Morrow et al., 2002).

The most important glucocorticoid metabolites measured in cow feces are 11,17-dioxoandrostanes (11,17-DOA; Palme and Möstl, 1997; Palme et al., 1999; Möstl et al., 2002). 11,17-Dioxoandrostane is measured utilizing an enzyme immune assay developed by Palme and Möstl (1997) and validated, for example, by Morrow et al. (2002). Several studies (e.g., Palme et al., 2000; Morrow et al., 2002; Palme, 2005) described a time lag of 8 to 16 h between an increase in blood cortisol concentration coinciding with the stressor and an elevated concentration of fecal 11,17-DOA.

The overall objective of this study was to evaluate the stress caused by drying-off dairy cows. Specifically, we set out (1) to quantify the changes of fecal 11,17-DOA concentration and udder pressure after a sudden dry-off, (2) to determine the effect of milk yield prior to dry-off on the fecal 11,17-DOA concentration and udder pressure, and (3) to evaluate the relationship between udder pressure and fecal 11,17-DOA concentration in the early dry period.

MATERIALS AND METHODS

Cows, Housing and Feeding

This study was carried out on a commercial dairy farm in Brandenburg, Germany from April 2011 to August 2011. A total of 80 healthy, late-lactating (343 ± 39 DIM; mean ± SD) and pregnant (49 ± 18 d before calving) Holstein-Friesian dairy cows were included in the experiment. All cows were managed according to the guidelines set by the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (Hellmann and Radeloff, 2000). The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee. Cows were housed in a straw-bedded freestall barn and fed a roughage mix delivered twice per day at 0830 and 1700 h. Late-lactating cows received (on a DM basis) 54.3% corn silage, 25.4% haylage, 16.3% distillers grains, 0.9% corn, 0.8% soy, 2.0% rapeseed, and 0.3% basic mineral mix. Dry cows received (on a DM basis) 64.7% haylage, 32.8% corn silage, 1.7% hay, 0.3% corn, and 0.5% mineral mix. Concentrate was available for each lactating cow individually via an automatic feeder (35% wheat, 35% rye, 24% rapeseed extract, 5% soy, 1% oil, on a DM basis). All cows had access to fresh water in their pen.

Lactating cows were milked twice daily in a 2 × 8 Herringbone milking parlor (Alpro System; DeLaval, Tumba, Sweden) from 0600 to 0900 h and 1600 to 1900 h. Cows were dried off once per week based on their estimated calving date (7 wk before calving) or daily milk yield (< 5 kg milk per day). All cows remained in the late-lactation pen until their last milking, received the same diet, and were milked twice daily. On the day of dry-off after the evening milking, cows scheduled for dry-off were treated with 150 mg of cefquinome (Cobactan DC; Intervet Deutschland GmbH, Unterschleißheim, Germany) administered into the teat canal and were transferred to the dry cow pen.

General Health Status and Milk Yield

Cows were enrolled 7 days before dry-off (54.7 ± 6.9 d to calculated calving date) and followed up for 9 d after drying off (Figure 1). A general examination was performed, including heart and breathing rate, rectal temperature, and rumination. Lameness was scored on a 5-point scale according to Sprecher et al. (1997). Udder quarters were palpated to diagnose pathological conditions (warmness, swelling, nodules, and changes in udder firmness). Additionally, the milk was visually examined on a dark surface. Examinations were repeated 1 d prior to dry-off and a California mastitis test was performed. Cows with signs indicative of mastitis, udder or teat lesions, alterations of the udder tissue, or cows with less than 4 functional quarters were excluded. Cows suffering from infectious or metabolic disease or lameness (i.e., lameness score > 3) were also excluded. General and udder examinations were repeated 9 d after dry-off, when the cow completed the study. Cows were retrospectively withdrawn if any of the signs mentioned above were observed.

Cows were assigned to 1 of 3 groups according to their average milk yield during the last 7 d before dry-off. High-yielding cows (n = 25) produced more than 20 kg of milk per day, medium-yielding cows (n = 29) 15 to 20 kg per day, and low-yielding cows (n = 26) less than 15 kg. Milk yield per cow per milking was recorded by a milk meter integrated into the milking parlor and documented.

Relevant cow data (i.e., age, parity, and DIM) were downloaded from the on-farm computer system. Test-day information (i.e., SCC, fat percentage, protein percentage, lactose percentage, and milligrams of urea per liter) was provided by the local DHIA (Landeskontrollverband Brandenburg e.V., Waldsieversdorf, Germany). Cows were reviewed for all relevant events (e.g., disease, culling, and euthanasia) up to 21 d after calving. Hourly ambient temperature (AT) and relative humidity (RH) data were downloaded from the local weather station 15 km from the farm. The temperature-humidity index (THI) was calculated according to the equation reported by Kendall et al. (2008): $THI = (1.8 \times AT + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times AT - 26)]$. Hourly values were averaged for each trial day individually.

Udder Pressure, Milk Leakage and Intramammary Infections

Udder pressure was measured using a hand-held dynamometer (Penefel DFT 14; Agro Technologie, Forges-les-Eaux, France) equipped with a 15-mm measuring tip and a plastic plate (70 × 100 mm) 20 mm behind and parallel to the surface of the measuring tip, as previously validated by Bertulat et al. (2012). The unit was programmed to a threshold of 0.3 kg and to display mean and coefficient of variation of 5 consecutive measurements. Mean values with a coefficient of variation exceeding 10% were discarded and the measurement repeated. Seven investigators were trained in handling the dynamometer according to a standard operating procedure based on previous recommendations (Bertulat et al., 2012). The penetration depth and the measuring point were defined by the plate attached to the dynamometer and a point marked in the middle of the udder with an animal marker pen, respectively. Measurements confounded by movements of the cow were repeated. Pressure measurements were always conducted by 2 investigators in the middle of the left hind quarter. Both investigators used the same dynamometer and recorded the pressure values independently within 2 ± 1 min. A mean pressure value was calculated based on values from both investigators.

On the day of enrollment and the day before dry-off, measurements were conducted in the barn 1 ± 0.5 h before the evening milking and a second time in the milking parlor directly after milking. During the experiment, udder pressure in dry cows was measured once per day in the dry-off pen. After dry-off, all measurements were carried out at 1400 ± 1 h. In addition to the pressure measurement, udders were visually examined and palpated. Milk leakage (i.e., milk observed dripping from 1 teat or more), signs of IMI, and udder pain (i.e., avoidance behavior during palpation) were evaluated.

Fecal Cortisol Metabolites

Fecal samples from each cow were collected on the day of enrollment, the day of dry-off and day 2, 3, 5, 7, and 9 after dry-off. About 50 to 100 g of feces was obtained manually from the rectum immediately after measuring the udder pressure. Disposable gloves were changed after every cow. According to Palme (2005), 10 to 15 g (equates 8 to 12 mL) of feces from different locations on the glove were filled into fecal sample tubes [Wirtschaftsgenossenschaft deutscher Tierärzte eG (WDT), Garbsen, Germany]. Samples were stored on ice immediately and frozen at −26° C within 2 h after collection.

For the extraction of the fecal glucocorticoid metabolites, samples were thawed at room temperature, stirred, and 0.5 g of feces was dispersed in 5 mL of 80% methanol (Palme and Möstl, 1997). Subsequently, the dispersion was vortexed for 30 min and centrifuged at 3,750 × g for 15 min (Palme et al., 1999). The supernatant was transferred into aliquots of 1.5 mL and stored at −18° C until further analysis. A group-specific enzyme immunoassay (i.e., 11-oxo-etiocholanolone enzyme immunoassay) was carried out to determine the 11,17-DOA concentration (Palme and Möstl, 1997; Palme et al., 1999; Morrow et al., 2002). All samples were analyzed in duplicate. Intraassay and interassay coefficients of variation were calculated. Concentrations are stated in nanograms of 11,17-DOA per gram of fresh feces.

Caused by the time lag between elevated stress level and increased 11,17-DOA concentration, 11,17-DOA concentrations are indicative of stress 12 to 18 h earlier. To avoid confusion between sampling and time of stress experienced, days of fecal sampling were designated with an “F” (e.g., $d2_F$, 3_F, 5_F, 7_F, and 9_F)

Statistical Analysis

Data were entered into Excel spreadsheets (version 2010; Microsoft Corp., Redmond, WA) and statistical analyses were performed with IBM SPSS Statistics for Windows software (version 20.0; IBM Deutschland GmbH, Ehningen, Germany). Homogeneity of the proportion of parity (i.e., first, second, or third-or-higher lactation) and yield group (i.e., low, medium, or high yield) was evaluated with a χ^2 test. The normal distribution of the 11,17-DOA and pressure values was assessed by plotting and visually examining the data and calculating a quantile-quantile (Q-Q) plot.

To summarize 11,17-DOA and udder pressure values measured before dry-off, 3 baseline values were calculated individually for every cow. The 11,17-DOA baseline averaged 11,17-DOA concentrations measured on d -7_F and 0_F. The first udder pressure baseline averaged values measured before milking on d −7 and 0; the second udder pressure baseline averaged pressure values measured after milking. To verify the validity of this approach, the association and difference between 11,17-DOA concentrations of d -7_F and 0_F and between pressure values of d −7 and 0, each before and after milking, were investigated using Pearson's correlation and paired t-test. Pressure values before milking (mean difference ± SD; 0.057 ± 0.34 kg, $P = 0.17$) and after milking (mean difference ± SD; −0.002 ± 0.12 kg, $P = 0.87$) did not differ between d −7 and 0. Also, 11,17-DOA concentrations between d -7_F and 0_F did not differ (mean difference ± SD; −10.3 ± 52.1 ng/g, $P = 0.091$). Thus, an 11,17-DOA baseline, a before milking udder pressure baseline, and an after milking udder pressure baseline were calculated accordingly.

Further analyses were carried out applying a linear mixed-model ANOVA. All mixed-model ANOVA were built according to the model-building strategies described previously (Dohoo et al., 2009). In brief, in a first step, each parameter considered for the mixed model was separately analyzed in a univariate model, including the parameter as a fixed factor (i.e., ordinal parameter) or covariate (i.e., continuous parameter). Only parameters resulting in univariate models with $P \leq 0.2$ were included in the final mixed model. Furthermore, all independent parameters were tested with Spearman's correlation (i.e., ordinal parameter) or Pearson's correlation (i.e., continuous parameter) for collinearity. If 2 parameters showed a high, significant correlation (r > 0.60), only the one resulting in the univariate model with the smaller P-value was used in the final mixed model. This final model was built in a manual backward stepwise manner by removing parameters resulting in $P > 0.05$ until all remaining parameters showed a significant effect. The covariance structure was chosen based on the model with lowest Akaike information criterion value. Post hoc comparison was carried out applying the least significant difference test. The significance level was set at $P \leq 0.05$.

The effect of dry-off on udder pressure values was evaluated in a linear mixed-model ANOVA, considering days as the repeated measure. The effect of yield group as fixed factor and the random effect of cows within yield groups were included in this model. Moreover, the diagonal covariance structure was used. The effects of parity, DIM, age, SCC, and their potential interactions on udder pressure were tested accordingly. Due to an interaction

($P < 0.001$) between days and yield groups we evaluated the within yield group between days effect and the within days between yield groups effect.

The effect of several parameters on the occurrence of milk leakage after dry-off was evaluated in a binary logistic regression model. A conditional backward stepwise manner was selected and significance levels of 0.1 and 0.05 were chosen to exclude and include terms, respectively. According to Peduzzi et al. (1996), we included a maximum of 7 parameters (i.e., yield groups, days after dry-off, udder pressure, parity, SCC, 11,17-DOA concentration, and DIM) and ensured at least 10 cases each with and without milk leakage to obtain reliable estimates. Odds ratios, 95% confidence intervals, and significance levels are reported.

The effect of dry-off on 11,17-DOA values was evaluated in a linear mixed-model ANOVA, considering days as the repeated measure. The effect of yield group and day as fixed factor and the random effect of cows within yield groups were included in this model. The diagonal covariance structure was used. The between groups within day and the within group between days effect was evaluated in the same model. Furthermore, the following parameters were tested as factors (i.e., ordinal data) and covariates (i.e., continuous data): udder pressure of the previous day, yield group, age, parity, DIM, SCC before dry-off, 21-d survival rate (i.e., culling or remaining in the herd), BW, milk leakage, and mean daily THI. Visually examining the 11,17-DOA values, a clear difference existed between baselines and days after dry-off. Therefore, the model was rerun twice covering only baseline values and excluding the baseline, respectively. In the model assessing effects on baseline 11,17-DOA, udder pressure values measured before milking were used. Cows within yield groups were included as random effect and the scale identity covariance structure was used in both ANOVA. Post hoc comparison was carried out applying the least significant difference test.

Baseline values were assigned to 3 equal groups using the percentile function in SPSS to visualize the heterogeneity of yield groups among cows with low, medium, and high baseline values. The time lag between stressor and elevated 11,17-DOA concentration was verified by calculating Pearson's correlation for 11,17-DOA and udder pressure of the same day the fecal sample was obtained and for 11,17-DOA and udder pressure of the day before the sample was obtained.

For better assessment of the variations in 11,17-DOA concentrations, the changes in 11,17-DOA concentrations after dry-off were calculated relative to the individual baselines (i.e., 11,17-DOA_{rel}). These were computed for each individual cow and day using the following formula:

$$11,17\text{-DOArel} = \frac{11,17\text{-DOA} - \text{baseline}}{\text{baseline}} * 100$$

The effect of dry-off on 11,17-DOA_{rel} values was evaluated in a linear mixed-model ANOVA, considering days as the repeated measure. The random effect of cows within yield groups was included in this model and the diagonal covariance structure was used. The effects of days after dry-off, yield group, udder pressure, parity, DIM, and udder pain on 11,17-DOA_{rel} values were tested in this model. The model was rerun to assess the within days between groups effect.

Because 11,17-DOA concentrations for cows after dry-off were not available for a sample size calculation, a post hoc power analysis was performed using the G*Power program (version 3.1.3; University of Düsseldorf, Düsseldorf, Germany) to verify the level of the effect of drying-off on the 11,17-DOA concentration. A post hoc repeated-measures ANOVA between factor analyses model was applied to calculate the power of analysis (1-β) and the effect size (f), accepting a null hypothesis error of 0.05.

RESULTS

Eighty cows in first (n = 31), second (n = 26), and third-or-higher (n = 23) lactation met the inclusion criteria and were enrolled in the study. The distribution of parity was homogeneous between the 3 yield groups (P = 0.21). Four cows had to be excluded from further analyses due to group change in the dry period (n = 2) or due to mastitis (n = 2). A total of 551 fecal samples were collected and analyzed and 1,024 udder pressure measurements were carried out. The intra- and interassay coefficients of variation for the 11-oxo-etiocholanolone enzyme immunoassay were 10.1 and 14.5%, respectively.

The power of analysis for the repeated measurement of 11,17-DOA concentration before and after dry-off in 3 yield groups was 0.9996, with an effect size of f = 0.318. The power of analysis was within the limits set by Cohen (1988) and Prajapati et al. (2010) and the effect size of this study was acceptable (Cohen, 1988). The chance of error in accepting the null hypothesis was 0.04%.

The threshold for heat stress (i.e., THI ≥72) established by Armstrong (1994) was not exceeded during the entire trial period. The mean THI for May, June, July, and August was 58.8 ± 4.6, 63.6 ± 3.2, 64.4 ± 3.1, and 64.1 ± 2.9, respectively. A significant difference in THI between the various months did not exist ($P = 0.097$).

Udder Pressure, Milk Leakage and Intramammary Infections

Udder pressure baseline values before and after milking averaged 0.72 ± 0.24 and 0.48 ± 0.10 kg for low-, 0.95 ± 0.25 and 0.56 ± 0.19 kg for medium-, and 1.01 ± 0.25 and 0.53 ± 0.104 kg for high-yielding cows, respectively. Mean pressure before milking differed between low- and medium- ($P = 0.001$) as well as between low- and high-yielding cows ($P < 0.001$). No difference existed between yield groups after milking ($P = 0.14$).

An overall effect of yield group ($P = 0.001$) and day ($P < 0.001$) on udder pressure could be evaluated in the linear mixed-model ANOVA. The post hoc comparison showed that udder pressure increased in all yield groups ($P < 0.001$) after dry-off and peaked on d 2 (Figure 2). But only in high-yielding cows was udder pressure after dry-off (i.e., d 2) higher than udder pressure measured in late lactation before milking ($P = 0.007$). After d 2, udder pressures declined in all 3 groups; however, baseline pressures measured in late-lactating cows after milking ($P < 0.05$) were not reached within 9 d. Considering the different yield groups, udder pressures after dry-off were highest in high-yielding cows. They differed between high- and low-yielding cows and between medium- and low-yielding cows for 9 (last sampling day) and 7 d after dry-off, respectively ($P < 0.05$). High-yielding cows had a higher udder pressure on d 3 and 4 ($P < 0.05$) compared with medium-yielding cows.

In addition to an effect of yield group and day on udder pressure, an interaction between day and yield group ($P = 0.003$) could be demonstrated. There was no effect, however, of parity ($P = 0.22$), DIM ($P = 0.076$), SCC ($P = 0.084$), or age ($P = 0.12$) on udder pressure after dry-off. The correlation coefficient between SCC and udder pressure after dry-off was −0.226 ($P = 0.042$).

Before dry-off, milk leakage was observed in 2 cows before milking; both were high yielding. After dry-off, 49 events of milk leakage in 27 different cows (33.8%) were recorded. Eight out of these 27 cows had milk leakage on more than 1 day after dry-off. The probability of the occurrence of milk leakage after dry-off was significantly associated with yield group (*P* < 0.001), parity (*P* = 0.006), and udder pressure (*P* = 0.016). Cows with a high udder pressure were more likely to show milk leakage than cows with low pressure values (odds ratio = 3.35; 95% CI = 1.26–8.93; *P* = 0.016). Additionally, animals in their third-or-higher lactation displayed 3.53-fold higher odds of having milk leakage than cows in first lactation (95% CI = 1.42–8.80; *P* = 0.007). No difference existed between cows in first and second lactation (*P* = 0.73). Furthermore, high-yielding cows were 5.07 times more likely to show milk leakage than low-yielding cows (95% CI = 1.83–14.04; *P* = 0.002; Figure 3). A difference between low- and medium-yielding cows was not significant (*P* = 0.69). The concentrations of 11,17-DOA (*P* = 0.70), DIM (*P* = 0.99), and SCC (*P* = 0.43) were not significantly associated with the likelihood of the occurrence of milk leakage. No difference was observed in the probability of milk leakage between d 1 and 9 after dry-off (*P* = 0.66).

Two cows developed clinical mastitis (i.e., firm, heated, and reddened quarter; abnormal milk with clots and pus) during the first 9 d after dry-off. Both cows were low yielding and did not show any signs of udder pain before the day of diagnosis (i.e., 4 and 6 d after dry-off) and no milk leakage. However, these cows were not included in the analyses described above.

Fecal Cortisol Metabolites

11,17-Dioxoandrostane baseline concentrations ranged from 30.0 to 184.9 ng/g. These baseline concentrations were affected by yield group (*P* < 0.001) and udder pressure before milking (*P* = 0.014). A difference was observed between low- and medium- (*P* = 0.003), low- and high- (*P* < 0.001), and medium- and high-yielding cows (*P* = 0.013). Interestingly, most high-yielding cows had low and most low-yielding cows had high 11,17-DOA baseline concentrations (Figure 4). Age, parity, and SCC had no effect on 11,17-DOA baselines. They were excluded from the final model, because they resulted in univariate models with $P \geq 0.2$. Furthermore, no effect was observed of DIM on the baseline 11,17-DOA concentration (*P* = 0.53).

After dry-off 11,17-DOA concentrations up to 412.39 ng/g were measured. For all cows, concentrations of 11,17-DOA increased significantly from d 2_F to 3_F, peaked on d 3_F, and decreased again subsequently (Table 1). In high- and medium-yielding cows, 11,17-DOA increased from baseline to d 2_F ($P < 0.001$) and in medium-yielding cows, a second increase occurred from d 2_F to 3_F ($P = 0.004$). In both yield groups, 11,17-DOA concentrations decreased from d 3_F to 5_F ($P < 0.05$) and remained at an elevated concentration compared with the baseline until d 9_F ($P < 0.05$). Subsequently, no differences were found between d 5_F, 7_F, and 9_F ($P > 0.05$) in medium- and high-yielding cows, respectively. In low-yielding cows, however, only 11,17-DOA concentration on d 3_F differed from the baseline ($P = 0.005$). In this group, there was neither a difference between baseline 11,17-DOA concentrations and concentrations measured on d 2_F, 5_F, 7_F, and 9_F, nor between 11,17-DOA concentrations measured on any day after dry-off ($P > 0.05$).

Besides the effect of day ($P = 0.005$) on the 11,17-DOA concentration after dry-off, the concentration was furthermore affected by udder pressure ($P = 0.05$). However, considering only the days after dry-off, 11,17-DOA concentrations did not differ ($P = 0.83$) between the 3 yield groups. The average 11,17-DOA concentration after dry-off (d 2_F–9_F) was 143.27 ± 65.0, 139.25 ± 70.1, and 128.2 ± 77.5 ng/g for low-, medium-, and high-yielding cows, respectively. The univariate models for age, DIM, BW, and THI were again not significant and these parameters were excluded from the final model. Also, no effect was observed of parity ($P = 0.39$) or milk leakage ($P = 0.26$) on the 11,17-DOA concentration after dry-off.

The 3 different yield groups showed diverging increases of 11,17-DOA concentrations after dry-off (11,17-DOA_{rel} values). Both the yield group ($P = 0.01$) and the experimental day ($P < 0.001$) had an effect on the change in 11,17-DOA concentration (Table 2). Although 11,17-DOA_{rel} values of low- and high-yielding cows differed ($P = 0.003$), no difference between low- and medium- ($P = 0.074$) and medium- and high-yielding cows ($P = 0.12$) was found. Within days, high-yielding cows had higher 11,17-DOA_{rel} values than low-yielding cows ($P < 0.02$) on all days after dry-off. Parity, DIM, and udder pain had no effect ($P > 0.05$) on 11,17-DOA_{rel} values.

Interestingly, udder pressure and 11,17-DOA concentrations showed a similar curve, but with a time lag of 1 d (Figure 5). The correlation coefficient between both parameters measured on the same day was 0.114 ($P = 0.027$). Considering the time lag and correlating 11,17-DOA values with the udder pressure measured on the previous day, the correlation coefficient increased slightly to 0.158 ($P < 0.001$).

Cows culled (i.e., slaughtered, euthanized, or died) within 21 d after calving (n = 11) due to metabolic disease or mastitis showed higher 11,17-DOA concentrations before (culled cows = 108.78 ± 41.4 ng/g; survived cows = 93.21 ± 37.1 ng/g; $P = 0.021$) and after dry-off (culled cows = 162.76 ± 83.4 ng/g; survived cows = 132.73 ± 68.0 ng/g; $P = 0.043$) compared with cows remaining in the study.

DISCUSSION

Milk yield has been increasing continuously since the beginning of the 20th century (Lucy, 2001). Management practices implemented to dry-off dairy cows, however, have stayed the same except for the approval of new drugs to decrease the risk of infection (e.g., antibiotic drugs and teat sealant; Berry and Hillerton, 2002; Godden et al., 2003). Considering the increased milk yield per cow, one might speculate that drying-off cows with considerable milk production could pose an animal welfare issue. To our knowledge, this is the first study evaluating the influence of milk yield on stress hormones directly after dry-off and correlating high milk yield in late-lactating cows with high extramammary udder pressure and elevated stress levels.

Udder Pressure and Milk Leakage

Two udder pressure baselines (i.e., before and after milking) were established in late-lactating cows before dry-off. The udder pressure baseline evaluated after milking was lower in all yield groups compared with before milking. This confirms data recently reported by Bertulat et al. (2012), who demonstrated a similar decrease in udder pressure due to milking. In the current study, udder pressure before milking differed considerably between yield groups and high milk yield was associated with high pressure values before milking. This observation underlines that udder pressure depends on the milk volume in the udder. A similar relationship between high milk yield and high intramammary udder pressure in lactating cows was reported by Tucker et al. (1961) and Graf and Lawson (1968).

After milking, udder pressures were similar in all cows irrespective of their milk yield. While the intramammary udder pressure is solely determined by the amount of milk within the udder (Tucker et al., 1961), extramammary udder pressure is also influenced by the firmness of the udder tissue (Bertulat et al., 2012). As the intramammary udder pressure after milking is negligible because all milk has been withdrawn, the remaining extramammary pressure measured after milking corresponds with the firmness of the udder tissue. Our results indicated that the firmness of the udder tissue was similar in all cows regardless of their milk yield. This observation warrants further research on the diagnostics of udder ailments.

Data on udder pressure after dry-off are sparse. Overall, in our study, the development of udder pressure after dry-off with an initial increase, a peak within 2 d, and a subsequent decrease was similar to results published previously (Tucker et al., 2009). A direct comparison of udder pressure values measured in both studies is not possible, however, because of different measuring devices, resulting in values with varying units. Furthermore, Tucker et al. (2009) compared cows with different feed rations and milking frequencies. Consequently, the milk yield averaged 9.3 ± 1.0 kg/d, which is comparable only to our low-yielding cows.

Anecdotal evidence from the field suggests that especially high-producing cows show firm and swollen udders. To our knowledge, however, studies are not available describing a relationship between milk yield and extramammary udder pressure after dry-off. Our study showed that udder pressure after dry-off was highest in high-yielding cows and lowest in low-yielding cows. The correlation between milk yield and udder pressure on d 2 ($r = 0.411$; $P < 0.001$) was within the range described by Graf and Lawson (1968) for milk yield and intramammary udder pressure. A recent study (Tucker et al., 2009) demonstrated a significantly lower udder pressure ($P \leq 0.012$) in cows with a lower (i.e., 8 kg of DM/d) compared with a higher feeding treatment (i.e., 16 kg of DM/d). The cows with 8 kg of DM/d also produced less milk ($P = 0.016$). The lower udder pressure and lower milk yield in cows with 8 kg of DM/d supports our results. The increase in udder pressure shown by Tucker et al. (2009) between pressure values measured for unmilked udders before dry-off and 2 d after dry-off was similar to our findings in low- and medium-yielding cows (average increase of 12.8%). The differences between before and after dry-off, however, in high-yielding cows (i.e., > 20 kg) were considerably higher. Therefore, we suspect that the higher milk secretion of high-yielding cows leads to a greater increase in udder pressures after dry-off.

In our study, udder pressure was measured once daily for 9 d after dry-off. For the whole period, pressure values in all 3 yield groups exceeded the baseline values after milking. This is in contrast to Tucker et al. (2009), who demonstrated that baseline pressure values were reached within 4 days after dry-off. Probably this discrepancy can be explained by the different milk yield of the cows enrolled (9.3 ± 1.0 vs. 17.6 ± 6.7 kg/d) and the different measuring methods. According to Hurley (1989), the total milk volume in udders decreased by 75% within 11 d after dry-off. Therefore, higher milk yield at the time of dry-off results in higher milk volume remaining in the udder after dry-off and a prolonged interval until complete resorption of the milk.

Our data did not show an influence of DIM, parity, and age on udder pressure. But similar to previous studies (Raubertas and Shook, 1982; Jones at al., 1984), an effect of DIM and SCC on milk yield was noted.

Milk leakage was diagnosed in 2 late lactating cows (2.5%) before dry-off, which confirms previous findings of 2% milk leakage before dry-off (Tucker et al., 2009). After dry-off, 31.6% of cows leaked milk within the first week after dry-off. The prevalence, however, varied between 56.0% in high-yielding and 15.4% in low-yielding cows. This yield-related prevalence confirms again the results of Tucker et al. (2009) who described up to 15% milk leakage after dry-off in cows with lower yield and up to 45% in higher-yielding dairy cows. Furthermore, our study indicated that a high extramammary udder pressure increased the risk of milk leakage. For lactating cows, a similar relationship between intramammary udder pressure and milk leakage has been demonstrated (Rovai et al., 2007). In contrast to Rovai et al. (2007), our data, however, also revealed a relationship between milk leakage after dry-off, parity, and udder pressure. A relationship between the decreasing integrity of the teat canal in higher-parity cows and enhanced risks for IMI was already demonstrated in a previous study (Dingwell et al., 2004). We speculate that these conditions of the teat canal in older cows provoke milk leakage, too. It remains unclear why this effect was not observed in peak-lactation cows (Rovai et al., 2007). We presume that the udder pressure plays an important role; nonetheless, further studies are warranted to elucidate this association.

Fecal Cortisol Metabolites

In order to verify a relationship between udder pressure and elevated stress levels we measured the concentration of 11,17-DOA in fecal samples before and after dry-off. First, baseline 11,17-DOA concentrations were established and compared between the varying yield groups. A clear relationship existed between average milk yield before dry-off and the baseline 11,17-DOA concentration. Interestingly, this relationship was negative, as low-yielding cows had high and high-yielding cows had low baseline 11,17-DOA concentrations. This observation contradicts results presented by Odensten et al. (2007), who showed similar blood cortisol concentrations in dairy cows with different yield classes (low = 5.0 – 11.4 kg/d; medium = 11.5 – 17.7 kg/d; high = 17.8 – 29.5 kg/d) before dry-off. Variations in the 11,17-DOA concentration could be caused by miscellaneous external triggers like transportation (Palme et al., 2000) or stressful handling (Saco et al., 2008). In our study, however, all cows were kept under identical conditions in the same pen. Clinical or subclinical diseases have been established by different authors as triggers for elevated stress levels (e.g., Peter and Bosu, 1987; Hockett et al., 2000). In this study, disease events are an unlikely reason for elevated 11,17-DOA concentrations because general health (i.e., body temperature, heart and breathing frequency, rumination, and BW) and udder health status were monitored multiple times throughout the study and cows with signs indicative of disease were withdrawn from analyses. Furthermore, an individual variability in the basal glucocorticoid concentration was already proven in cats (Graham and Brown, 1996) and is suspected also in cows (Palme et al., 2000; Morrow et al., 2002). A relationship between higher feed efficiency and, therefore, better performance in steers with higher baseline 11,17-DOA concentration was demonstrated by Montanholi et al. (2010). Our study, however, provides the first evidence that baseline 11,17-DOA concentrations in dairy cows could be yield related. A similar relationship between high milk yield and lower blood cortisol was demonstrated in dairy cows 30 and 90 d postpartum (Sartin et al., 1988). Those authors hypothesized that high milk yield may be correlated with a faster hormone metabolism and, thus, lower cortisol levels. Further evidence was provided by Wiltbank et al. (2006), who evaluated a relationship between milk yield, elevated steroid metabolism, and as an extension elevated metabolic activity. Both papers related high milk yield to a faster metabolism, but were unable to substantiate this assumption and demand further research. As there is a lack of controlled studies investigating 11,17-DOA concentrations during peak and mid lactation, the reasons for those differences in baseline 11,17-DOA concentrations remain speculative. A relationship between high milk yield, faster metabolism, and lower 11,17-DOA concentrations could neither be validated nor rejected by our results.

Despite the fact that sudden dry-off is a common management practice, there is a dearth of information about the intensity of stress cows might suffer as a consequence of this procedure. The current study was able to demonstrate that fecal 11,17-DOA, an established indicator of stress, increased following dry-off. A similar increase in blood cortisol concentration after dry-off was reported previously (Odensten et al., 2007). In their study, however, stress levels were evaluated over a 4-wk period before and after last milking, including a 5-d dry-off regimen with prolonged milking intervals combined with a feed change (i.e., reduction in energy density) before the last milking. Regardless of the type of dry-off, both studies were able to demonstrate an effect of milk yield on stress levels after dry-off. In agreement with the results of the present study Odensten et al. (2007) showed an increase in blood cortisol concentration in high- (17.8 – 29.5 kg/d) and medium- (11.5 – 17.7 kg/d) yielding cows after dry-off. In contrast to the current study, however, no effect was evident in low-yielding (5.0 – 11.4 kg/d) cows. The latter might be due to different thresholds for the classification of the 3 yield classes. In our study, the threshold between low- and medium-yielding cows was 15 kg/d, whereas in the study cited above, the threshold between low and medium milk yield was set at 11.4 kg/d. Consequently, 10 out of 26 cows (i.e., 38.5%) classified in the low-yield group in our study would have been in the medium-yield group defined by Odensten et al. (2007).

The increases (11,17-DOA_{rel} values) between baseline 11,17-DOA concentrations and values measured after dry-off differed considerably between yield groups. Whereas high-yielding cows had the lowest 11,17-DOA concentrations before and the highest increase after dry-off, low-yielding cows had the highest baseline and only a slight increase. The measurement of stress hormones to estimate discomfort and pain is an established method (Anil et al., 2002; Rutherford, 2002). The significant increase of 11,17-DOA in high-yielding cows might indicate discomfort due to high udder pressure.

This assumption is substantiated by the similarity of udder pressure and 11,17-DOA profiles (Figure 5). Both parameters peaked within a few days after dry-off and decreased subsequently. Levels of both pressure and 11,17-DOA were elevated until the end of the study period in medium- and high-yielding cows. As reported earlier for fecal 11,17-DOA determinations (Palme et al., 2000; Morrow et al., 2002; Palme, 2005), a time lag of 8 to 16 h between stress exposure and elevated 11,17-DOA concentrations existed. The highest 11,17-DOA concentrations on d 3_F indicate that the stress was most intense on d 2, on which the udder pressure peaked as well. Low-yielding cows with low pressure experienced elevated 11,17-DOA levels only on d 3_F after dry-off. A relationship between high intramammary pressures after dry-off and an increase in stress hormones has been assumed previously (Odensten et al., 2007). Our results confirm this hypothesis, although the correlation between pressure and 11,17-DOA was low ($r = 0.158$).

As our study was conducted on a commercial dairy farm, drying-off was accompanied by a group and ration change, which are common management practices in modern dairy farms (Bushe and Oliver, 1987; Dingwell et al., 2001; Tucker et al., 2009). These changes, however, might have influenced the 11,17-DOA concentrations. The concentration of 11,17-DOA increased in all yield groups after dry-off, but the increase was greatest in high-yielding cows. This difference cannot be explained by a group or ration change, because all cows were exposed to identical management procedures and had to adjust to the same changes irrespective of their yield group. Several studies evaluated the effect of regrouping on dairy cows (von Keyserlingk et al., 2008; Schirmann et al., 2011). Schirmann et al. (2011) showed that an effect of regrouping on the feeding, social, rumination, and lying behavior of dairy cows lasted only for 1 d after regrouping. In our study, however, stress levels peaked only 2 d after dry-off and remained elevated for at least 9 d in medium- and high-yielding cows, indicating that other factors than a group or ration change, presumably elevated udder pressure, were prevalent. Nevertheless, an effect of regrouping could neither be validated nor rejected. Especially in low-yielding cows, the group and ration change might have contributed to the increase in 11,17-DOA concentration.

In addition to indicating stress, elevated 11,17-DOA concentrations before calving can be a predictor for adverse events (Huzzey et al., 2011). Those authors described a relationship between elevated 11,17-DOA concentrations 3 to 2 wk before calving and the probability of culling within the first 30 DIM. A similar association could be demonstrated in our study. Cows culled within the first 21 d after calving had elevated 11,17-DOA concentrations before and after dry-off. Due to the long interval between elevated 11,17-DOA concentration and event, this relationship should be interpreted carefully and further research is warranted to substantiate these findings. Several studies established that low milk yield in the previous lactation influenced treatment decisions and increased the risk of culling (Gröhn et al., 1998; Weigel et al., 2003; Norman et al., 2007). Considering the relationship between high 11,17-DOA baseline concentrations and low milk yield, it could be speculated that cows were not culled due to high 11,17-DOA but due to their low milk yield.

CONCLUSIONS

The results of the current study indicate that a reevaluation of the well-established dry-off procedures in dairy cows is warranted by demonstrating a relationship between a sudden dry-off and an increase in udder pressure and fecal stress hormones. High-yielding cows showed higher udder pressure and a greater increase in their stress levels after dry-off. The effect of a sudden dry-off on low-yielding cows was negligible. Further research should focus on long-term effects on stress and metabolism, particularly in high-yielding cows and subsequently assess animal health and performance parameters. Considering a reevaluation of current dry-off strategies, especially a reduction of milk yield before dry-off should be researched (e.g., by applying different dry-off strategies such as gradual feed restriction and cessation of milking).

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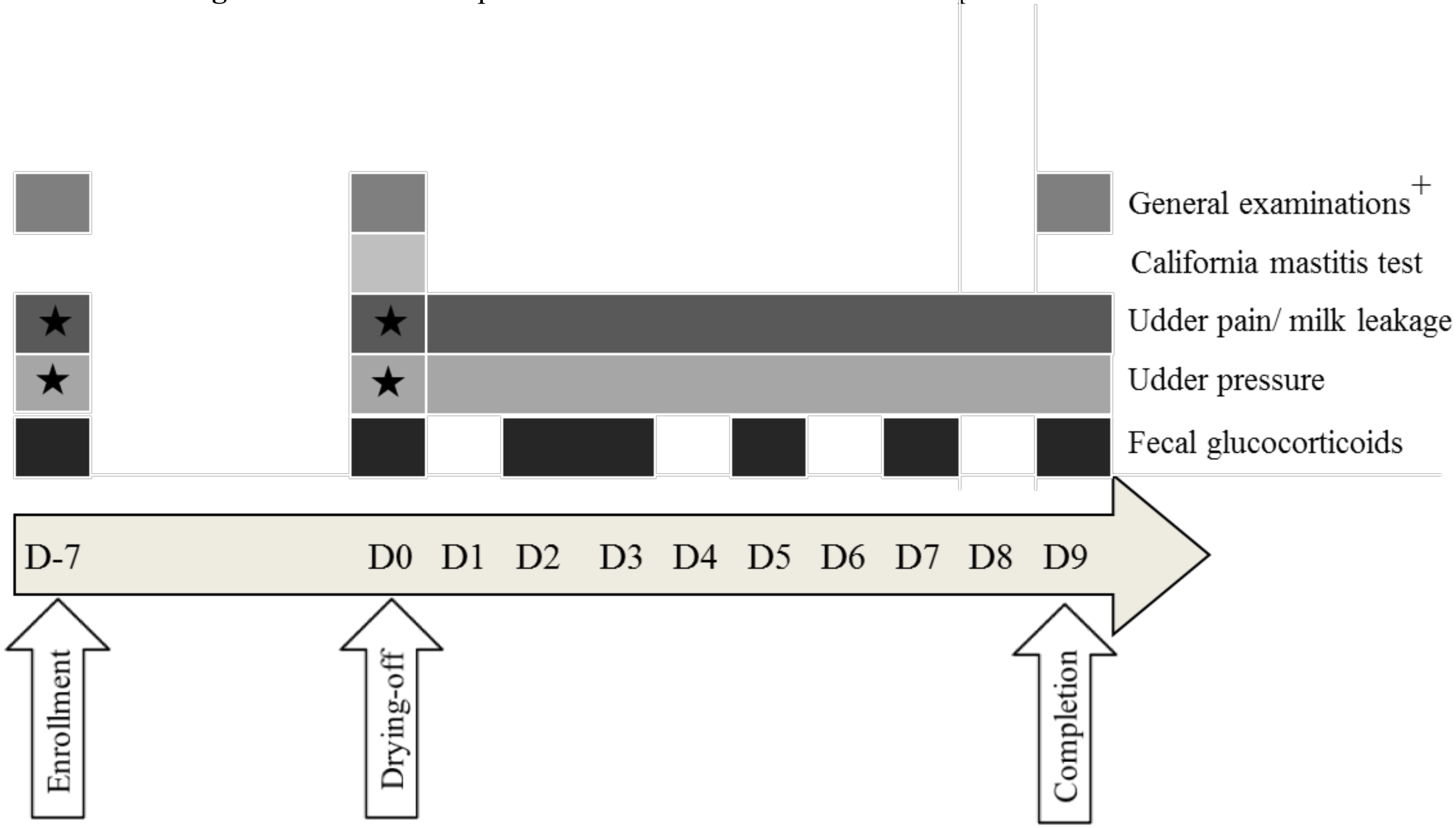
Figure 1. Schematic representation of examinations and sample collection.



+ including clinical and udder examination and locomotion scoring (Sprecher et al., 1997)

★ before and after milking

Figure 2. Udder pressure (in kg) after dry-off considering low (n = 25; < 15 kg/d; ····), medium (n = 27; 15 – 20 kg/d; - - -), and high (n = 24; > 20 kg/d; ──) yielding cows.

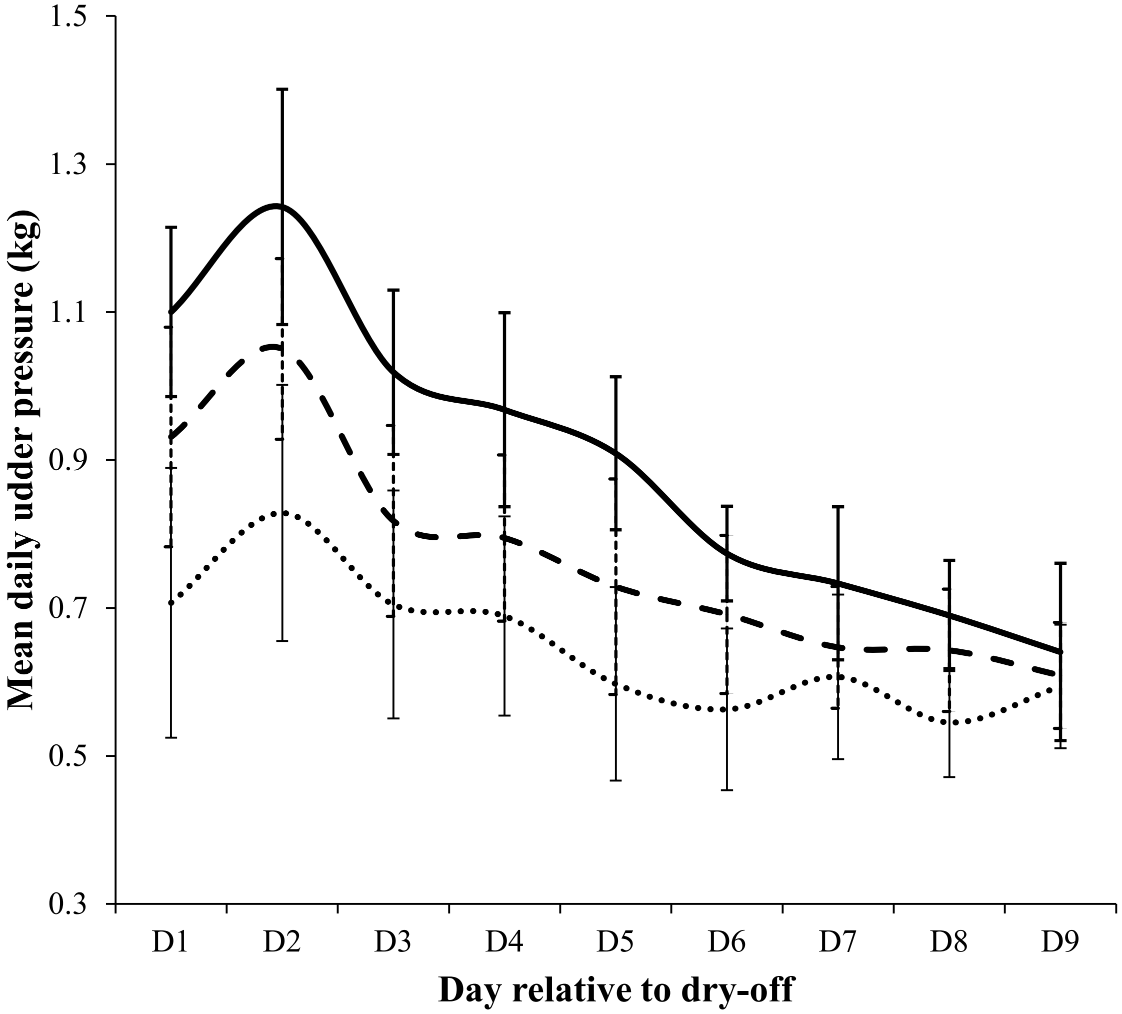


Figure 3. Number of cows with milk leakage after dry-off considering milk yield: low (n = 25; < 15 kg/d; ····), medium (n = 27; 15 – 20 kg/d; - - -), and high (n = 24; > 20 kg/d; ——).

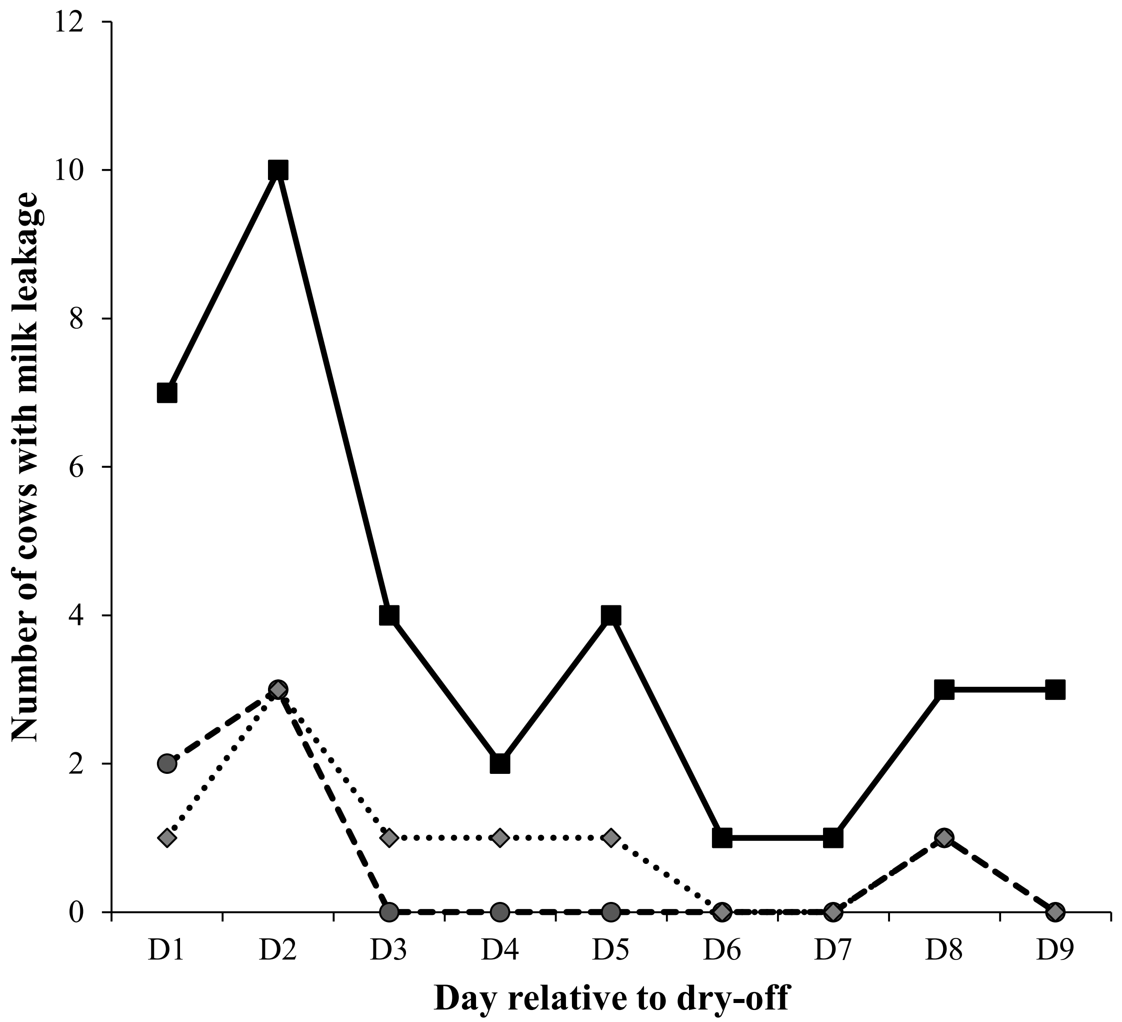


Figure 4. Distribution of daily milk yield in cows with different baseline 11,17-dioxoandrostane concentrations.

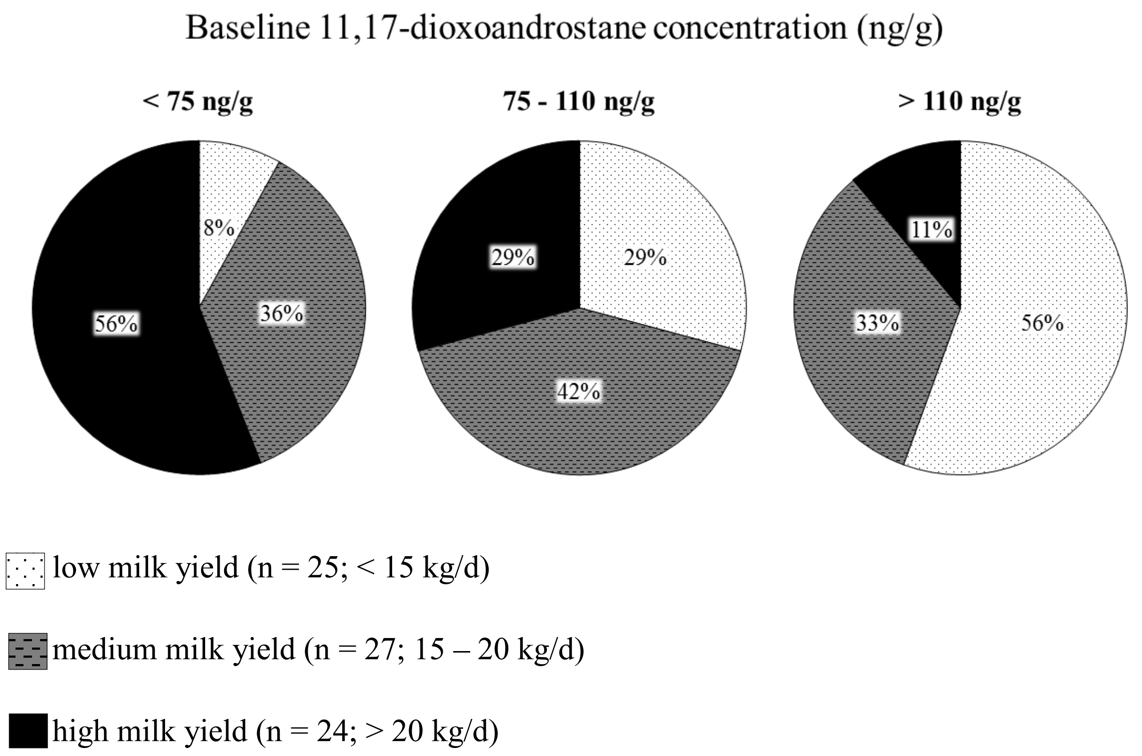


Table 1. Mean daily 11,17-dioxoandrostane concentration (mean ± SE; ng/g) on different days of fecal sampling (Dx_F) before and after dry-off in 76 cows with varying milk yield.

	Yield group		
Day relative to dry-off	Low (< 15 kg)	Medium (15 – 20 kg)	High (> 20 kg)
baseline	121.7 ± 6.8	94.0 ± 6.3	71.1 ± 6.1
$D2_F$	132.8 ± 12.1	118.9 ± 10.9	129.8 ± 19.8
$D3_F$	163.2 ± 14.1	164.6 ± 16.1	136.6 ± 14.0
$D5_F$	131.9 ± 14.8	134.1 ± 13.0	113.3 ± 11.9
$D7_F$	140.1 ± 11.1	143.3 ± 13.7	125.2± 14.8
$D9_F$	148.4 ± 14.0	135.2 ± 11.6	135.9 ± 18.1

Figure 5. Mean (± SE) daily 11,17-dioxoandrostane concentration (——) and udder pressure (— — —) after dry-off in 76 dairy cows.

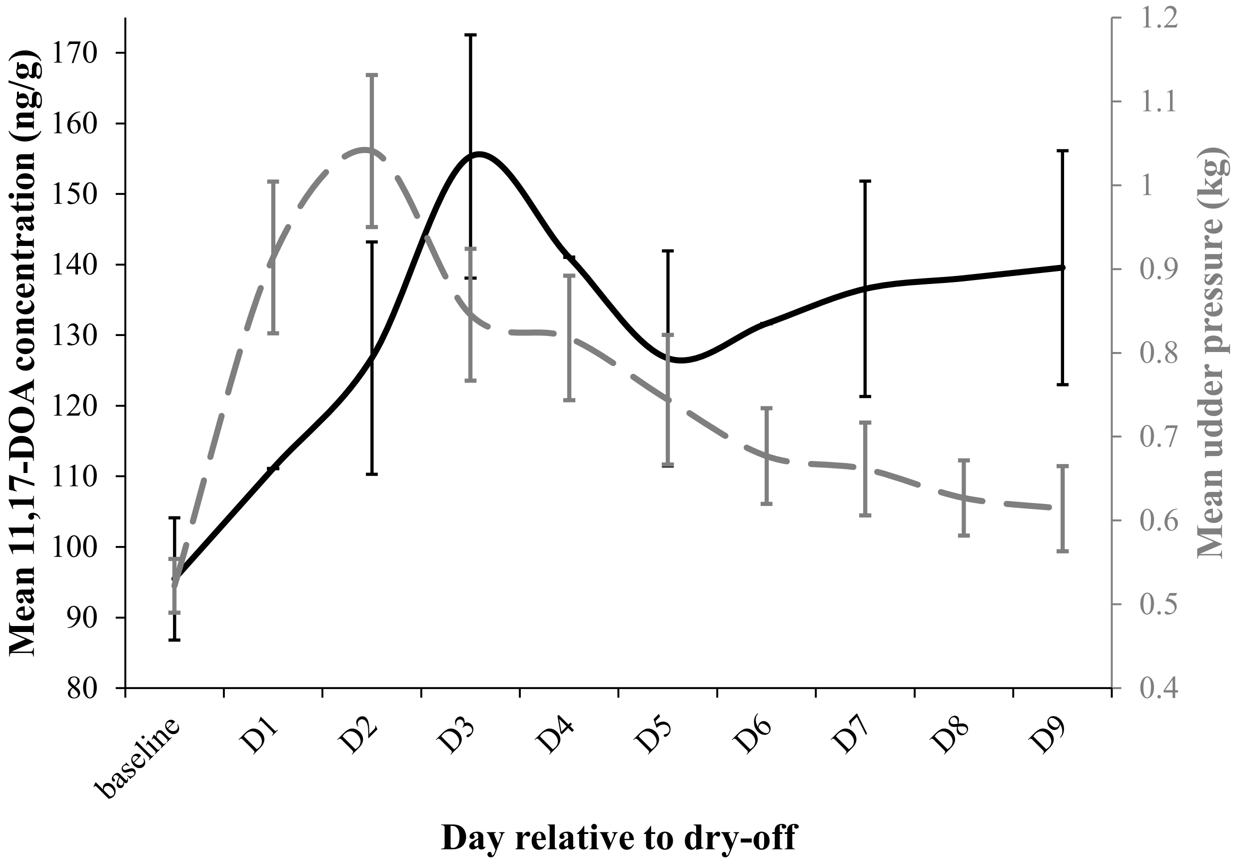


Table 2. Percentage increase[1] (mean ± SE; %) of 11,17-dioxoandrostane (11,17-DOA) concentration in relationship to the baseline in different yield groups and on different days of fecal sampling after dry-off in 76 dairy cows.

Day of fecal sampling relative to dry-off	Yield group			
	Low (< 15 kg)	Medium (15 – 20 kg)	High (> 20 kg)	*P*-value[b]
D2	10.6 ± 8.3	33.9 ± 11.3	117.7 ± 41.4	0.008
D3	40.1 ± 13.1	81.2 ± 16.1	129.1 ± 37.5	0.044
D5	14.7 ± 12.5	55.5 ± 15.1	83.9 ± 29.6	0.060
D7	17.9 ± 9.0	60.4 ± 13.0	94.2 ± 27.7	0.017
D9	24.0 ± 10.4	48.5 ± 9.3	128.8 ± 44.1	0.015
P-value[a]	0.060	< 0.001	0.01	

[a] within group between days effect

[b] within day between groups effect

[1] $\text{DOArel} = \frac{\text{11,17-DOA} - \text{baseline}}{\text{baseline}} * 100$

3. ADDITIONAL UNPUBLISHED DATA

a. A survey of dry-off management practices on commercial dairy farms in northern Germany and a comparison to science-based recommendations

For the sake of consistency, the additional contribution is formatted in an identical style as both research papers.

a. A survey of dry-off management practices on commercial dairy farms in northern Germany and a comparison to science-based recommendations

INTRODUCTION

It is well documented that dry cow management and the dry period are important for animal health (Kim et al., 2003), milk production (Annen et al., 2004; Andersen et al., 2005) and fertility (Beever, 2006) of dairy cows in the following and further lactations.

A recent study analyzed the effect of different feeding strategies during dry-off on animal health measured by clinical findings (i.e., heart rate, rectal temperature, rumen contraction), intramammary infections, somatic cell count (SCC), and blood parameters (Odensten et al., 2007). Green et al. (2007) investigated the influence of herd management practices during the dry period on the prevalence of clinical mastitis after calving. Animal welfare parameters such as behavioral changes and concentrations of stress hormones during dry-off were evaluated recently by Tucker et al. (2009) and in the second study of my thesis (Bertulat et al., 2013). Dry-off procedures described in these studies, however, differed considerably. Procedures like a sudden dry-off (Annen et al., 2004; Bertulat et al., 2013), prolonged milking intervals in preparation of the dry-off (Odensten et al., 2007) and changes of the feed ration or feed restriction before last milking (Valizaheh et al., 2008; Tucker et al., 2009) were described. While these dry-off strategies are well known and have been applied for decades (Wayne et al., 1933; Steyn, 1940), more recently the question has been addressed whether drying-off dairy cows is necessary (Rémond et al., 1992; Madsen et al., 2008). Advantages and disadvantages of continuous milking with omitting a dry period (Fitzgerald et al., 2007; Schlamberger et al., 2010) and varying dry period lengths were investigated (Watters et al., 2008; Santschi et al., 2011). Furthermore, studies focused on benefits of antibiotic dry cow treatment in combination with (Berry and Hillerton, 2007, Bradley et al., 2011) or without (Bradley and Green, 2001; Dingwell et al., 2002) teat sealer. Despite considerable research efforts to improve current dry-off strategies (Ollier et al., 2013; Zobel et al., 2013) there is a dearth of information on the actual dry-off procedures implemented on commercial dairy farms.

Therefore, the objectives of this study were 1) to evaluate current dry-off management procedures on dairy farms in northern Germany using a questionnaire, 2) to quantify behavior indicative of stress after dry-off on commercial dairy farms in northern Germany, and 3) to compare management strategies used on commercial dairy farms to recommendations given in the current literature on dry-off procedures.

MATERIALS AND METHODS

A comprehensive questionnaire was developed focusing on farm characteristics, dry cow management and the perception of the farmer considering animal welfare aspects of the dry-off procedure.

Five open-ended questions covered general farm information like farm size, milk production and bulk milk SCC. Furthermore, 23 closed-ended questions with the option to add comments were asked in order to obtain information regarding the management of late lactating cows (5 questions), the general dry-off management and preparation before dry-off (10 questions) and the management of cows in the early dry period (8 questions). The last set of questions (8 questions) covered the perception of the dry-off procedure concerning animal welfare aspects. A 5-point Likert scale was used for these questions.

The questionnaires were distributed using a convenience sample of 370 farmers attending a continuing education event organized by a German cattle breeding organization (Rinderzuchtverband Schleswig-Holstein e.G., Neumünster, Germany). Attendants were dairy farmers from northern Germany, a region dominated by farms holding an average of 97.5 cows per farm with an average milk production of 8,471 kg (German Cattle Breeders' Federation, 2013). The participation in the survey was voluntary and anonymous. A total of 200 questionnaires were distributed and the farmers were asked to fill the out the survey during the event.

Data were entered into Excel spread sheets (Version 2010, Microsoft, Redmond WA, United States) and statistical analyses were performed using IBM SPSS Statistics for Windows (Version 20.0, IBM Deutschland GmbH, Ehningen, Germany). Means and corresponding standard deviations were calculated for continous and ordinal variables. Frequencies were computed for binary and categorial variables. The interrelation between two categorial variables was summarized using Cross tabulations. Binary and multinomial logistic regression models were calculated to verify the association between different responses (i.e. categorial variables). Odds ratio and 95% confidence intervall were calculated to determine the association between different management procedures and opinions of the farmers. Percentages were rounded to the nearest first decimal place. The significance level was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

A total of 98 questionnaires were returned. The response rate of the presented survey was relatively high (i.e., 49.0%) compared to similar questionnaires (Caraviello et al., 2006; Heuwieser et al., 2010; Gottardo et al., 2011) and most probably caused by my presence, at the time participating farmers completed the questionnaire (Caraviello et al., 2006). Three out of 98 survey forms (3.1%) had more than half of the questions unanswered and thus were excluded from further analysis. Additionally, 4 duplicates (i.e., survey forms with identical answers) were excluded as well, leaving 93 survey forms for final analyses.

In the first and second block covering farm data and the dry-off management 95.7% of all questions were answered. The response rate for the last block focusing on animal welfare aspects of the dry-off procedure, was 87.0%. This result is similar to that of a survey on fresh cow management (Heuwieser et al., 2011), mentioning that 70% to 91% of questions were answered depending on the type of questions.

General farm data and management of late lactating cows

The number of cows dried off annually on participating farms ranged from 35 to 1000 dairy cows. A median of 3 full time equivalents (minimum 1; maximum 19) were employed in the milk production. One employee cared for an average of 52 ± 27 cows. The farms had a mean 305 d production of 8949 ± 1154 kg milk with 4.2 ± 0.28% fat and 3.5 ± 0.18% protein. The average annual bulk milk SCC was estimated at 172,000 ± 63,500 cells/mL.

The information considering housing and management of late lactating cows are shown in Figure 1 and Table 1, respectively. Before dry-off, cows were mostly housed in freestalls with cubicle housing systems (89.2%) and milked twice daily (96.7%) in a milking parlor (89.2%). While cows on 2 farms were exclusively held on pasture (2.2%), 31.2% of the farms offered access to pasture for late lactating cows at least part of the day.

Time of dry-off

Cows were dried off approximately 7 weeks (minimum 4 weeks; maximum 10 weeks) before the calculated calving date. While 3.7% of the farms favored a short dry period length of 35 d or less, only 18.3% had a defined dry period length of 56 to 63 d. The majority (64.5%) dried off their dairy cows 40 to 55 d before the calculated calving date. The optimal dry period length is a subject of controversial and ongoing discussions. While an optimal lifetime production has been described for a dry period length between 40 and 60 d (Bachman and Schairer 2003; Kuhn et al., 2006), most recently Pinedo et al., (2011) suggested a dry period length between 53 and 76 d considering udder health and milk yield in the following lactation. Several studies have demonstrated that a shortened dry period of 35 to 40 d was associated with reduced milk yield in the subsequent lactation (Pezeshki et al., 2007; Watters et al., 2008), but higher milk persistency (Atashi et al., 2013). Prevalence of intramammary infections (Church et al., 2008) and postpartum disease (Watters et al., 2008) were not affected by a shortened dry period. Completely omitting the dry period also reduced the milk production in the next lactation (Annen et al., 2004; Andersen et al., 2005; Madsen et al., 2008; Schlamberger et al., 2010; Steeneveld et al., 2013) and affected the colostrum quality (Rastani et al., 2005; Caja et al., 2006). However, risks for metabolic diseases were reduced (Schlamberger et al., 2010) and milk protein increased (Madsen et al., 2008; Schlamberger et al., 2010). Not a single farm participating in this study omitted the dry period and favored a continuous lactation.

An extended dry period of more than 70 d was shown to have a negative effects on lifetime yield (Kuhn et al., 2006), on the calving to conception interval (Pinedo et al., 2011), and on the culling rate caused by subclinical mastitis and infertility (Pinedo et al., 2011). Only 1 farm (1.1%) in this survey had a regular dry period of more than 70 d.

Several authors (Kuhn et al., 2006; Pezeshki et al., 2007) suggested adapted dry period lengths for individual cows. Dry period lengths of 60 d were recommended for cows in 1st and 35 d for overconditioned cows in 2nd and higher lactation (Pezeshki et al., 2007). The farms participating in this survey, however, did not differentiate between primi- and multiparous cows, but implemented a general dry period length for all cows regardless of age. On average cows were dried of 42 to 49 d before calculated calving date, what is slightly later than the optimum of 60 d. A negative effect of this shortened dry period length, however, is not substantiated by recent publications.

Interestingly, the majority (76.3%) of the farms did not implement a preplanned schedule for dry-off and replied that cows were dried off as needed. Only 14.0% and 9.7% of the farms had a weekly or bi-weekly dry-off routine. Science-based recommendations for an optimal dry-off routine are not available.

Preparation before dry-off

The majority of the farmers (73.0%) performed a sudden dry-off without any previous preparation of the cows. The second study of my thesis, however, has demonstrated that high milk yield at dry-off caused elevated stress levels after sudden dry-off (Bertulat et al., 2013). Furthermore, Rajala-Schultz et al. (2005) showed increased odds of a cow having an environmental intramammary infection after calving when an abrupt cessation of milking was implemented.

Besides a sudden dry-off, various dry-off preparation strategies (i.e., reducing milking frequencies, adjusting feed rations, limiting water supply) have been known for decades (Wayne et al., 1933). Though, it is still being controversially discussed if a reduction of milk yield before dry-off is advantageous or not. Tucker et al. (2009) compared the effect of feed restriction and reduced milking frequencies on behavior and udder health aspects in dairy cows before and after dry-off, respectively. While milk yield was reduced with both strategies, only the reduction of feed intake was able to reduce milk leakage and the prevalence of intramammary infections after dry-off. Cows treated with a reduced feed allowance, however, vocalized significantly more than control cows without feed restriction. The authors speculated that these cows might suffer from hunger and thus feed restriction may pose an animal welfare concern. A gradual cessation of milking had no effect on milk leakage and behavior in this study (Tucker et al., 2008) while in another trial cows with a gradual dry-off had less milk leakage and spent less time anticipating the milking (Zobel et al., 2013). Odensten et al. (2005; 2007) compared different feeding strategies in cows prepared for dry-off by a reduction of milking frequencies 5 d before dry-off. A more drastic feed restriction in form of a straw diet caused increased cortisol levels, indicating stress, effected non-esterified fatty acid (NEFA), beta-hydroxybutyrate (BHBA) and urea concentrations, but did not improve udder health (Odensten et al., 2005; 2007). In my study only 11.8% and 15.0% of the farms attempted to lower milk yield prior to dry-off by reducing the milking frequency and adjusting the ration, respectively. While milking intervals were prolonged 2 to 14 d (mean ± SD; 6.7 d ± 4.3 d) before dry-off, changes in the feeding routine were established up to 60 d before dry-off (22.0 d ± 18.7 d). Seven out of 89 farmers (7.9%) each, reduced the feed quantity and changed the feed composition mostly by reducing concentrate in the mixed ration, respectively. A combination of reduced milking frequency and adjusted ration was described by only 3.3% of the farmers. Furthermore, 4.5% had a separate dry-off preparation group to which the cows were transferred to 14 to 70 d before dry-off.

All dry-off management procedures implemented by farmers participating in this survey were shown to have certain advantages, but also distinctive negative effects either on animal welfare or udder health. Especially the sudden dry-off, the procedure predominantly used by farmers participating in this survey, might increase stress levels and heighten risks for intramammary infections (Rajala-Schultz et al., 2005; Bertulat et al., 2013). Therefore, it is recommended that this procedure should include an examination of cows after dry-off, especially of those cows with high milk yield. According to recent literature, it is important to consider risks and benefits and customize the dry-off procedure to the farm conditions. The ability to implement various dry-off procedures in a given herd might vary depending on the facilities, available labor and management structure of the farm (Dingwell et al., 2001).

Antibiotic dry cow treatment and teat sealer

A more recent meta-analysis compared the effects of antibiotic and non-antibiotic dry cow treatment (Halasa et al., 2009a, b). The authors showed that cows treated with antibiotics had a lower risk (RR = 0.61) for new intramammary infections and a higher risk for curing existing intramammary infections (RR = 1.78) compared to cows without antibiotic dry cow treatment.

Principles of the prudent and rational use of antimicrobials in animals and guidelines for the antimicrobial use in cattle are well researched (Guardabassi et al., 2009). According to the guidelines for the prudent use of antibiotics in veterinary medicine, antibiotic usage should be limited and the susceptibility of pathogens ensured before treatment (Federation of Veterinarians of Europe, 1999). In 2012, Teale and Moulin published a review on existing guidelines for the prudent use of antibiotics. They emphasized that the selection of an antimicrobial should be based especially on previous antimicrobial restistance profiles. Therefore, a blanket dry cow therapy and antibiotic usage without previous bacteriological examination is critical.

A blanked antibiotic dry cow treatment was conducted on 79.6% of the farms in this survey, whereas a bacteriological examination of milk before dry-off was less common on farm agendas (i.e., 31.0%). Bacteriological examinations of milk samples of all cows before dry-off were conducted on 6.6% of the farms, while 24.4% of the farmers mentioned them for selected cases such as high yielding cows. A relationship between the use of antibiotics and bacteriological examinations was not found ($P = 0.307$). A total of 64.9% of all antibiotic dry cow treatments were conducted without preceding bacteriological examination. A selective dry cow treatment was not mentioned by any farmer.

Internal teat sealer were less frequently used at dry-off by farmers participating in this survey (i.e., 33.3%). This is in agreement with previous results describing the usage of milking gloves and teat sealer in Germany (Fischer-Tenhagen et al., 2011). The authors demonstrated that 18.7% of German dairy farmers always and 11.0% sometimes used teat sealer for dry-off, respectively.

The positive effect of teat sealer on new intramammary infections in the dry period and early lactation has been demonstrated in several studies (Berry and Hillerton, 2002; Halasa et al., 2009b; Bhutto et al., 2011). Huxley et al. (2002) showed that an internal teat sealer can significantly reduce the number of new intramammary infections with major pathogens acquired during the dry period compared to an antibiotic dry cow therapy (i.e., 250 mg cephalonium, Cepravin Dry Cow) under UK field conditions. Nevertheless, several publications advise exclusive use of teat sealer only in cows with low SCC and without subclinical mastitis (Crispie et al., 2004; Rabiee and Lean, 2013). The only reliable method to rule out subclinical mastitis is a bacteriological examination of the milk (Crispie et al., 2004). Data of this survey, however, did not show a relationship between performing a bacteriological examination and the decision to use an internal teat sealer ($P = 0.240$).

Most studies (Rabiee and Lean, 2013) used either a teat sealer or an antibiotic drug at the time of dry-off. But a combination of internal teat sealer and antibiotic dry cow treatment has been demonstrated to lower the prevalence of new intramammary infections and clinical mastitis in contrast to a single antibiotic dry cow therapy (Godden et al., 2003; Halasa et al., 2009b, Runciman et al., 2010). Therefore, it is recommended to combine both treatments for an optimal prevention of new intramammary infections during the dry period. This recommendation was also followed by farmers participating in my survey. Farms that used antibiotics were 2.8 times more likely to use internal teat sealer as well (CI 95% = 0.998 – 7.876; $P = 0.05$). While 22.6% of the farms used a combination of internal teat sealer and antibiotic dry cow treatment, 9.7% did not implement any dry cow treatment at all. Two farmers mentioned the application of homeopathic drugs at the time of dry-off to influence the dry-off procedure.

Management and housing after dry-off

Several changes concerning the housing and management of dairy cows after dry-off were mentioned by the farmers in this survey. After last milking most famers transferred cows to a separate dry cow pen (94.1%) that was often located in a different barn. While compared to late lactating cows, more dry cows were housed in free stalls with deep bedding, the number of farms keeping dry cows in tie stalls doubled as well (Figure 1). After dry-off more cows (45.7%) were provided part time access to pastures in comparison to late lactating cows ($P = 0.01$). In addition, two farms offer grazing for dry cows during the summer month. This management practice has the potential to reduce the prevalence of lameness (Haskell et al., 2006) and thus prevent milk production loss (Huxley, 2013).

The majority of farmers (85.9%) changed feed rations at the time of dry-off. While 76.5% changed their ration to a low energy density roughage mix, 9.4% fed a hay or hay-straw-mix after dry-off. Only 7.1% did not change the ration, but reduced the feed quantity. While 7.1% mentioned that cows before and after dry-off received the same ration, only one of them had a ration change before dry-off in order to prepare cows. Eight (8.6%) farmers did not answer this question. Similar to ration changes in preparation to the dry-off procedure, feed changes at the time of dry-off shall reduce milk yield and milk leakage, prevent intramammary infections and hasten the mammary involution (Dingwell et al., 2004; Odensten et al., 2007; Tucker et al., 2009).

Limited water access for 1 and 3 d and a reduction of the daily lighting period for 1 and 4 d after dry-off were mentioned by 2 farmers, respectively. The negative effects of those dry-off strategies on health and animal welfare parameters are well known today (Battaglia, 1998; Rushen et al., 2007;Valizaheh et al., 2008) and, therefore, this procedures cannot be recommended anymore.

Deviations from the standard dry-off protocol

While standard operating procedures are useful tools to implement dairy management practices efficiently and consistently, it might be necessary in some instances to deviate from such guidelines and implement adjustments. Therefore, in the 4th part of the questionnaire, the farmers were asked under which conditions they alter their dry-off protocols, change the dry-off schedule or even omit the dry period.

One reason for abandoning a dry-off protocol mentioned by participating farmers was low milk yield. Most farmers (77.4%) preponed the dry-off date, if milk yield dropped below an individual threshold. This level, however, varied considerable between farms (mean ± SD; 9.8 ± 3.3 kg). About one-third (35.3%) mentioned 10 kg as a cut-off value. But thresholds below 10 kg milk yield per day (38.2%) and between 10 and 15 kg (26.5%) were used, as well. Natzke et al. (1975) demonstrated that cows with an average milk yield of less than 4 kg at the time of dry-off were more likely to have new intramammary infections during the dry period. While an earlier dry-off of low yielding cows was frequently mentioned, an altered dry-off procedure in high yielding dairy cows was rare (9.7%). Thresholds for high milk yield were set between 18 and 35 kg per day and changes to the dry-off protocol varied. Strategies mentioned were a reduction of milk yield by feed change or restriction, shortening of the dry period to 4 weeks or the application of a higher dosage of the intramammary antibiotic dry cow treatment. While on 2 farms quarters of high yielding cows were treated with 2 syringes of antibiotic dry cow treatment, I assume that most farmers implementing such practices are not aware of pharmacological (i.e., extended withdrawl time) and legal (i.e., extra lable drug use) ramifications.

Negative effects of high milk yield at the time of dry-off are well documented (Rajala-Schultz et al., 2005; Bertulat et al., 2013). Management practices to reduce milk yield, however, have negative side effects as well, i.e., elevated stress levels (Odensten et al., 2007), increased risk for mastitis (Tucker et al., 2008; Zobel et al., 2013) or pronounced metabolic responses (Odensten et al., 2007). A shortening of the dry period could be advantageous but might interfere with the required dry period length for milk withdrawal after antibiotic dry cow treatment. While at least some farmers are aware of the challenge to dry off high yielding dairy cows, specific science-based recommendations for this subpopulation of cows are not available.

The second most important factor to adjust the dry-off procedure was udder health. The majority (78.5%) of farmers participating in this survey forewent the dry-off in cows with clinical mastitis, 16.7% even delayed drying off cows with a case of subclinical mastitis. Interestingly, farmers that conducted a bacteriological examination before dry-off were 5.1 times more likely to consider a subclinical mastitis a reason to adapt the dry-off procedure ($P \leq 0.001$) compared to farmers that did not use bacteriological examinations.

Whereas, it is obvious that cows with clinical mastitis should not be dried off, several studies proved that the application of an antibiotic dry cow therapy is efficacious to cure subclinical mastitis during the dry period (Hallberg et al., 2006; Arruda et al., 2013). To achieve adequate cure rates, however, the selection of an effective antibiotic drug considering the guidelines for the prudent use of antibiotics is mandatory (Ungemach et al., 2006). Farmers that do not implement a blanket antibiotic dry cow treatment should test cows before dry-off for subclinical mastitis and select cows with a positive bacteriological finding for an antibiotic dry cow therapy (Halasa et al., 2009a; Cameron et al., 2014). A subclinical mastitis left untreated is likely to become clinical during the dry period and early lactation (Green et al., 2002; Arruda et al., 2013). Furthermore, cows with subclinical mastitis are at risk to infect other cows during the next lactation and increase the bulk milk SCC (Deluyker et al., 2005; Salat et al., 2008; Bhutto et al., 2012). In this survey, however, farmers that forwent antibiotic dry cow therapy were not more likely to treat subclinical mastitis before dry-off ($P = 0.313$).

Another reason to postpone the dry-off was a high SCC (20.4%). While the National Mastitis Council set a threshold of 200.000 cells per mL as indicative of infections (National Mastitis Council, 2001), thresholds between 100.000 and 300.000 cells per mL have been used in previous studies to differentiate infected mammary quarters from uninfected (Deluyker et al., 2005; Berry and Meany, 2006; Schwarz et al., 2010; Malek dos Reis et al., 2011). Thresholds mentioned by farmers participating in this survey varied considerably between 100,000 and 600,000 cells per mL (296,000 ± 134,000 cells per mL). An increased SCC is a valid indicator for subclinical mastitis (Bhutto et al., 2012; Rajala-Schultz et al., 2012) and used in many protocols for selective dry cow therapy instead of bacteriological examinations (Torres et al., 2008). In this data set, however, an association between postponed dry-off due to high SCC and bacteriological examinations before dry-off ($P = 0.544$) or antibiotic dry cow treatment ($P = 0.265$) did not exist.

Dry cow monitoring

All participating farmers monitored their dry cows, but the monitoring schedules varied considerably. Most farms (67.7%) implemented a daily dry cow monitoring, 9.7% and 2.2% of the farmers checked their cows once or twice weekly. Only 20.5% of the farms did not regularly implement a dry cow monitoring.

A total of 95.6% of the farmers examined the cows in the dry cow pen. Of these, 68.9% checked their cows whilst they were free in the pen, on 26.7% of the farms, cows were fixed in headlocks or kept in tie stalls, respectively. The milking parlor was mentioned 4 times (4.4%). The intensity of monitoring, however, differed considerably. A total of 38.2% of the farmers specified that one of their parameters for the dry cow examinations was the general behavior of the dry cow group (i.e., disproportionate restlessness). Furthermore, 92.1% evaluated the general health status of the cow, e.g., body condition score, lameness score, general behavior. An inspection of the udder (i.e., for swelling and redness) was done by 87.1% of the farmers, while 40.4% especially looked for milk leakage. Only 29.2% of the participating farmers regularly touched the udder and checked for udder pain.

During the early dry period cows are most susceptible to clinical mastitis (Cousins et al., 1980; Oliver and Mitchell, 1983). Therefore, a sufficient monitoring of the cows is important in this period. To the best of my knowledge, there is no study available addressing monitoring of dairy cows after dry-off.

Perception of dry-off

The second study of my thesis (Bertulat et al., 2013), as like as several other studies demonstrated that cows suffer stress after dry-off and might show behavioral changes (Leitner et al., 2007; Valizaheh et al., 2008; Tucker et al., 2009). Therefore, the last section of this survey aimed at studying farmers' awareness of behaviors indicative of stress and asked to estimate the frequency of such observations. While agitation, reduced feed intake and increased vocalization were mentioned by nearly all farmers, an increase of aggressive behavior, increased licking of the udder, and waiting in front of the gates to the milking parlor were less frequently seen (Figure 2). Overall, each farmer reported at least one behavior that is related to stress.

Strength and Limitations of the study

I am well aware that the present study has several limitations that should be considered, when interpreting the results. Like most surveys, this study is based on a convenience sample and therefore results are not representative. Similar to Caraviello et al. (2006) who questioned farmers participating in an progeny testing program of Holstein sires, I questioned farmers attending an education event organized by a cattle breeding organization. The number of participating farms in the current study was limited, but similar to that of Caraviello et al. (2006) who evaluated 103 surveys from large United States commercial farms. Participating farms were located only in the northern part of Germany. Previous studies, that similar to my survey, questioned farmers in a circumcised area, however, had mostly fewer responses. In Pennsylvania, Kehoe et al. (2007) analyzed 55 surveys on colostrum management, Heinrichs et al. (2013) 44 surveys on dairy heifer production. Adams et al. (2014) only had a sample size of 20 farms, who answered a questionnaire on dairy beef quality assurance in Colorado.

Nevertheless, this is the first survey addressing current dry-off management practices implemented on commercial dairy farms. Exept for one older proceeding (Dingwell at al.,

2001) no data are available considering the use of different dry-off strategies. This study, provides a good overview of the most important and most common dry-off procedures used in commerial dairy farms and also considers the rationale of antibiotic dry cow treatment.

CONCLUSIONS

Despite a limited number of questionnaires, the data of this survey provided insight in the dry-off procedures currently applied on commercial dairy farms. It was shown that recommendations made by scientists are recognized by farmers and implemented in the daily routine e.g., consideration of milk yield for the dry-off procedure and the combination of teat sealer and antibiotic dry cow treatment. Selective dry cow therapy has not become a common management tool, yet. Obsolete practices like the limitation of water access were applied only sporadically. As critical management practices have the potential to influence the perception of the dairy industry by the general public, implementation of research results into daily routines must be improved. On the one hand, it is important to report scientific results in a way that they are accessible and understandable for farmers. On the other hand, future studies on dry-off procedures should be oriented on the reality of daily dry-off management practices on commercial dairy farms. For example, increasing milk yields and preparation strategies to reduce milk yield prior to dry-off, should be considered in studies in order to increase the value of possible results.

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Table 1. Percentages of responding farm managers to questions related to management of late lactating cows (n = 93).

Survey question and answer category	Percentages
What kind of milking system are you using?	
Milking parlor	89.2
Rotary parlor	5.4
Milking robot	3.2
Pipeline milking system	2.2
How often are cows milked per day?	
Once daily	1.1
Twice daily	96.7
Three times daily or more	2.2
What do you feed cows in late lactation?	
Total mixed ration	49.5
Roughage mix + concentrate	50.5
Concentrate per hand	41.1
Concentrate per automat	8.8
Do you feed cows individually according to milk yield?	
Yes	31.2
No	68.8

Figure 1. Percentages of responding farm managers to questions related to housing of late lactating and dry cows (n = 93).

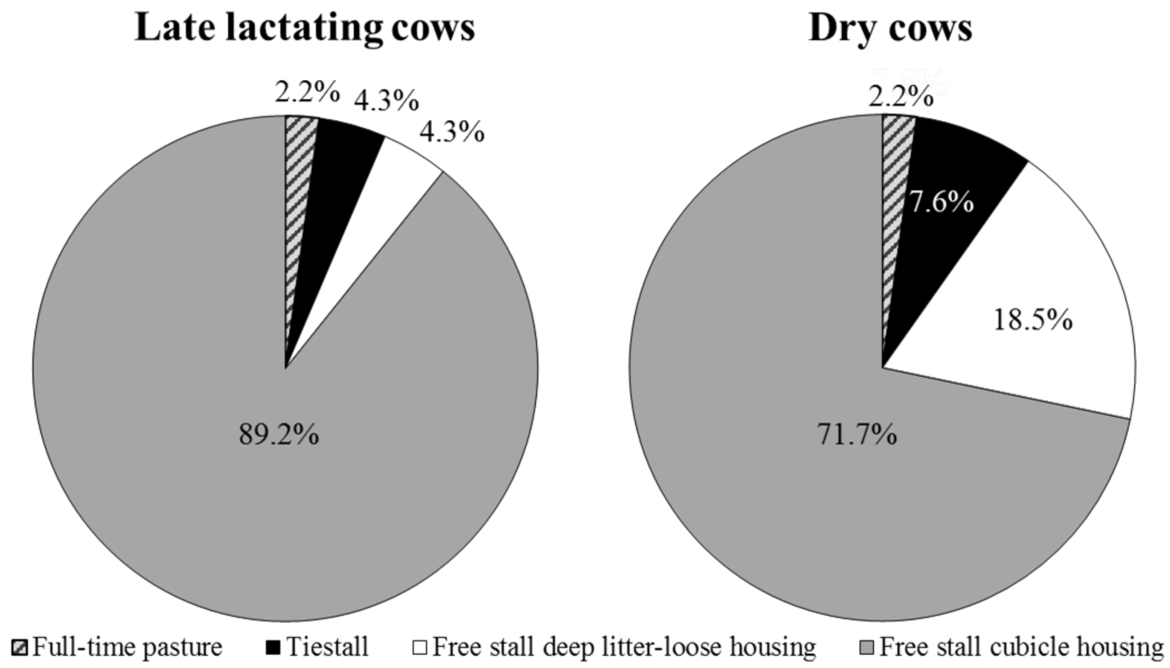
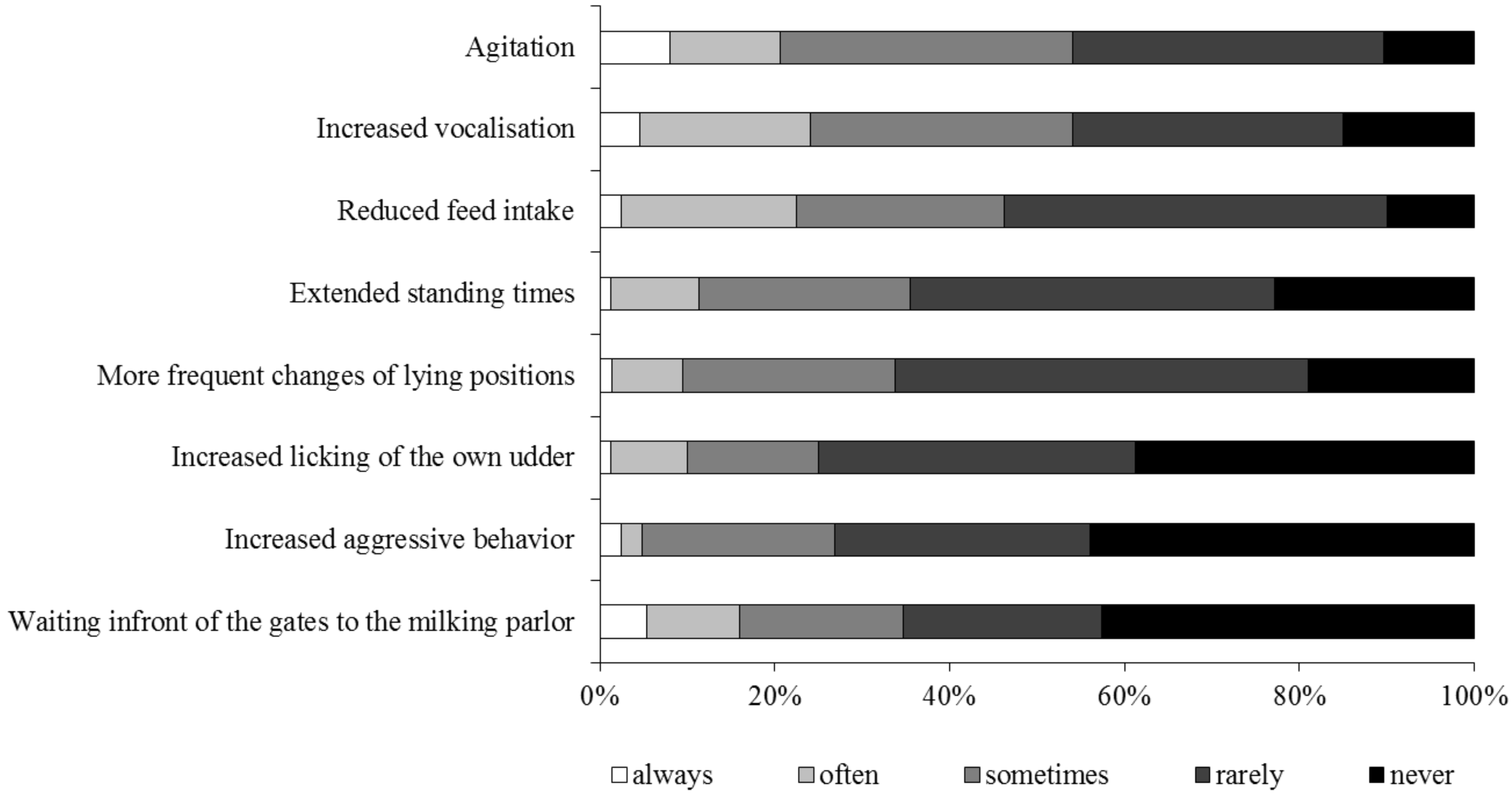


Figure 2. Percentages of responding farm managers to questions related to the perception of the dry-off procedure (n = 93).



4. DISCUSSION

While milk yield has been increasing continuously since the beginning of the 20^{th} century (Lucy, 2001), animal welfare aspects of management practices in general and the dry-off procedures in particular were rarely considered. Especially in recent years, however, animal welfare in dairy cows has become a major public concern (von Keyserlingk et al., 2009).

To the best of my knowledge, this is the first scientific approach evaluating stress, caused by drying-off dairy cows and relating stress hormone levels to milk yield at dry-off and udder pressure after dry-off.

In order to allow an objective comparison of udder pressure values, the first study of this thesis was designed to validate the Penefel DFT 14 – a dynamometer – for the measurement of udder pressure in dairy cows. In 2004, the Global Harmonization Task Force recommended that each medical device should be validated. Especially the importance of an independent validation by a disinterested third party, verifying that a system meets requirements and specifications and fulfills its intended purpose, was emphasized. In contrast to human medicine, validation paper in dairy science, however, are still not as common. While Tucker et al. (2007, 2009) acknowledged the significance of a defined penetration depth and marked measuring point to measure udder pressure, the device used for their study was not validated. In most previous studies, evaluating udder pressure several different devices were used. The authors rarely provided information concerning the measuring protocols and none of the used pressure measuring devices were validated (Phillips, 1954; Tucker et al., 2007, 2009). A reliable comparison of udder pressure values measured in different studies was and is hardly possible. For example, the average decrease of udder pressure during milking measured with the Penefel was 36.5% and distinctively smaller than the value (i.e., 91%) determined by Graf and Lawson (1968), who used a cannula inserted into the teat canal to measure udder pressure.

Operation procedures from former studies, evaluating fruit firmness (Gamrasni et al., 2010; Sabban-Amin et al., 2011) are hardly transferable. In these studies specifications of the penetration depth and the measuring point were unnecessary, because the Penefel DFT 14 was used stationary, attached to an adjustable stand that limited the penetration depth of the measuring tip. Therefore, the validation of the Penefel DFT 14 for the measurement of udder pressure in dairy cows was a necessary step to guarantee reliable data.

The results of the validation experiments indicated that the Penefel DFT 14, albeit imperfect, warrants an adequate measurement of udder pressure. It facilitates a non-invasive, extramammary udder pressure measurement and provides reliable results, given that a standardized protocol is followed in order to minimize confounding. Similar to the assessment of body condition scores (Vasseur et al., 2013), an application of the Penefel DFT 14 in daily field practice as like as in scientific studies is prone to influence especially under field conditions. The following recommendations should be considered in order to guarantee a precise udder pressure measurement: 1) investigators have to be trained to handle the dynamometer, 2) movement of the cow during the measurement has to be prevented, 3) the measuring locations, including quarter and level, have to be defined, 4) the measuring point has to be marked 5) the penetration depth of the measuring tip has to be defined and 6) time of milking has to be considered to acquire comparable measures.

The validation of the Penefel is the first step to objectify udder pressure measurement. If the measuring protocol provided in this study is followed in future studies, comparable and reliable results can be achieved. Such a validated device can be a useful tool in research related to animal health and welfare. It could be used for studies investigating, e.g. the diagnostic of chronic mastitis. An application for the measurement of udder pain was already suggested by Fitzpatrick et al. (2013).

The following study determined the relationship between milk yield, udder pressure and stress levels before dry-off and in the early dry period. Udder pressure after dry-off increased in all cows, but was highest in high and lowest in low yielding cows. An elevated 11,17-dioxoandrostane concentration after dry-off was evaluated in high and medium yielding cows. The effect in low yielding cows was negligible. Evidence for a causal relationship between milk yield, udder pressure and 11,17-dioxoandrostane concentration was found. The reasons for varying 11,17-dioxoandrostane baseline concentrations before dry-off, however, remain unclear. It seems likely that the faster hormone metabolism of high yielding cows (Sartin et al., 1988) might lower the blood cortisol level and thus the 11,17-dioxoandrostane concentration. Further research is required to verify this assumption.

Whereas few studies investigated animal welfare aspects of the dry-off procedure, a relationship between udder pressure and stress after dry-off was only suspected by Tucker et al. (2007) and Odensten et al. (2007). Milk yield, however, was lower and different dry-off methods were used in these studies. Moreover, Odensten et al. (2007) measured blood cortisol before and after dry-off as an indicator for stress, Tucker et al. (2007) recorded behavioral changes and measured udder firmness utilizing a non-validated and not commercially available device. Consequently, the interpretation and comparison of these results is difficult. In my study, however, the Penefel DFT 14 was utilized and the standard operation procedure established in the first study was followed. Hence, the measurements allow a reliable comparison of pressure values between and within groups and cows.

Although, the measurement of stress during the dry-off procedure is a new approach and only few studies assessed stress caused by management procedures (Schirmann et al., 2011; Silva et al., 2013), stress measurement in dairy cows is not uncommon. Many studies were conducted evaluating heat stress (Burfeind et al., 2012), social stress (Huzzey et al., 2012), stress caused by lack of resources (Ronchi et al., 2001), disease (Hopster et al., 1998) or mere handling (Leroy et al., 2011). Therefore, the consequences of stress exposure are well researched. Cows suffering from stress have a lower fertility (Boni et al., 2014; Schüller et al., 2014), a compromised immune status (Doherty et al., 2007; Ballou et al., 2013) and altered energy metabolism (Wheelock et al., 2010). Tao and Dahl (2013) even showed that the placental development in late-gestation cows is compromised by heat stress, resulting in fetal hypoxia, malnutrition, and eventually fetal growth retardation. In the second study of this thesis, cows culled due to metabolic disease or mastitis after calving had higher 11,17-dioxoandrostane concentrations before and after dry-off than cows remaining in the study. But although Huzzey et al. (2011) described a relationship between elevated 11,17-dioxoandrostane concentrations 3 to 2 weeks before calving and the probability of culling after calving, this relationship should be interpreted carefully considering the long timespan between 11,17-dioxoandrostane measurement and culling.

While this is the first study that provided evidence of a relationship between milk yield before, udder pressure and stress levels after dry-off, it demonstrated that an abrupt cessation of milking in high yielding dairy cows is a stressful event and a re-evaluation of this dry-off procedure is warranted. Further research should focus on ways to reduce udder pressure and stress during dry-off especially in high yielding dairy cows.

The third study of this thesis was carried out in order to proof the relevance of this research for the dairy husbandery in Germany. A total of 73.0% of the farmers participating in a survey conducted in northern Germany performed an abrupt cessation of milking at the time of dry-off. Only 26.8% lowered milk yield prior to dry-off by varying management procedures (i.e., feed restriction or reduced milking frequencies). Dingwell et al. (2001) reported that about 75% of dairy cows produced 10 kg or more at the time of dry-off. Considering the results of the second study, showing that cows with more than 10 kg milk yield suffer stress after dry-off and the high portion of farmers in northern Germany that perform an abrupt cessation of milking, even at a rough estimate 55% of dairy cows might suffer stress during dry-off. Bearing in mind that milk yield has been increasing continously during the last 50 years (German Cattle Breeders' Federation, 2013), this percentage might rise further in the future.

While the measurement of cortisol and its metabolits is an established method to estimate stress, the recording of behavioral changes is just as well researched (Grandin, 1997; Bristow and Holmes, 2007; Tucker et al., 2009). Several authors (Leitner et al., 2007; Valizaheh et al., 2008; Tucker et al., 2009) described stress-related behavioral changes after dry-off. Each farmer participating in the survey reported at least one stress related behavior like reduced feed intake and increased vocalization after dry-off. A fact that furthermore substantiates the relevance of the second study and its results.

While a sudden dry-off is a well-known management tool and was recommended in the past (Wayne et al., 1933; Steyn, 1940), increasing milk yield and the increased importance of animal welfare require a rethinking. Several approches are concivable. A reduction of milk yield by breeding selection might be an optimal solution considering animal welfare and health. Economic aspects, however, prevent such a strategy. Management tools like a restriction of feed intake and intermittent milking or restricted access to water can be applied to reduce milk yield before dry-off. Though, the animal welfare aspects of these methods are questionable. Cows suffer from hunger (Tucker et al., 2009) and stress levels increase (Odensten et al., 2007). Intermittent milking causes elevated blood cortisol concentrations for several days before and after last milking (Odensten et al., 2007). A new approach is the administration of prolactin inhibitors before and after dry-off to reduce milk yield. Quinagolide administered 4 days before and 3 days after dry-off reduced milk yield significantly (Ollier et al., 2013). Preliminary results of a study using a single injection of cabergoline even showed an effect on udder pressure after dry-off and a hastened involution of the mammary gland (Bertulat et al., 2013). Long-term effects of prolactin inhibitors on milk production and udder health, however, need further investigation.

5. SUMMARY

Evaluation of stress caused by drying-off dairy cows and its relation to milk yield and udder pressure

An abrupt cessation of milking at the time of dry-off is a most common and a well-proven management procedure that was already established at the beginning of the 20th century. But whereas cows even in the 1970s rarely produced more than 9 kg milk per day at the time of dry-off, in recent years milk yield exceeding 30 kg and more is not uncommon. In addition to an increased udder firmness and udder swelling after an abrupt cessation of milking, cows frequently show certain behavioral changes, e.g., reduced feed intake and increased vocalization. These behavioral changes might be an indicator for stress, pain and discomfort. Considering these evidences, I hypothesized that there is a relationship between milk yield at the time of dry-off, udder pressure and elevated stress levels after dry-off. Thus, the overall objective of this thesis was, to evaluate stress caused by drying-off dairy cows and to relate milk yield at dry-off, udder pressure and stress levels after dry-off.

While udder pressure has been measured for multiple reasons, the equipment used in previous studies was cumbersome and had diverse technical limitations. Manual palpation, however, might be subjective. A new device, a dynamometer, developed to measure fruit crisp, facilitates an objective, non-invasive measurement of udder pressure. While these dynamometers have been already validated for the measurement of fruit crisp, there is a dearth of information about their applicability to measure udder pressure. Therefore, the objective of the first study of my thesis was to validate a dynamometer (Penefel DFT 14) for the measurement of udder pressure in dairy cows.

Two experiments were conducted in order to establish a measuring procedure that guarantees an excellent inter-investigator repeatability. In both experiments udder pressure was measured multiple times ($n = 2838$) by two independent investigators. According to operation procedures implemented for the measurement of fruit crisp, an initial protocol utilized in experiment 1 was developed. The basic handling of the device was determined, the penetration depth was roughly defined, and a general definition of the measuring location was given. Following this protocol, the agreement between investigators was mediocre and repeatability was not sufficient (i.e., $r = 0.80$, $P < 0.001$). Therefore, it was improved and experiment 2 was conducted. This protocol featured the usage of a spacer to define the

penetration depth more precisely and the marking of the measuring point. In this experiment there was no disagreement between investigators ($P > 0.05$) and the coefficient of correlation exceeded the one calculated for experiment 1 clearly (i.e., r = 0.94, $P < 0.001$). Experiment 1 and 2 demonstrated that udder pressure measurements with a sufficient inter-investigator repeatability could be achieved with an exact measuring protocol.

A third experiment was conducted in order to quantify the effects of location within a given quarter and between quarters on udder pressure. Therefore, udder pressure was measured in 6 different locations – at the upper, middle, lower third of the left hind quarter (n = 198) and in the middle of each quarter at the same level (n = 56). Udder pressure differed significantly between the three locations within the left hind quarter. Between quarters udder pressure was lower in the front than in the hind quarters ($P < 0.05$). Based on these results, it was recommended to carry out udder pressure measurements at the same quarter and at the same level in order to achieve comparable results.

In the last experiment, the change of udder pressure before to after milking and its relationship to milk yield was investigated. Measurements were carried out 1 h ± 30 min before and directly after the evening milking. Udder pressure decreased after milking in 91.5% of the udders. In average, pressures after milking were 36.5% lower than before. The predictive value of udder pressure, however was limited (i.e., correlation udder pressure change – milk yield r = 0.42, $P < 0.001$). Nevertheless, this experiment showed the importance of measuring udder pressure every day at the same time before or after milking in order to allow an objective comparison of values.

The first study provided evidence that the dynamometer provides reliable results, given that a standardized protocol is followed in order to minimize confounding.

The second study determined the relationship between milk yield, udder pressure and stress levels before dry-off and in the early dry period. While the dynamometer presented an adequate method to measure udder pressure, the evaluation of stress in dairy cows was challenging. The blood cortisol concentration is a common, albeit highly sensitive indicator of stress that is not usable to determine chronic stress levels. 11,17-dioxoandrostane, a fecal cortisol metabolite is less susceptible to acute stress and provides a reliable alternative for the measurement of chronic stress. Therefore, the objectives of the second study were 1) to quantify changes of udder pressure and fecal 11,17-dioxoandrostane concentrations after a

sudden dry-off, 2) to determine the effect of milk yield prior to dry-off on udder pressure and the fecal 11,17-dioxoandrostane concentration, and 3) to evaluate the relationship between udder pressure and fecal 11,17-dioxoandrostane concentration in the early dry period.

Seventy-six healthy, late-lactating Holstein-Friesian dairy cows were enrolled in the study 7 days before dry-off. They were grouped based on their average daily milk yield in low (< 15 kg/d, n = 25), medium (15-20 kg/d, n = 26) and high (> 20 kg/d, n = 25) yielding. Udder pressure was measured daily at the same time utilizing the Penefel DFT 14. Fecal samples were collected twice within the last week before dry-off and 2, 3, 5, 7 and 9 days after dry-off. An 11-oxo-etiocholanolone enzyme immunoassay was carried out to determine the 11,17-dioxoandrostane concentration.

For all cows, an effect of yield group ($P = 0.001$) and day ($P < 0.001$) on udder pressure could be proven. Udder pressure increased in all yield groups ($P < 0.001$) after dry-off, peaked on the second day after dry-off and declined afterwards. Considering different yield groups, udder pressure after dry-off was highest in high yielding cows. Values differed between high and low yielding and between medium and low yielding cows for 9 and 7 days after dry-off, respectively ($P < 0.05$). Milk leakage was recorded as one factor associated with udder pressure. While a total of 27 cows (33.8%) had milk leakage after dry-off, cows with high udder pressure were more likely to show milk leakage than cows with low pressure values ($P = 0.021$).

After dry-off, 11,17-dioxoandrostane concentrations were effected by day ($P = 0.005$) and udder pressure ($P = 0.05$). They increased after dry-off in medium and high yielding cows, peaked on day 3 after dry-off and remained at an elevated level afterwards ($P < 0.05$). In low yielding cows, however, only the 11,17-dioxoandrostane concentration on the third day after dry-off was higher than the baseline ($P = 0.005$). While 11,17-dioxoandrostane concentrations after dry-off did not differ between yield groups ($P > 0.05$), the increase of 11,17-dioxoandrostane compared to the baseline diverged clearly ($P < 0.05$). It was highest in high yielding cows ($P < 0.001$). The increase in low yielding cows, however, was negligible.

Interestingly, udder pressure and 11,17-dioxoandrostane concentrations after dry-off showed a similar profile, but a time lag of 1 day. Former studies evaluated a time lag of 8 to 16 h between an increase in blood cortisol coinciding with the triggering stressor and an elevated concentration of fecal 11,17-dioxoandrostane. Therefore, a causal relationship

between udder pressure and 11,17-dioxoandrostane concentration could be suspected. This hypothesis is furthermore supported by the fact that high yielding cows had the highest udder pressure values and the highest increase in 11,17-dioxoandrostane.

Overall, the second study showed that an abrupt cessation of milking causes an increase in udder pressure and fecal stress hormone concentration. While high yielding cows showed higher udder pressure and a greater increase in their stress levels after dry-off, the effect of a sudden dry-off on low yielding cows was negligible.

The effect of a sudden dry-off on udder pressure and stress levels in high yielding cows was clearly demonstrated in the second study of this thesis, the relevance of these data for the dairy husbandry and animal welfare, however, remained unclear. In order to prove the importance of this research results, especially for German dairy farms and to substantiate the relevance of this thesis a third experiment was conducted. The objectives of this third study were 1) to evaluate current dry-off strategies on German dairy farms using a questionnaire, 2) to quantify behavior indicative of stress after dry-off, and 3) to compare dry-off strategies used on commercial dairy farms to recommendations given in the current literature.

A questionnaire was developed and distributed among participants in a continuing education event organized by a German cattle breeding organization. Two hundred questionnaires were distributed and data from 91 farms (35 to 1,000 lactating cows) with an average milk yield of 8,949 kg 305-day lactation were analyzed.

Farmers participating in the survey mentioned that cows were dried off approximately 7 weeks before the calculated calving date. Only 9.9% of the farms had a dry period length of 5 weeks or less. A continuous milking regime without dry period was not established on any farm participating in the survey.

The majority (73.0%) of the farmers performed a sudden dry-off without any previous preparation. Only 11.8% and 15.0% of the farms attempted to lower milk yield prior to dry-off by reducing the milking frequency and adjusting the feed ration, respectively.

Most farmers (94.1%) transferred cows after last milking to a separate dry cow pen. At the same time the feed ration was mostly (76.5%) changed to a low energy density roughage mix. Only 7.1% of the farmers reduced the feed quantity instead of changing the ration. A blanked antibiotic dry cow treatment was carried out on 79.6% of the farms, whereas 64.9%

of all antibiotic dry cow treatments were conducted without preceding bacteriological examination. A selective dry cow treatment was not mentioned by any farmer.

Milk yield was an important factor considering the dry-off management. Most farmers (77.4%) preponed the dry-off date, if milk yield fell below an individual threshold, an altered dry-off procedure in high yielding dairy cows, however, was rare (9.7%). Besides milk yield, udder health was the most important factor to adjust the dry-off procedure. Reasons to forgo the dry-off were clinical (78.9%) and subclinical mastitis (16.7%).

All participating farmers monitored their dry cows, 92.1% by evaluating the general health status of the cow. Only 29.2% of the participating farmers, however, regularly touched the udder and checked for udder pain.

The last section of the questionnaire covered questions about behavioral changes associated with stress after dry-off. Each farmer participating in the study reported at least one stress related behavior like reduced feed intake and increased vocalization.

Regarding the overall hypothesis of my thesis, the results of the three studies demonstrated that the sudden dry-off is a stressful management procedure in high yielding dairy cows. Considering increasing milk yield, this finding is relevant as the sudden dry-off currently is one of the most common dry-off procedures. Therefore, a re-evaluation of present dry-off methods especially in high yielding cows is indicated. Strategies to reduce udder pressure and stress caused by an abrupt cessation of milking are necessary and further research is warranted in this field.

6. ZUSAMMENFASSUNG

Bestimmung von Stress verursacht durch das Trockenstellen bei Milchkühen und dessen Zusammenhang mit der Milchleistung und dem Euterdruck

Das abrupte Trockenstellen ist eine allgemein etablierte und allseits bewährte Methode, welche bereits seit Beginn des 20. Jahrhunderts Anwendung findet. Während das Tagesgemelk zum Zeitpunkt des Trockenstellens jedoch noch in den 70er Jahren selten 9 kg überstieg, sind heute Milchleistungen von 30 kg und mehr keine Seltenheit. Als Folge zeigen Kühe heute nach einem abrupten Trockenstellen nicht nur eine vermehrte Euterschwellung und gesteigerte Euterfestigkeit, sondern auch Verhaltensänderungen wie verminderte Fresslust und vermehrt Lautäußerungen. Diese Verhaltensänderungen sind als Ausdruck von Stress, Schmerz und Unbehagen bekannt. Daher lag die Vermutung nahe, dass es einen Zusammenhang zwischen hoher Milchleistung zum Zeitpunkt des Trockenstellens, hohem Euterdruck nach dem Trockenstellen und einem erhöhten Stresslevel gibt. Deshalb war es das Ziel dieser Doktorarbeit, den durch das abrupte Trockenstellen möglicherweise verursachten Stress bei Milchkühen zu quantifizieren und diesen Stress mit der Milchleistung vor dem Trockenstellen sowie dem Euterdruck nach dem Trockenstellen in Beziehung zu setzten.

Zur Beantwortung dieser Fragestellung war jedoch eine objektive Methode den Euterdruck zu messen essentiell. Zwar wurde der Euterdruck bereits in der Vergangenheit im Rahmen von Studien gemessen, das benutze Equipment war jedoch unhandlich und unterlag diversen technischen Einschränkungen. Im Gegensatz dazu ist die manuelle Palpation ein eher subjektives Verfahren. Ein neues Gerät, ein sogenanntes Dynamometer, welches ursprünglich für die Bestimmung des Reifegrades von Früchten entwickelt wurde, ermöglicht objektive, nicht invasive Messungen von Festigkeiten. Während Dynamometer bereits für die Messung der Fruchtreife und -festigkeit validiert sind, fehlen jedoch Informationen zu ihrer Anwendbarkeit bei der Euterdruckmessung. Daher war es das Ziel der ersten Studie, ein Dynamometer (Penefel DFT 14) zur Messung des Euterdrucks bei Milchkühen zu validieren.

Im ersten Schritt wurde dazu eine Messmethode entwickelt, die bestmögliche Wiederholbarkeit zwischen 2 Untersuchern garantiert. Hierfür wurden 2 Experimente durchgeführt. Bei beiden Experimenten wurde der Euterdruck mehrmals (n = 2838) jeweils von 2 unabhängigen Untersuchern gemessen. Das ursprüngliche Protokoll, welches im ersten Experiment zum Einsatz kam, wurde in Anlehnung an die Arbeitsanweisungen zur Messung der Fruchtreife entwickelt. Der generelle Umgang mit dem Messgerät wurde erklärt, die Eindringtiefe der Messspitze grob definiert und die Messlokalisation bestimmt. Entsprechend dieses Protokolls war die Übereinstimmung zwischen den Untersuchern eher mäßig und die Wiederholbarkeit nicht ausreichend ($r = 0{,}80$; $P < 0{,}001$). Aus diesem Grund wurde das Messprotokoll überarbeitet und das zweite Experiment unter Berücksichtigung des verbesserten Protokolls durchgeführt. Dieses umfasste das Anbringen eines Abstandshalters um die Eindringtiefe der Messspitze präziser zu definieren sowie die Kennzeichnung der Messlokalisation. Mit diesem Protokoll konnte kein Unterschied zwischen den Untersuchern festgestellt werden ($P > 0{,}05$). Der Korrelationskoeffizient überstieg den für das erste Experiment berechneten deutlich ($r = 0{,}94$; $P < 0{,}001$). Die Experimente 1 und 2 haben gezeigt, dass Euterdruck unter Berücksichtigung eines exakten Messprotokolls mit einer ausreichenden Wiederholbarkeit gemessen werden kann.

Ein drittes Experiment wurde durchgeführt um den Einfluss der Messlokalisation innerhalb eines Viertels und zwischen verschiedenen Vierteln auf den Euterdruck zu bestimmen. Dazu wurde der Euterdruck in 6 verschiedenen Lokalisationen am Euter gemessen. Zum einen wurde im oberen, mittleren und unteren Drittel des linken Hinterviertels gemessen ($n = 198$), zum anderen in der Mitte jedes der vier Viertel jeweils auf gleicher Höhe. Im Ergebnis differierte der Euterdruck innerhalb des linken Hinterviertels deutlich ($P < 0{,}05$). Zudem zeigten sich Druckunterschiede zwischen den Vorder- und Hintervierteln ($P < 0{,}05$). Basierend auf diesen Ergebnissen sollten Euterdruckmessungen für eine optimale Wiederholbarkeit immer am selben Viertel und auf derselben Höhe durchgeführt werden.

Im letzten Experiment wurde der Zusammenhang zwischen Euterdruckänderung von vor zu nach dem Melken im Vergleich zur Gemelksmenge untersucht. Die Messungen wurden jeweils 1 h ± 30 min vor sowie direkt nach dem abendlichen Melken durchgeführt. Im Ergebnis sank der Euterdruck nach dem Melken bei 91,5% der Tiere und fiel im Mittel um 36,5%. Der Vorhersagewert des Euterdrucks für die jeweilige Gemelksmenge ist jedoch begrenzt ($r = 0{,}42$; $P < 0{,}001$). Nichtsdestotrotz unterstreicht dieses Experiment die Bedeutung des Messzeitpunktes im Vergleich zur Melkzeit. Für einen objektiven Vergleich von Druckwerten sollte der Euterdruck täglich annähernd zur gleichen Zeit gemessen werden.

Insgesamt zeigt die erste Studie, dass eine verlässliche Messung des Euterdrucks mittels eines Dynamometers möglich ist. Voraussetzung dafür ist jedoch die Einhaltung eines standardisierten Messprotokolls.

Während für die Messung des Euterdrucks somit eine adäquate Methode zur Verfügung stand, war die Quantifizierung von Stress bei Kühen eine Herausforderung. Zwar ist die Messung der Kortisolkonzentration im Blut ein etabliertes, jedoch auch hoch sensibles Verfahren, sodass dieser Parameter für die Bestimmung chronischer Stresszustände nur bedingt geeignet ist. 11,17-Dioxoandrostan hingegen ist ein im Kot verzögert ausgeschiedener Kortisolmetabolit, der weniger empfindlich auf akute Stresszustände reagiert und daher eine zuverlässige Alternative zur Messung bei chronischen Stresszuständen bietet.

Entsprechend dem Ansatz dieser Doktorarbeit einen Zusammenhang zwischen Euterdruck und Stress nach dem Trockenstellen nachzuweisen, war es das Ziel der zweiten Studie 1) Änderungen des Euterdrucks sowie der 11,17-Dioxoandrostankonzentration im Kot nach dem Trockenstellen zu quantifizieren, 2) den Einfluss der Milchleistung vor dem Trockenstellen auf den Euterdruck sowie die 11,17-Dioxoandrostankonzentration zu bestimmen und 3) den Zusammenhang zwischen Euterdruck und 11,17-Dioxoandrostan-konzentration in der frühen Trockenstehphase zu untersuchen.

Sechsundsiebzig klinisch gesunde, spätlaktierende Holsteinrinder wurden jeweils 7 Tage vor ihrem geplanten Trockenstelltermin in die Studie aufgenommen und entsprechend ihrer Milchleistung in 3 Gruppen unterteilt. Die durchschnittliche Milchleistung niedrigleistender Tiere lag bei unter 15 kg/d (n = 25), mittelleistender Tiere zwischen 15 und 20 kg/d (n = 26) und hochleistender Tiere bei über 20 kg/d (n = 25). Der Euterdruck wurde täglich zur gleichen Zeit und mit Hilfe des Penefels DFT 14 gemessen. Kotproben zur Bestimmung des 11,17-Dioxoandrostan wurden zweimal direkt vor dem Trockenstellen sowie an den Tagen 2, 3, 5, 7 und 9 nach dem Trockenstellen genommen. Ein 11-Oxo-Etiocholanolone-Enzymimmunoassay wurde zur Bestimmung der 11,17-Dioxoandrostan-konzentration durchgeführt.

Sowohl die Milchleistungsgruppe ($P = 0{,}001$) als auch der Tag nach dem Trockenstellen ($P < 0{,}001$) hatten einen Einfluss auf die Höhe des Euterdrucks. Dieser stieg in allen drei Gruppen direkt nach dem Trockenstellen an ($P < 0{,}001$), erreichte seinen Maximalwert am Tag 2 nach dem Trockenstellen und sank im Anschluss kontinuierlich ab.

Die höchsten Druckwerte wurden bei hochleistenden Tieren nachgewiesen. So unterschieden sich hoch- und niedrigleistende Tiere bis zum 9. Tag, mittel- und niedrigleistende Tiere bis zum 7. Tag nach dem Trockenstellen ($P < 0{,}05$). Milchtröpfeln wurde als ein mit dem Euterdruck in Verbindung stehender Parameter bei 27 Tieren (33,8%) nach dem Trockenstellen festgestellt. Die Wahrscheinlichkeit für dessen Auftreten war jedoch bei hochleistenden Tieren deutlich höher als bei niedrigleistenden ($P = 0{,}021$).

Nach dem Trockenstellen wurde sowohl ein Einfluss des Versuchstages ($P = 0{,}005$) als auch des Euterdrucks ($P = 0{,}05$) auf die 11,17-Dioxoandrostankonzentration nachgewiesen. Diese stieg bei hoch- wie auch mittelleistenden Tieren nach dem Trockenstellen an und erreichte einen Maximalwert an Tag 3 nach dem Trockenstellen. Im Anschluss blieben die Werte auf einem annähernd konstant hohen Level ($P < 0{,}05$). Bei niedrigleistenden Tieren überstieg die 11,17-Dioxoandrostankonzentration nur an Tag 3 nach dem Trockenstellen den Basalwert ($P = 0{,}005$). Während sich nach dem Trockenstellen die absoluten 11,17-Dioxoandrostankonzentrationen nicht zwischen den Leistungsgruppen unterschieden, zeigten sich deutliche Divergenzen bei den Kurvenanstiegen im Vergleich zu den Basalwerten ($P < 0{,}05$). Bei den mittelleistenden Tieren konnte ebenso wie bei den hochleistenden Tieren nach dem Trockenstellen ein deutlicher Anstieg verzeichnet werden ($P < 0{,}001$). Der Anstieg bei niedrigleistenden Tieren war hingegen vernachlässigbar gering.

Interessanterweise zeigten Euterdruck und 11,17-Dioxoandrostankonzentration, abgesehen von einer zeitlichen Verschiebung um einen Tag, einen ähnlichen Kurvenverlauf auf. Vorhergehende Studien haben nachgewiesen, dass zwischen auslösendem Stressor und Anstieg der Konzentration von Kortisol im Blut nur wenige Sekunden, bis zum Nachweis erhöhter 11,17-Dioxoandrostankonzentrationen im Kot jedoch mindesten 8 – 16 h vergehen. Daher lag die Vermutung nahe, dass der Anstieg des Euterdrucks Auslöser für die Erhöhung der 11,17-Dioxoanstrostanwerte war. Diese Annahme wurde zudem vom Sachverhalt gestützt, dass hochleistende Tiere sowohl die höchsten Euterdruckwerte als auch den größten Anstieg an 11,17-Dioxoandrostan aufwiesen.

Im Ergebnis beweist diese zweite Studie, dass der Effekt eines abrupten Trockenstellens bei niedrigleistenden Tieren zwar vernachlässigbar ist, es bei hochleistenden Tieren jedoch zu einem erheblichen Anstieg des Euterdrucks sowie der Stresshormonkonzentration kommt.

Obwohl ein Zusammenhang zwischen Milchleistung und Stress eindeutig aufgezeigt wurde, bleibt die Bedeutung dieser Ergebnisse für die Milchviehhaltung insgesamt unklar. Um die Relevanz dieser Forschungsergebnisse besonders auch für die deutsche Milchviehhaltung zu verdeutlichen und den Stellenwert der hier vorliegenden Arbeit zu unterstreichen, wurde im Rahmen dieser Doktorarbeit eine dritte, bislang unveröffentlichte Studie durchgeführt. Ziel dieser Studie war es 1) aktuelle Trockenstellmethoden in deutschen Milchviehbetrieben mittels eines Fragebogens zu erfassen, 2) nach dem Trockenstellen auftretende, stressimplizierende Verhaltensänderungen zu quantifizieren und 3) angewendete Trockenstellmethoden mit Empfehlungen aus der aktuellen Literatur zu vergleichen.

Dazu wurde ein Fragebogen entwickelt und unter den Teilnehmern einer Fortbildungsveranstaltung für Landwirte verteilt. Zweihundert Fragebögen wurden ausgegeben und 91 Betriebe (35 bis 1000 laktierende Tiere) mit einer durchschnittlichen 305-Tage Leistung von 8.949 kg konnten in die Analysen einbezogen werden.

Teilnehmende Landwirte gaben an, ihre Milchkühe durchschnittliche 7 Wochen vor dem errechneten Abkalbetermin trocken zu stellen. Nur 9,9% der Betriebe hatten eine Trockenstehdauer von weniger als 5 Wochen. Das zuletzt häufig thematisierte, durchgängige Melken wurde in keinem der teilnehmenden Betriebe praktiziert.

Der Großteil (73,0%) der Landwirte gab an, die Tiere ohne vorhergehende Vorbereitung abrupt trocken zu stellen. Nur jeweils 11,8% und 15,0% versuchten durch eine Futterumstellung vor dem Trockenstellen oder das Herabsetzen der Melkfrequenz die Milchleistung zu reduzieren.

Insgesamt 91,4% der Betriebe stellten die Tiere nach dem letzten Melken in eine separate Trockenstehergruppe. Gleichzeitig erfolgte bei 76,5% eine Umstellung der Fütterung auf eine Rauhfuttermischration mit geringerer Energiedichte. Nur 7,1% der Betriebe reduzierten lediglich die Futtermenge. Ein generelles Trockenstellen mittels antibiotischer Trockensteller wurde in 79,6% der Betriebe durchgeführt. Der Großteil (64,9%) verzichtete jedoch auf eine vorhergehende bakteriologische Untersuchung. Keiner der teilnehmenden Betriebe nutzte ein selektives Trockenstellregime.

Die Milchleistung war bei den befragten Landwirten ein wichtiger, die Trockenstellentscheidung beeinflussender Faktor. Während 77,4% der Landwirte angaben, niedrigleistende Tiere vorzeitig trocken zu stellen, waren angepasste Trockenstellregimes bei hochleistenden Tieren eine Seltenheit (9,7%). Gründe um auf das Trockenstellen zu verzichten, waren unter anderem klinische (78,9%) und subklinische (16,7%) Mastitiden.

Eine Trockensteherkontrolle wurde auf allen Betrieben durchgeführt. 92,1% beurteilten hierbei den generellen Gesundheitszustand der Tiere. Nur 29,2% der teilnehmenden Landwirte kontrollierten das Euter palpatorisch auf Wärme und Schmerzhaftigkeit.

Der letzte Abschnitt des Fragebogens umfasste das Auftreten stressassoziierter Verhaltensänderungen nach dem Trockenstellen. Interessanterweise berichtete jeder Landwirt über mindestens eine dieser stressbedingten Verhaltensänderungen, wie etwa verminderte Futteraufnahmen oder vermehrte Lautäußerungen.

Zusammenfassend zeigen die im Rahmen meiner Doktorarbeit von mir durchgeführten Studien, dass das abrupte Trockenstellen bei hochleistenden Milchkühen zu einem messbaren Anstieg von Stress führt. Vor dem Hintergrund kontinuierlich steigender Milchleistungen und kritisch geführter Diskussionen zur artgerechten Nutztierhaltung ist dies ein relevantes Thema. Dieses gewinnt zusätzlich an Bedeutung, bedenkt man, dass das abrupte Trockenstellen derzeit eine der am weitesten verbreiteten Trockenstellmethoden ist. Daraus folgend ist eine Überprüfung gängiger Trockenstellmethoden besonders bei hochleistenden Kühen indiziert. Strategien, den Euterdruck und dadurch auch den mit dem Trockenstellen assoziierten Stress zu reduzieren, sind notwendig und weitere Forschung auf diesem Gebiet ist sinnvoll.

7. REFERENCES FOR INTRODUCTION AND DISCUSSION

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8. PUBLICATIONS

a. Research articles

S. Bertulat, C. Fischer-Tenhagen, V. Suthar, E. Möstl, N. Isaka, W. Heuwieser. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yield. 2013. Journal of Dairy Science 96:3774-3787.

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M. Pilz, S. Bertulat, G. Thiele, C. Fischer-Tenhagen. Genauigkeit der rektalen Palpation zur Trächtigkeitsdiagnostik bei pathologisch veränderter Gebärmutter bei Milchkühen – ein Fallbericht. 2014. Praktischer Tierarzt 95:462 – 469

b. Book chapters

C. Fischer-Tenhagen, S. Bertulat, M. Grau, W. Heuwieser. Usage of milking gloves and teat sealer on German dairy farms. In: Hogeveen, H. and T. J. G. M. Lam. 2011. Udder Health and Communication. Wageningen Academic Publishers. ISBN: 978-90-8686-185-9

c. Oral presentations

S. Bertulat, C. Fischer-Tenhagen, W. Heuwieser. Trockenstellmanagement von Milchkühen – Eine Umfrage zu Trockenstellmethoden auf kommerziellen Milchvieh-haltungsbetrieben in Norddeutschland. DVG Vet Congress. Berlin. November 06th – 10th 2013. In: Referatzusammenfassungen: Deutsche buiatrische Gesellschaft – Schweinekrankheiten - Krankheiten kleiner Wiederkäuer, p 9-11, ISBN: 978-3-86345-172-1

W. Heuwieser, S. Bertulat. Stress durch Trockenstellen bei hochleistenden Milchkühen und erste Ansätze zur Minderung. bpt Kongress 2013. Mannheim. September 26th – 29th 2013. In: bpt-Kongress 2013: Vortragsband – bpt-Akademie GmbH (Hrsg.), p. 154–159, ISBN: 978-3-937266-47-3

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S. Bertulat, N. Isaka, A. Deflandre, A. Lopez, T. Hetreau, W. Heuwieser. Efficacy of cabergoline to reduce udder pressure and milk leakage after dry-off in dairy cows. ADSA – ASAS Joint Annual Meeting 2013. Indianapolis, United States. July 8th – 12th 2013. Proceedings

S.Bertulat, C. Fischer-Tenhagen, V. Suthar, E. Möstl, W. Heuwieser. Effect of dry-off on stress levels of differently yielding dairy cows. Dairy cattle welfare symposium. October 24th – 26th 2012. Guelph, Canada. Proceedings.

S.Bertulat, C. Fischer-Tenhagen, V. Suthar, E. Möstl, W. Heuwieser. Evaluating stress caused by dry-off in dairy cows. 16th annual meeting of the European Society of Domestic Animal Reproduction. August 29th – September 1st 2012. Dublin, Ireland. In: Reproduction in Domestic Animals 47 Suppl. 5, 2012, p.70.

S. Bertulat, C. Fischer-Tenhagen, W. Heuwieser. Udder Pressure Measurement in Dairy Cows before and after milking. 7. Doktorandensymposium u. DRS Präsentationsseminar "Biomedical Sciences. July 13th 2012. Berlin. Proceedings p29. ISSN:978-3-86387-153-6.

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d. Poster presentations

S. Bertulat, C. Fischer-Tenhagen, W. Heuwieser. Drying-off dairy cows – a brief survey on dry-off procedures on commercial dairy farms in northern Germany. 8th PhD-Symposium & DRS Presentation Seminar. Berlin July 15th 2013. Proceedings, p.58, ISBN: 978-3-86387-326-4

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10. DECLARATION OF INDEPENENCE

Hiermit bestätige ich, Sandra Bertulat, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Tabelle 1. Eigener Anteil[1] an den Forschungsprojekten der vorliegenden Dissertation.

	Studie 1[a]	Studie 2[b]	Studie 3[c]
Studienplanung	+++	+++	+++
Datenerhebung	+++	+++	+++
Datenanalyse	+++	+++	+++
Verfassen des Manuskripts	+++	+++	+++
Editieren des Manuskripts	++	++	++

[1]Legende: +++: > 70%
++: 50 – 70%
+: < 50%

[a] Technical note: Validating a dynamometer for non-invasive measuring of udder firmness in dairy cows

[b] Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yield

[c] A survey of dry-off management practices on commercial dairy farms in northern Germany and a comparison to science-based recommendations

Berlin, den 26. Februar 2014

Sandra Bertulat