Behavior of dairy cattle queuing in the waiting area before the milking parlor in a pasture based system

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Content

Abstract

Monitoring stress in dairy cows is important, as it is suggested to decrease milk production and welfare. Queuing in the waiting area before the milking parlor could cause stress in dairy cows, and it is therefore an interesting subject for research. A suggested way to monitor stress in animals is by observing the frequency of changes in behavior. Behavioral diversity can be calculated using the Shannon-Weaver-Diversity-Index (SW-index). This study investigated whether queuing for the milking parlor causes stress in dairy cattle by scan sampling the behavior of 107 dairy cows using an ethogram. The behavioral diversity was calculated using the SW-index. Several factors possibly influencing the behavior of dairy cows were monitored and compared with the SW-indices in a multiple linear regression model. The results show that waiting time influence the SW-index positively. Assuming that stress can be indicated by the diversity of an animal's behavior, this study show that queuing before the milking parlor is a causal factor for stress in dairy cows.

Introduction

In dairy farming, the milking process is one of the most important daily activities. For example, half of the labor costs are related to milking[1]. The last decades, improvement of this process has become an important subject for research, and automatic milking systems increasingly replace traditional milking parlors. The determining factor of the daily milking process is, of course, the milk production itself, and knowledge of factors possibly influencing this milk production is crucial for a profitable dairy farm. Stress is suggested to decrease milk production[1-3]. Factors influencing this stress, and thus the daily milk production, are for this reason an important subject for research. These factors can be divided into two groups: endogenous and environmental. Endogenous factors are inherited (breed, age) or acquired (influenced by e.g. hierarchical order) traits[1]. Animals cope with certain conditions (stressors) by using behavioral and physiological stress responses, trying to maintain homeostasis. If these responses are unsuccessful or impossible to express, typical behavioral and physiological symptoms of chronic stress occur. In such situations there is an obvious decrease in welfare[3]. Considering the effect of stress, it is, in order to have an efficient production system, important to have detailed knowledge of these stress responses and factors influencing them.

An environmental element that has been suggested to be important in the milking process is queuing in the waiting area before entering the milking parlor[1,2]. Several aspects of queuing in a waiting area could be seen as stress inducing, i.e. by limiting the cow in behaving normally: there are less to no possibilities for feeding and drinking, lying down is usually impossible, and locomotion activities are restricted. Being unable to carry out these normal behaviors, a stress response arises, and thereby a decrease in welfare[2,4]. Besides that, during lying down, the blood flow to the udder doubles, ensuring a higher milk production[5]. Despite the suggested importance, queuing for the milking parlor has not been studied intensively yet and the few published studies vary in their outcomes. A study of Varlyakov et al. (2011) suggests a relationship between waiting time and stress: queuing for more than 30 minutes influenced the milk release reflex negatively[1]. Another study, however, showed that queuing for an automatic milking system does not lead to an increase of concentrations of adrenaline

or cortisol[6] and thus does not indicate it as being stressful. Since different studies have different outcomes, the relation of queuing for the milking parlor and stress needs further research.
Several ways to monitor stress in cows have been suggested[3], one of them being the frequency of changes in an animal's behavior. A study of Raussi et al. (2005) showed this by demonstrating that cows that have been socially stressed change their activity more often, and move more. A greater behavioral diversity could, therefore, be an indicator of stress[7]. However, several studies on the behavior of zoo animals use behavioral diversity as a positive indicator of welfare: an increase can be interpreted as an increase in species specific behavior and a decrease in stereotypical behavior[8,9]. A study of Miller et al. (2016) showed that this is accompanied with a decrease in fecal glucocorticoid levels[8], and thus it is concluded that a higher behavioral diversity indicates less stress in zoo animals[8,9]. The different outcomes of these studies suggest that an optimum for behavioral diversity exists; a too high or too low diversity could indicate stress. Since stress leads to a decrease in welfare, it is suggested that behavioral diversity can be used as a measure of welfare[8-10]. Behavioral diversity can be calculated using the Shannon-Weaver-Diversity-index (SW-index), which quantifies the diversity of observed behavior[8-10]. A higher value of the SW-index indicates a greater behavioral diversity; meaning that the number of different behaviors increased, or that the same number of behaviors were performed but the time spent in displaying these was more evenly distributed. Thus, the SW-index does not just use the number of different behaviors performed, but also takes into account the amount of time devoted to them. Intensive study of the behavior of cows queuing for the milking parlor could determine if this process causes more or less behavioral diversity, and thus possibly stress.
In this study the behavior of cattle queuing for the milking parlor was scan sampled using an ethogram, designed using different behavioral studies of dairy cows[2,11,12] and observations done by the researchers. The behavioral diversity was calculated using the SW-index. Observations were made on an experimental farm of the University of Montevideo, Uruguay, where cows are housed continuously outdoors in pasture, except during the milking. The goal of the study was to investigate whether queuing for the milking parlor causes stress in dairy cattle. This was done by assuming that stress can be indicated by the diversity of an animal's behavior, calculated with the SW-index. Several factors possibly influencing the behavior of the cows were determined, assuming that waiting time is the most important factor in this process. The main question for this study was therefore 'how does waiting time before getting milked influence the SW-index in dairy cows?'. It was hypothesized that waiting time influences the SW-index positively: the longer the waiting time, the higher the SW-index. Other factors that were determined to possibly influence the SW-index were temperature and rain. Furthermore, the relationship of SW-index and milk production was compared using milk production results monitored monthly on the farm. The calculated SW-indices and different factors were compared in a multiple linear regression model, drafted using IBM SPSS Statistics.
Understanding the behavior of cows in the milking process and pointing out components in this process that lead to stress could result in improvements in this process. It could thereby point out how to increase (a part of) the welfare of dairy cattle.

Materials and methods

Location and time of data collection

The observations were done on the experimental farm of the Veterinary Faculty of the University of Montevideo, Campo Experimental No. 2 in Uruguay. This was done during the early spring period of 2012 in the months October and November.

Experimental animals

The group of cows used for this study consisted of about 150 lactating cows (total size differed during the study because of temporarily removal of non-lactating cows), mainly Holstein Friesians and Jerseys and a few other (mixed) breeds. In total 120 different cows were observed during waiting to get milked, resulting in 107 different observations (due to some double observations of a few animals and missing milk productions).

Housing

The experimental group of dairy cows were held in a pasture based system, meaning that except during milking the cows were kept outside on the pasture. Here they had ad libitum access to grass and drinking water. There were several different pastures, which all differed in size. This semi-natural environment for the cows to live in makes it possible for the animals to show natural behavior.

Milking parlor

The cows were milked twice a day, in the morning around 05:00 am and in the afternoon around 03:00 pm. Cows were milked in a 2 x 4 herringbone parlor. The floor was made of grooved concrete. During milking cows were fed silage.

Waiting area

The waiting area (fig. 1), where the cows queued before getting milked, consisted of a corral of about 120m^2 (A) and an extra part for the beginning of the milking process of about 30m^2 (B), because of lack of space in the corral at the beginning of milking. The floor of the waiting area was made of concrete.

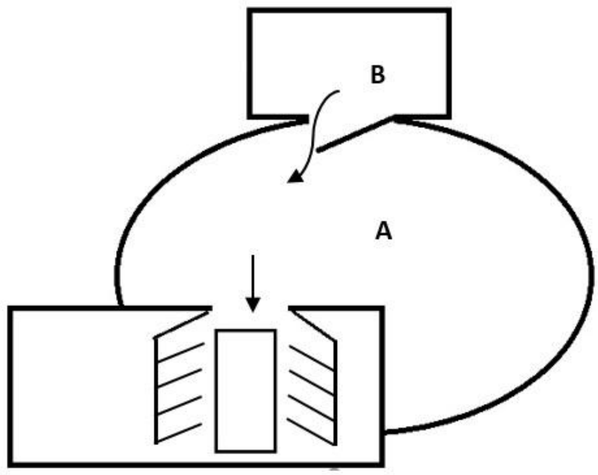


Fig. 1. Schematic overview of waiting area and milking parlor

Observations - Observations were made by two students of the faculty Veterinary Medicine (Utrecht University). The observers were always at 2-3 meters distance from the cows in order not to disturb the behavior. An ethogram, created during the first two days of the study, was used to record the observed behavior. Observations were done always at the 03:00 pm milking process, using a scan sampling method, and were noted in time periods of 1 minute. On average 5 cows per milking session during 24 days were observed, resulting in 107 observations (due to a few accidental double observations and some missing milk production results). The amount of minutes each observed animal waited before entering the milking parlor was registered using a digital watch.

Ethological methods

The ethogram (table 1) used in this study was created using different behavioral studies of cows[2,12] and by observing the behavior of cows in the Netherlands during queuing for the milking parlor for one day. The created prototype for an ethogram was validated and completed in Uruguay during two days prior to the observations, assuring that the ethogram contained all relevant behaviors.

Category	**Element**	**Definition**
Eating behavior	Ruminate	Combination of regurgitating, chewing and swallowing regurgitated food
	Eat	Intake of food
	Drink	Intake of fluids
Resting behavior	Lie down	Position in which the body weight of the animal rests on the torso
	Sleep	Period of lower to no conscience, eyes are closed
Social behavior	Sniff other cow	Stretching the head towards or touching another cow with head
	Lick other cow	Licking another cow on head, neck -or shoulder area
Reproductive behavior	Estrous behavior	Flehmen, mounting, resting head on other cow, standing when mounted
Agonistic behavior	Push head	Pushing head against other cow, or between two other cows
	Push body	Pushing body against other cow, or between two other cows
	Bump/fight	Pushing head against other head, sometimes followed by pushing head against other cow’s neck and maneuver to take a position
	Threaten	Showing dominance to other cow in a lateral or frontal position, head held low
	Kick	Movement of legs whereby another cow gets hit
	Scrape hoof	Intentional behavior, scraping hoof over ground
	Submissive behavior	Head held low, neck in a stretched position
	Pant	Heat stress
	Sexual behavior without heat	Flehmen, mounting, resting head on other cow
	Agitation	Disturbance of normal behavior, resulting in restlessness, alertness and locomotion behavior
Excretion behavior	Defecate	Excretion of feces
	Urinate	Excretion of urine
Explorative behavior	Ear play	Movement of one or both ears
	Alertness	Turning head towards sound or movement
	Sniff environment	Sniffing environment without eating
Locomotion behavior	Stand	All legs in normal stretched position, body weight resting on legs

	Toddle	Lifting legs without moving forward
	Walk forward	Lifting legs while moving forward
	Walk backward	Lifting legs while moving backward
	Run	Lifting legs in a fast speed while moving forward
	Jump	Propelling one or more limbs from the ground in a quick movement
Comfort behavior	Scrape surroundings	Moving a body part against an object in the surroundings
	Scrape oneself	Moving a body part against own body
	Stretch	Stretching of body(part)
Self-hygiene behavior	Lick nose	Licking with tongue over nose
Vocalization	Moo	Normal, low throat noise of a cow
	Cough	Sound that arises by exhaling with a closed glottis
Discomfort by surrounding behavior	Fight off flies	Fight off flies by whipping tail, moving head or kicking hoof(s)
Other behavior	Yawn	Opening mouth and breathing in heavily

Table 1. Used ethogram

Daily outside temperature and rain
As no thermometer was available during the study, the daily outside temperature was estimated using the daily weather forecast from multiple websites during the measurements. The presence of rain (yes or no) was registered.
Milk production
The milk production (liters) per cow was registered on the farm once a month for one day (i.e. 2 milking sessions). The productions of the months August, September and October were used for the present study.
SW-index
The SW-index was calculated using the following formula: $H' = -\sum_{i=1}^{S}(p_i \ln p_i)$, calculated using the computer program Microsoft Excel 2010. The index 'H' is a quantification of the diversity of observed behaviors. In the equation, 'S' is the amount of different behaviors, 'i' the displays of one behavior, and 'p' the proportion of displays of one behavior divided by the total number of behaviors.
Statistical methods
Statistics were calculated using the computer program Microsoft Excel 2010 and IMB SPSS Statistics. The response variable (SW-index) and explanatory variables (waiting time, temperature, rain and milk production) were always first displayed by the use of descriptive statistics. Each variable was checked for normal distribution by a histogram and a Q-Q plot and the mean, range, standard deviation and standard error were calculated.
Then the explanatory variables were compared with the response variable in a multiple linear regression model. With this, the relationship between the different variables (waiting time, temperature, rain, milk production) and the response variable is tested by fitting a linear equation to

the observed data. First a model with all the explanatory variables was formed. The variable with the least significance was then removed from the model, repeating this until a model with only significant variables was formed. The level of significance (P) was 0,05.

Results

Overall descriptive statistics are shown in table 2.

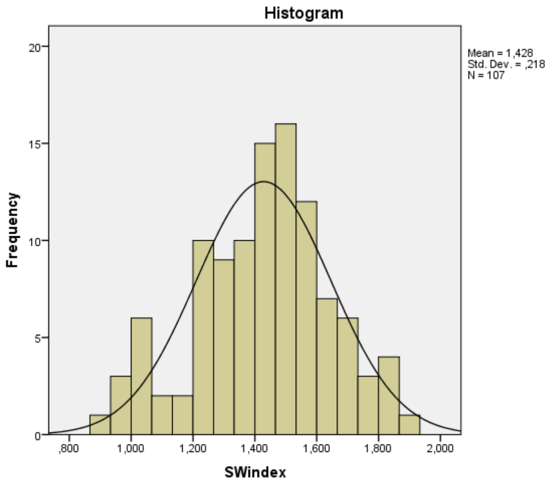
Descriptive	SW-index	Waiting time	Temperature	Milk production
Mean	1.428	107.09	22.29	24.148
95% CI mean lower	1.386	96.93	21.35	22.980
95% CI mean upper	1.470	117.25	23.22	25.315
Median	1.448	108.00	22	24.500
Variance	0.048	2809.746	23.84	37.097
Std. deviation	0.218	53.007	4.88	6.091
Minimum	0.912	20	15	8.500
Maximum	1.867	235	33	40.100
Range	0.955	215	19	31.600

CI = confidence interval

Table 2. overall descriptive statistics

SW indices

The SW-indices were normally distributed (as shown on the histogram and Q-Q plot, fig. 2). The mean SW-index was 1,428, standard deviation was 0,218 and 95% confidence interval for the mean 1,386 to 1,470. Minimum and maximum SW-index values were 0,912-1,867.



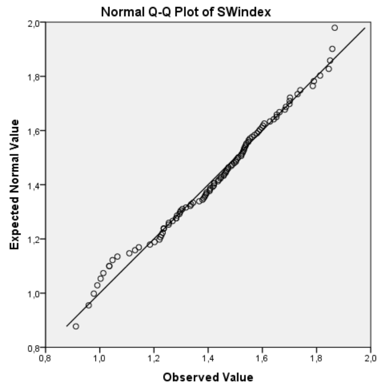
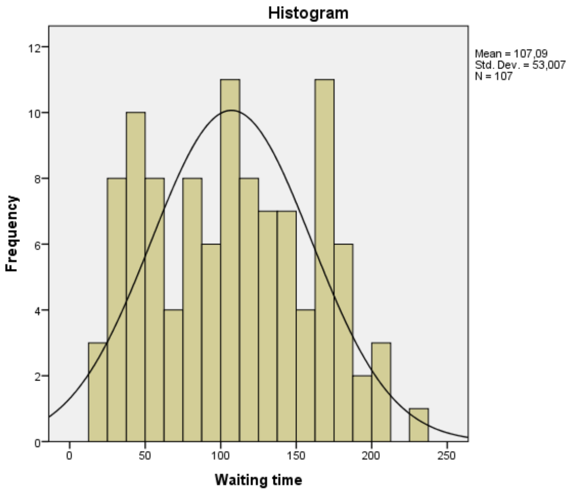


Fig. 2. Histogram and Q-Q plot of SW-indices

Waiting time

Waiting time was normally distributed (as shown on the histogram and Q-Q plot, fig. 3). The mean waiting time was 107,09 minutes. The standard deviation was 53,007 minutes and 95% confidence interval for the mean was 96,93 to 117,25 minutes. There was a wide range between the cows' waiting times; the shortest period was only 20 minutes, the longest lasted 235 minutes.



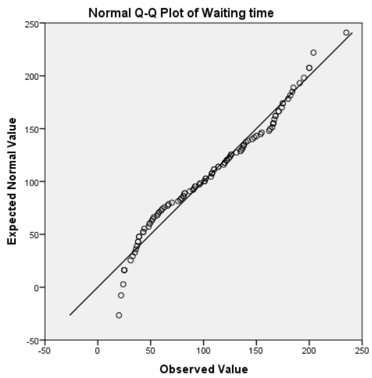
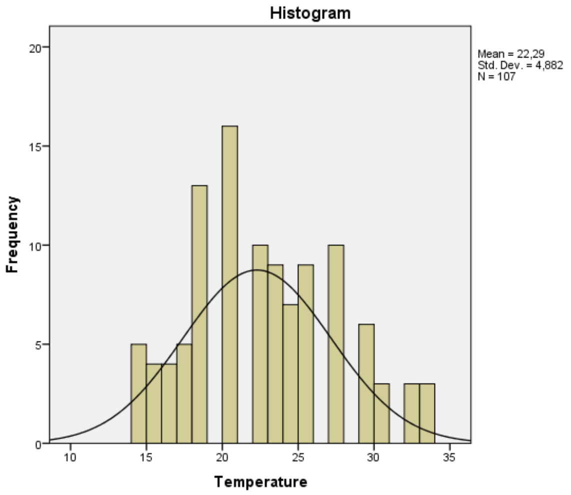


Fig. 3. Histogram and Q-Q plot of waiting times

Temperature

The registered temperature during the observations was normally distributed (as shown on the histogram and Q-Q plot, fig. 4). The mean temperature was 22,29 degrees Celsius, standard deviation was 4,882 and 95% confidence interval for the mean was between 21,35-23,22. During the research there were a few hot (maximum temperature being 33 degrees Celsius) and colder (minimum temperature being 15 degrees Celsius) days. This was due to the time of the year the study took place, during the Uruguayan spring.



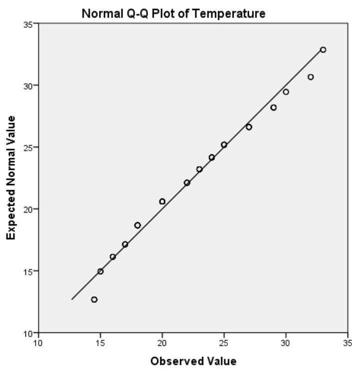


Fig. 4. Histogram and Q-Q plot of temperature

Milk production

The milk production was normally distributed (as shown on the histogram and Q-Q plot, fig. 5). Mean milk production was 24,15 liters, standard deviation 6,09 liters and 95% confidence interval of the mean 22,98 to 25,32 liters. The amount of milk production varied extremely between cows, minimum amounts being 8.5 liters, while the maximum milk production was more than 40 liters.

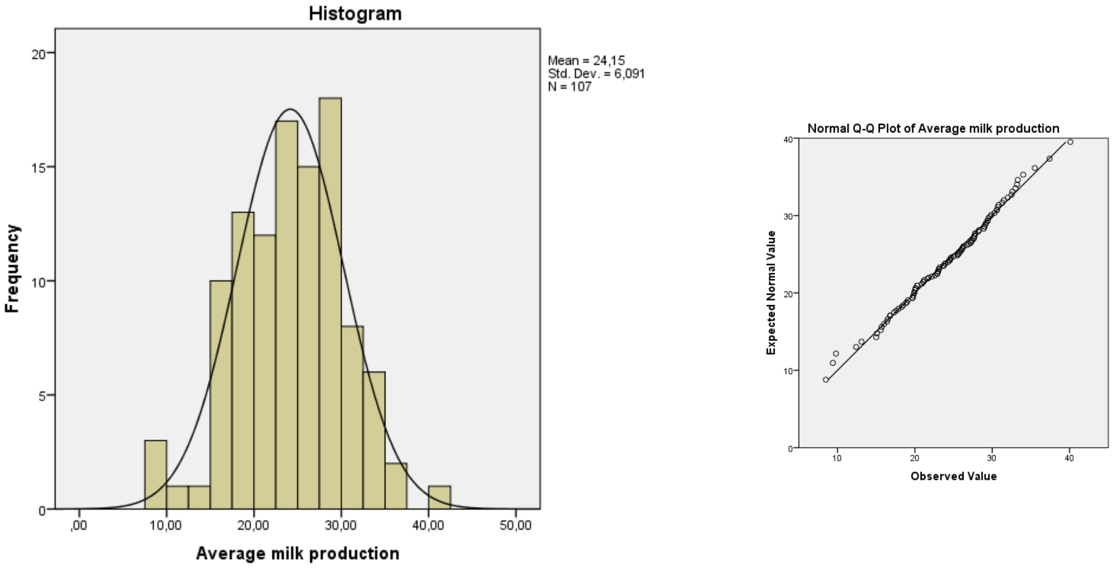


Fig. 5. Histogram and Q-Q plot of milk production

Multiple linear regression model

In the multiple linear regression model with all explanatory variables (waiting time, milk production, temperature and rain) waiting time was found to be significant (P = 0,000). Temperature was marginally significant (P = 0,057), and rain and milk production were found to be insignificant (P = 0,183 and 0,205 resp.). Milk production, being the least significant, was then removed from the model. In the model with waiting time, rain and temperature as explanatory variables, waiting time was again significant (P = 0,000). Temperature was not significant anymore (P = 0,060) and rain was again insignificant (P = 0,170). Rain, being the least significant, was then removed from the model. In the linear regression model with waiting time and temperature, the significance of temperature was P = 0,123, and waiting time was again significant (P = 0,000). Finally, the simple linear regression model for SW-index and waiting time showed waiting time to still be significant (P = 0,000) (table 3). Waiting time, being significant every time the model is produced, significantly influences the SW-index. Using the linear regression model, a formula calculating the SW-index can be drafted. The standard formula for a linear relation is y = ax + b, ‘a’ being the slope of the variable (0,001) and ‘b’ being the constant term for the calculated variable (in the model 1,275) (table 3). SW-index can thus, according to this model, be calculated with SW-index = 0,001 x W(aiting time) + 1,275. Thus, the minimal SW-index of a cow in this model is 1,275, and every minute it has to wait longer, the SW-index increases 0,001, as shown in fig. 6.

Coefficients[a]

Model		Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
1	(Constant)	1,275	,045		28,319	,000
	Waiting time	,001	,000	,347	3,792	,000

a. Dependent Variable: SWindex

Table 3. Simple linear regression of SW-index and waiting time

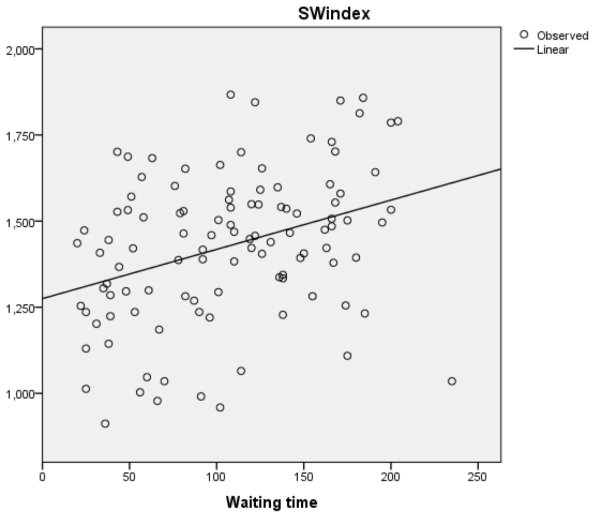


Fig. 6. Linear relation of SW-index and waiting time

Discussion

In this study the amount of stress dairy cows experience during queuing for the milking parlor was investigated, using an ethogram to observe behavioral diversity. By calculating the SW-index for 107 dairy cows these data could be compared to several possible stress influencing factors (waiting time, temperature, rain, milk production) in a multiple linear regression model. It was hypothesized that waiting time influences the SW-index positively. The only significant factor appeared to be waiting time; the longer a cow had to wait before getting milked, the higher its SW-index got (0,001 per minute). The present study thus suggests a positive relation between waiting time and stress. It is, however, important to realize that in this study a derivate of stress, the SW-Index, is investigated. Several studies[8-10] suggest the SW-index to be a reliable indicator of stress. These studies have used the SW-index as a positive indicator of stress in (stereotypic) behavior of zoo animals, assuming that more species specific natural behavior indicates less stress. Miller et al. (2016) showed that when the SW-index increased in zoo cheetahs, fecal glucocorticoids decreased[8]. The present study, however, suggested the opposite. The longer cows had to queue before getting milked, the higher the SW-index got. The positive relation between stress and a greater behavioral diversity is a finding that a study of Raussi et al. (2005) concluded as well in socially stressed heifers[7]. As stated in the introduction, the contrary outcomes of different studies suggest that an optimum for behavioral diversity exists. Furthermore, one has to realize that performing stereotypic behavior in a zoo is not the same as the behaviors that were recorded in the ethogram in the present study. Further research to the influence of behavioral diversity on physiological and biochemical factors such as heart rate or stress hormones (glucocorticoids) is needed to further determine the exact effect.

As stated in the introduction, multiple environmental and endogenous factors may have an influence on the presence of stress. In the present study, a few of these factors were investigated; waiting time, temperature, rain and milk production. However, multiple other factors could have influenced the behavior of the cows. Examples of environmental factors influencing the behavior of the cows during this study could have been e.g. the farmer working next to the waiting area with different machines, which disturbed the cows more or less, the different walk that the cows had to undertake every day from the pasture to the milking parlor (differing in length; from 2 to sometimes 30 minutes), the presence of the observers during queuing, the presence of the gaucho trying to get the cows in as soon as possible using both verbal and manual ways for this, etcetera. Also endogenous factors could have influenced the behavior and stress level of animals; health in general, reproduction cycle activities, but also characteristics of an individual and the hierarchy in the group[13].

During the study, it was sometimes impossible to start the measurements directly from the beginning of the milking process. This could of course influence the results.

The outside temperature used as a factor was estimated using the daily weather forecast from multiple websites, and subsequently the estimated temperature by being outside during the measurements for the research. This could mean that it differed from the real outside temperature. The milk production of the used cows was measured only once a month, so it cannot be excluded that this has an influence on the SW-index of dairy cows.

Overall, it can be concluded that more research is necessary to determine whether the SW-index is a true indicator of stress, and to investigate other factors that influence stress during queuing for the milking parlor.

Conclusion

Having studied several factors possibly influencing stress of dairy cows during queuing for the milking parlor, the results of the present study show that waiting time influences the SW-index significantly. The main question of the present study, 'how does waiting time before getting milked influence the SW-index in dairy cows?' is thereby answered. The SW-index can, according to this model, be calculated with H = 0,001 x W(aiting time) + 1,275. As can be seen in the results of the regression model the slope of the waiting time is 0,001, indicating that waiting time influences the SW-index positively. Every minute a dairy cow had to wait longer, the SW-index would increase with 0,001. Thus the hypothesis, stated in the introduction, was right. The other factors used in this research, temperature, rain and milk production, had no significant influence on the SW-index.
The SW-index is a calculation of behavioral diversity. Assuming that stress can be indicated by an increase in the diversity of an animal's behavior, this study showed that queuing before the milking parlor is a causal factor for stress in dairy cows. Understanding the behavior of cows in the milking process and pointing out components in this process that lead to stress could result in improvements in this process.

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