Is the incidence of clinical mastitis associated with changes of weekly average dry matter intake in lactating dairy cows?

Julia Brennecke [1], Ulrike Falkenberg [2], Nicole Wente [3] and Volker Krömker [4]

[1] Department of Microbiology, Faculty of Mechanical and Bioprocess Engineering, University of Applied Sciences and Arts, Hanover 30453, Germany; brenner3108@googlemail.com
[2] Cattle Health Service of the Mecklenburg Vorpommern Animal Disease Fund, Neustrelitzer Str. 120/C, D-17033 Neubrandenburg, Germany; u.falkenberg@tskmv.de
[3] Department of Microbiology, Faculty of Mechanical and Bioprocess Engineering, University of Applied Sciences and Arts, Hanover 30453, Germany; nicole.wente@hs-hannover.de
[4] Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark, volker.kroemker@sund.ku.dk

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Abstract

The aim of this cross-sectional study was to test whether there is an association between clinical mastitis incidence and variations in dry matter intake in lactating dairy cows. Data were collected and analyzed from two voluntarily participating dairy herds (1,000 -1,200 cows) between 2017 and 2018. Lactating cows were assigned to seven "effective" husbandry groups (HGeff), considering important performance parameters such as lactation number, lactation day, reproductive status, and health status. The average daily dry matter intake of a cow in a husbandry group was determined once a week. Dry matter was determined using dehydration equipment that dried the fresh masses of the total mixed ration (TMR) in a standardized way. The incidence of clinical mastitis was calculated for different aetiological groups (environment associated mastitis pathogens, cow-associated mastitis pathogens, NAS (non-aureus staphylococci) and no growth cases). Dry matter intake (DMI) per individual cow was calculated as the averaged value plus the associated standard deviation (DMI (sd)) from weekly examinations of each husbandry group (HGeff). The average dry matter intake per cow per day was 23.6 kg +/- 3.7 kg. Environment associated pathogens were found in about half of all clinical mastitis cases (49.4 %). Cow-associated pathogens were found in 4.8 % of clinical mastitis cases. In all models, the different clinical incidences of mastitis studied were significantly associated with HGeff. In most cases, the incident rates were significantly higher in the fresh milking and high milking groups compared to the other groups. The incidence of clinical non severe mastitis cases (only mild and moderate cases) caused by environment associated microorganisms was further associated with variation in dry matter intake, with higher variation related to higher clinical mastitis incidence. Further studies are needed to verify this association.

Keywords: Mastitis, DMI, Cow

Introduction

Anecdotal reports have shown a correlation between feed intake and the incidence of mastitis. A common measure of feed intake in dairy cows is dry matter intake (DMI) [1]. As there are few reports in the literature on dry matter intake and mastitis, it seems even more important to further investigate the issue of feeding and the resulting incidence of mastitis. In their study, Becker et al. describe that there is little knowledge about phenotypic and genetic correlations between disease susceptibility and DMI, as recording feed intake and accurately recording diseases is expensive and, moreover, the joint evaluation of both types of data is not trivial [2]. Cows are capable of devouring at least 4 % of their live weight in dry matter (DM) [3]. The mean DMI of cows is related to lactation number, lactation status, and body condition score (BCS) [4]. A reduced DMI is a risk factor for postpartum diseases (postpartum paresis, metritis, abomasal dislocation) and associated losses in milk production [5]. According to studies by Grummer et al. [6], only 18 % of the variation in DMI intake in cows is due to the importance of parity, body condition score, and various feed components. Obviously, there are many other factors affecting DMI that need to be identified. Aspects of farm management that may influence animal stress need to be investigated, especially around calving when cows are naturally susceptible to reduced feed intake [6].

It is unclear to what extent feeding and, in particular, DMI or variations in DMI are related to the occurrence of mastitis. However, such a relationship has been reported anecdotally in the past. Feeding-related issues that may be associated with decreased mammary gland defense mechanisms include feed quality, feeding management, ration changes or modification of individual feeding [7]. Good basic forage qualities and silage qualities that are as consistent as possible are associated with better udder health [8]. Avoiding unstable feeds or those with a high proportion of very rapidly digestible starch are associated with better udder health [9]. A small bunk space increases the number of aggressive interactions between the animals and reduces feed intake, which has a negative effect on milk production [10]. According to Barkema et al. [11], DMI is higher and mastitis rates are lower when number of feeding places per cow is > 0.8 to < 1.1 versus ≤0.8. Another aspect is lactation stage-adapted feeding; for example,

avoidance of obesity in heifers and dry-cows is associated with lower rates of new mastitis [12]. Systematic monitoring of the risks described above, as well as careful feed selection, feeding, and animal control, can limit the resulting udder health disorders [13]. In addition, the dry matter content of the total mixed ration (TMR) is critical because if a ration is suboptimal, intake will also be reduced due to slowed rumen fermentation [14]. Inadequate water supply or poor drinking water quality in addition to affecting health leads to reduced feed intake and reduced milk yields as well as udder health disorders [15].

At the beginning of lactation, an energy deficit is almost always present in high-yielding cows. This energy gap is due to inadequate feed intake compared to milk yield [16]. Cows do not reach their maximum feed intake capacity of > 20 kg dry matter until the 12th week of lactation, with a peak milk yield as early as the 8th week of lactation [17]. If an attempt is made to compensate the energy deficit, rumen acidosis can occur very quickly. Rumen acidosis can be accompanied by secondary diseases in the claw and hock area, which, in turn, have a negative effect on feeding performance, especially in loose housing.

Variations in DMI are associated with increased risk of undesirable acid-based imbalance and signs of adverse rumen physiology (diarrhea or left abomasal displacement) [18]. This significantly affects the immune system of the animal [19]. Farm management continues to be a crucial factor, as the feeding person, the quality of the basic feed, and the ranking of the animals are significantly decisive for DMI fluctuations [18]. Another study suggests that there may be greater variation in DMI, rumination time, reticulorumen pH, and milk production in early lactation when animals are fed a diet of longer straw particles [20]. In addition to the influences on the clinical incidence of mastitis, which are mapped by the husbandry group (including the differences in DMI), we explicitly looked at the variation in dry matter intake over time, independent of diseases in a husbandry group. For this purpose, the weekly mean dry matter intake for each husbandry group and the variation thereof in a three-week rhythm were used.

The aim of this study was to investigate whether average DMI or variations in average DMI from week to week of a mean individual cow in a husbandry group are associated with the occurrence of clinical mastitis in the individual animal.

Material and Methods

Study design - farm demographics: A cross-sectional study was carried out from February 2017 to January 2018 on two commercial dairy farms in Mecklenburg-Western Pomerania, Germany. The herds consisted of 1,000 and 1,200 Holstein-Friesian dairy cows. Annual milk yield (305d) ranged from 11,000 to 13,202 kg energy corrected milk (ECM)/cow, with a bulk milk somatic cell count of 164,000 to 280,000 cells / mL. All study animals were housed in free-stall barns with cubicles. A modern outside rotary milking parlor with 60 places was located in the middle of the barn on both farms. The cows received a TMR depending on their production level and all lactating cows were milked three times a day. On both farms, the barn was equipped with overhead belt feeding. The prepared forage was transported from the mixer to the individual feeding tables via belts and evenly distributed in the barn. This was ensured by a metering unit that loaded the exact amount of TMR over to the belts by computer. The exact amount of feed was determined by lactation performance and group. Fresh cows, mid lactation, and old lactation cows were fed serval times per day. The belt feeding system used in the experimental farms controlled the DMI concerning the time interval between two feed presentations and not the residual amount. Both farms made an effort to maximize the dry matter intake. The lactating cows were housed in individual husbandry groups (HG) including 45 – 90 animals, considering lactation number, days in milk (DIM), reproductive status and state of health. Cows of the same performance class and of the same feeding ration were grouped in one HG. Animals from the 11 individual farm-specific husbandry groups were assigned to seven husbandry groups (HGeff) considering important performance parameters such as lactation number, lactation day, reproductive status, and health status (Table 1). The effect of diseases that only occur in certain lactation phases was taken into account in the HGeff.

Data collection and sampling: Clinical mastitis was detected by fore-milking by trained milking personnel immediately prior to milking. Each clinical mastitis case was assigned a corresponding mastitis grade according to the International Dairy Federation (IDF) [21]. If clinical mastitis was detected, a quarter milk sample was collected aseptically from the affected quarter for cytomicrobiological examination. The sample tubes contained a boric acid-based preserving agent ("Ly20") as a stabilizer and were stored cool [22]. According to recognized studies, the stabilizer "LY20" is suitable and approved for both cytological and cultural examination of udder pathogens [22]. Each animal was treated appropriately based on the farm-specific treatment plans after mastitis has been detected. All milk samples were sent to the laboratory at the University of Applied Sciences and Arts Hannover once a month for cytomicrobiological examination. Once a week, a dry matter sample was taken from the appropriate feeding belt for each husbandry group. The exact procedure is described in the section Dry matter determination of the TMR.

Laboratory procedures - milk samples: Microbiological analysis of the milk samples was performed in accordance with the GVA guidelines [23]. A total of 10 µL of each milk sample was cultured on esculin blood agar (Oxoid Deutschland GmbH, Wesel, Germany). . The plates were analyzed after a 24-hour and 48-hour incubation period at 37 °C. The grown colonies were initially differentiated by their hemolysis status, esculin hydrolysis, cell morphology, and Gram staining. The exact mastitis diagnostic has already been described [24].

Dry matter determination of the TMR: Once a week, the dry matter of the presented full TMR per husbandry group was determined and converted to the mean DMI per cow and feeding group by weighing and backweighing the leftover ration. For this purpose, 100 g of the fresh masses of the full TMR of each of the different husbandry groups were taken and dried in duplicate at 70°C for four hours using a drying apparatus (Concept, Gobi). The dried material was weighed and the dry matter content was calculated from the fresh matter and dry matter values. The fresh mass of the submitted TMR was recorded and documented weekly for each husbandry group. From the total dry matter, the average DMI for the individual animal could be calculated. Thus, the following parameters of DMI were available: absolute average DMI per animal; week-to-week variation in DMI (standard deviation). The mean DMI variation and the standard deviation of the DMI variation of the husbandry group were calculated from the DMI of the previous week, the current week and the following week of a group, respectively.

Clinical mastitis incidence: Various clinical mastitis incidences were calculated per 100 cow weeks under risk. The total clinical mastitis incidence(MI) included mild, moderate, and severe cases (M1-M3) for all pathogens, and the clinical mastitis incidence caused by different pathogen groups (environment-associated, cow-associated, and no growth/opportunistic MI.) Since a disease-related decrease in DMI can be assumed for severe mastitis, the incidences of mild and moderate mastitis were also combined as another possible outcome variable.

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Table 1: Description of the different husbandry groups in the 2 experimental farms + weekly averaged dry matter intake (dry matter intake, mean and standard deviation) per husbandry group

HG(eff)[1]	Group characteristics	Farm 1 [2]	Farm 2 [2]	DMI (mean)[3]	DMI (sd)[4]
1	hospital pen	45	73	23.6	2.3
2	fresh cows	80	174	24.1	2.0
3	high lactation	139	178	24.2	2.0
4	high lactation	0	344	24.1	3.6
5	mid + late lactation	191	268	23.2	2.2
6	late lactation	184	166	21.9	3.0
7	mid lactation	187	0	24.2	2.2

[1] : Husbandry group - cows of one performance class and the same feeding ration combined
[2] : mean number of animals per HG/farm
[3] : average DMI per cow (mean)
[4] : DMI variation per cow measured over three weeks (standard deviation)

The effective husbandry groups (HGeff) consisted of a total of seven groups and meant that cows with the same feeding and lactation from both farms were grouped together (Table 1).

Statistical analysis: Data were collected using Excel 2010 (Microsoft Inc., Redmond, WA, USA) and analyzed using SPSS (SPSS 26.0, Chicago, IL, USA). The statistical unit was the husbandry group per week (repeated measurements). Since the groups were dependent on lactation numbers and lactation stages, effects on DMI (diseases close to birth) as well as effects on clinical mastitis incidences were also mapped by this group classification. The classification of the groups reflected the lactation period. The diseases were ordered according to the lactation stage they occurred in and thus grouped under the aspect of HGeff. Generalized mixed models were calculated for statistical analysis. The dependent variables were the total number of clinical mastitis incidences (clinical mastitis cases per week per cows under risk in a husbandry group; MI), total mild and moderate MI; environment-associated MI; cow associated MI and no growth/opportunistic MI. Explanatory variables were the effective husbandry group (HGeff), mean DMI per husbandry group and standard deviation (sd) as variation of DMI. The predictor effective husbandry group was used to group according to the results of lactation stage (early, medium, late, and special groups (heifers, long milkers, mastitic cows, health status). Farm and husbandry group on a farm were included as (nested) random effects. The multivariable analysis was performed using a backward stepwise selection and elimination procedure. After each run, the variable with the highest p-value was excluded from the model until all variables had $p \leq 0.05$. The most optimal model was evaluated using the Akaike information criterion (AIC), where an AIC with the lowest value indicated the best model. Confounding was monitored by the change in the coefficient of a variable after re-moving another variable from the model. If the change of the estimates exceeded 25 %, the removed variable was considered a potential confounder and was included again in the model. In the final models, biologically plausible two-way interactions were tested. Model fit was evaluated by checking normality of the residuals. Least square means from the model were calculated for the HGeff groups. A p-value < 0.05 was considered indicative of a statistically significant difference.

Results

The objective of this study was to determine whether there is an association between parameters of DMI and mastitis incidence in dairy cows.

DMI: DMI was calculated as the averaged value plus the associated standard deviation (DMI (sd)) from the weekly examinations of the husbandry groups (HG). This resulted in an average DMI of 23.6 kg +/- 3.7 kg. The mean DMI per husbandry group of the respective current week, the previous week, and the post-week over a total of 52 weeks were considered. The maximum average DMI per cow was 24.2 kg (high lactation) and the minimum was 21.9 kg (late lactation, Table 1).

Table 2: Mean incidence of mastitis per husbandry group and 100 cow weeks at risk

		Pathogen group/ mastitis incidence			
HG(eff)	Mean mastitis incidence[1]	Environment[2]	Cow[3]	NaS/ (others)[4]	No growth[5]
1	0.82	0.54	0.00	0.10	0.18
2	2.20	1.03	0.00	0.64	0.53
3	2.28	0.99	0.00	0.77	0.52
4	1.60	0.72	0.01	0.49	0.38
5	1.13	0.45	0.04	0.36	0.28
6	0.62	0.25	0.01	0.19	0.17
7	0.84	0.38	0.04	0.21	0.21
Total	1.35	0.59	0.02	0.40	0.34

[1] : mastitis incidence is the rate of new disease in animals under risk and time (mastitis incidence per 100 cow weeks under risk)
[2] : incidence of mastitis with environment-associated (IMI)
[3] : incidence of cow-associated mastitis pathogens
[4] : incidence of minor pathogen e.g. NaS (non-aureus staphylococci) and others such as Coryneforms
[5] : incidence of no bacterial growth

Mastitis incidence: Mastitis incidence is the rate of clinical mastitis of animals under risk and time (per week). There were 1.35 clinical mastitis cases per 100 cow weeks under risk in an HGeff. The incident rate of mastitis per HGeff during the experimental period is shown in Table 2. A total of 1090 clinical cases of mastitis were enrolled in the study from February 2017 to January 2018. Most cases of clinical mastitis were caused by environmental pathogens. Environment-associated pathogens occurred in 49.4 % of mastitis cases. Cow-associated pathogens occurred in 4.8 % of cases. NAS were found in 5.4 % of mastitis cases. No bacteriological growth was detected in 33.2 %. A total of 0.4 % of the samples were contaminated and two different microorganisms (mixed) were detected in 4.9 % of the samples (Table 3).

Table 3: Microbiological findings of the mastitis samples

Microbiological Findings	Farm1_n	Farm1_%[1]	Farm2_n	Farm2_%[1]	Total_n	Total_%[1]
Environment-associated[2]						
S. uberis	180	32.0	113	21.4	293	26.9
E. coli	63	11.2	66	12.5	129	11.8
S. dysgalactiae	44	7.8	36	6.8	80	7.3
Other Streptococci	2	0.4	1	0.2	3	0.3
Coliforms	4	0.7	7	1.3	11	1.0
Coryneform bacteria	7	1.2		0.0	7	0.6
Enterococcus spp.	6	1.1	10	1.9	16	1.5
Cow-associated[3]						
S. aureus	14	2.5	20	3.8	34	3.1
T. pyogenes	12	2.1	7	1.3	19	1.7
Others						
Pseudomonas spp.		0.0	4	0.8	4	0.4
Bacillus spp.	2	0.4	1	0.2	3	0.3
Yeasts	6	1.1	6	1.1	12	1.1
Klebsiella spp.		0.0	1	0.2	1	0.1
NaS[4]	19	3.4	40	7.6	59	5.4
Mixed	27	4.8	26	4.9	53	4.9
Contaminated[5]	2	0.4	2	0.4	4	0.4
No growth	175	31.1	187	35.5	362	33.2
Total	563	100.0	527	100.0	1090	100.0

[1] : percentage of mastitis of the total mastitis cases (within farm 1, within farm 2 or for both farms together)
[2] : environment-associated mastitis-causing microorganism
[3] : cow-associated microorganism
[4] : non-aureus staphylococci
[5] : more than two different pathogens were detected

Associations - multivariable analyses: We applied models with different mastitis incidences (MI) as target variables. These variables were the overall mastitis incidence, MI caused by environmental associated pathogens and the MI caused by cow associated pathogens and MI caused by opportunistic pathogens and mastitis cases without detection of causing pathogens. We also applied the models for the mastitis incidence rates respecting only mild and moderate mastitis cases.

Except for the clinical mastitis incidence models of cow-associated mastitis pathogens, which could not be formed, all models showed that mastitis incidence was significantly associated with HGeff. Significantly higher mastitis rates (in all variants) were found in HGeff of fresh cows and high yielding cows compared to all other HGeff (Table 4). Since the groups were dependent on lactation numbers and lactation stages, effects on dry matter intake (diseases close to birth) were also mapped by this group classification. Thus, the variable HGeff included the known lactation-dependent risks on the clinical incidence of mastitis.

An association of DMI, and in particular the influence of DMI variation per cow measured over three weeks (DMIsd) on mastitis incidence could be shown for environmental pathogen-associated mild and moderate mastitis incidence ($p = 0.03$) only. The incidence of mastitis increased with increasing variation in DMI (measured over three weeks).

Discussion

The aim of the present study was to investigate whether the occurrence of mastitis in dairy cows is related to DMI. The data from our study showed that in the case of environmental mastitis, regardless of all already known risk factors which are compromised in the husbandry groups, variation in dry matter intake was a significant factor. This possibly means that beyond the lactation-dependent influence on the clinical incidence of mastitis, this is influenced under certain conditions by the variation in dry matter intake. This applies to mild and moderate cases of clinical mastitis caused by environment associated mastitis

Table 4: Results of multivariable analyses (mastitis incidence for non-severe mastitis with environment-associated mastitis pathogens only)

	Estimates[a]					
Variables	**Average**	**Std. Error**	**df**	**Sig.**	**95% Confidence Interval**	
					Lower	**Upper**
HG_eff_1	0.003[b]	0.0013	425.17	0.172	0.002	0.004
HG_eff_2	0.006[b]	0.0003	424.74	0.001	0.005	0.008
HG_eff_3	0.007[b]	0.0011	426.33	0	0.006	0.008
HG_eff_4	0.007[b]	0.0013	401.76	0.036	0.005	0.009
HG_eff_5	0.004[b]	0.0011	423.95	0.781	0.003	0.005
HG_eff_6	0.002[b]	0.0011	417.23	0.136	0.001	0.003
HG_eff_7	0.004[b]	0.0011	420.31	0	0.002	0.005
DMIsd	0.002	0.0009	2.172	0.03	0	0.004

a. Dependent variable: incidence of non-severe clinical mastitis with environmental IMI
b. The covariates in the model were calculated using the following values: DMIlog_sd = 1.265

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pathogens. The dry matter intake of each group was not significantly associated with the incidence of mastitis.

In our study the mastitis incidences were calculated for effective husbandry groups (HGeff). For the formation of these groups lactation number, days in milk (DIM), reproductive status and state of health were considered. Cows in the same performance class and with the same feeding ration were grouped. The aforementioned parameters were risk factors for mastitis which were unevenly distributed in the husbandry groups. The risk of a clinical mastitis occurring is higher in fresh cow groups and high yielding cows as described by Schmenger and Krömker [25].

A negative energy balance in early lactation occurs frequently in dairy cows [26]. With negative energy balance, cows are under metabolic stress, which, in turn induces immune suppression. This also leads to a higher susceptibility to diseases such as mastitis, hoof and leg diseases and metabolic diseases [27]. Any decrease in average DMI as a percentage of body weight increases the likelihood of ketosis and clinical mastitis [28]. Consequently, there are phases in lactation when low DMI occurs due to poor feed supply and insufficient feed quality or sick cows that do not eat worsens the energy balance and further negatively affects health [29]. These effects are mapped in our study by HGeff.

The effect of dry matter variation on clinical mastitis rates that we found cannot be explained by the above arguments alone. This effect is also present in later lactation. Furthermore, absolute dry matter intake is not significantly associated with clinical mastitis incidence, only variation.

High quality and accurate ration calculation are crucial for an adequate dry mater intake [30]. If the dry matter of the TMR is insufficient, it will lead to a shift in the energy, protein, and crude fiber content of the total mixed ration. The passage rate is too slow and the feed intake of the cow decreases. Overfeeding of some minerals, too much degradable protein or degradable starch in the ration, not infrequently results in a very liquid slurry with a consistency like pea soup (mushy and watery) [31]. The resulting contamination of the udder and manipulation of the teat canal by daily milking pave the way for invading bacteria. These results emphasize the importance of efficient management and the use of protocols to monitor DMI [32]. These aspects are possible explanations for the found association. Determining the causes of this effect is reserved for further research.

The mastitis incidence in the present study was 1.35 % per week with a total of 1090 cases during the trial period. Compared to the usual international target values for mastitis incidence, these values are high [33]. Possible explanations for this are that first and recurrent cases were enrolled and not registered separately. Moreover animal husbandry in old barn buildings, difficult hygienic conditions in the barn and many chronic mastitis cows were contributory factors. On the other hand, detection rates of mastitis were good. When recording mastitis, the milkers proceeded according to a defined scheme for the assessment. It is common knowledge that different personnel influence the registration of mastitis cases. However this was not the case in the current study as we had the same milking personnel during the entire trial period. Furthermore, the laboratory work was standardized and unaffected by variations.

DMI variation was a significant factor in the models with and without severe mastitis cases. This is important because significance in all mastitis cases could also be interpreted as an effect of reduced dry matter intake in the animal severely affected by mastitis in HGeff. Thus, the model is valid for mild and moderate cases of mastitis.

It would be beneficial to conduct a follow-up study, preferably in an experimental barn with weighing troughs and installed cameras, so that an accurate statement can be made regarding dry matter intake of individual animals in relation to mastitis incidence.

Conclusion

The present study has shown that on the experimental farms the incidence of clinical mastitis caused by environmental pathogens is associated with variation in dry matter intake. The incidence increased with increasing variation in dry matter intake. The results indicate that uniform dry matter intake should be strived for in herd management and that monitoring the mean dry matter intake of animal groups is useful for identifying disease risks. DMI monitoring offers the farm manager the possibility of quickly and easily checking his own feedings and reacting accordingly to the results to ensure optimal rationing of the herd.

Disclosure of conflicts of interest

The authors declare no conflict of interest.

Compliance with Ethical Standards

This study has been conducted in compliance with ethical standards. All applicable guidelines for the care and use of animals were followed. The study was performed in accordance with the guidelines of the Declaration for the Care and Use of Animals, and was reviewed by the Animal Welfare Commission of the University of Applied Sciences and Arts Hannover, Hannover, Germany, and approved by the Animal Welfare Officer (file number of the Animal Welfare Office TVO-2017-V-79; date of approval July 5, 2017).

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