1 2	Association of body condition with lameness in dairy cattle, a single-farm longitudinal study
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6	SUPPLEMENTARY FILE
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8	Supplementary Material & Methods
9	Because all the assessments for the current data set were conducted as a standard procedure on
10	the farm, this data collection is not considered an animal experimentation under German law
11	and did not, therefore, need a special permit.
12	This project was conducted with the 288 German Holstein dairy cows kept on the LVAT –
13	Institute for Animal Breeding and Husbandry (Groß Kreutz, Brandenburg, Germany) from Feb-
14	ruary to October 2019. Cows were milked using a unit of an automatic milking system for each
15	group (Lely Astronaut, Lely Holding S.à.r.l., Maassluis, The Netherlands). They were fed a
16	maize-grass-silage-based total-mixed-ration dependent on the lactation stage, received individ-
17	ual performance-based quantities of concentrate feed at milking, and had ad-libitum access to
18	water.
19	All barns provided a cubicle housing system. In a first barn, one of two high-yielding groups
20	(approx. 55 cows) was kept on a grooved solid floor, with deep lime-straw cubicles and an
21	animal-to-cubicle ration of 1:0.9. In a second barn, the second high-yielding group (approx. 55
22	cows) and the late-lactating cows (approx. 65 cows) were located on a slatted floor (partly cov-
23	ered with rubber for the high-yielding group) and elevated cubicles with a rubber cover at ani-
24	mal-to cubicle ratios of 1:1.2, and 1:1.1, respectively. Cows were moved to the late-lactating
25	group with a performance of < 30 l/d, after at least day 100 into lactation and with a BCS of at
26	least 3. A pen for cows due for calving, the calving pens, and a pen for freshly-lactating cows
27	were also located in the second barn. All these pens had a grooved solid floor and deep-bedded
28	group areas for lying. About 8 weeks before expected calving (and with a performance of < 30
29	l/d), the cows were dried-off. Manure was regularly scraped in the activity areas by fixed
30	scraper, by hand, or by robot.

The claws of all lactating cows were routinely treated by a professional claw trimmer three times yearly. Around the time of the current observations, treatments took place in November 2018, on April 8-10 2019, August 8-9 2019, and November 2019. In addition, cows with claw problems were treated about once a month. In acute cases, specially trained personnel of the farm treated claws and, a veterinarian was consulted if medical treatment seemed necessary.

At the end of each month (except for March), all cows were scored for lameness and body condition by the first author (MK). At each of eight monthly scoring time-points 235 ± 8 (mean \pm SD) cows were scored. A total of 1877 scorings were completed (6.5 ± 2.3 per cow). A preliminary visual inspection of the data revealed that dry cows had a clearly higher BCS than the cows in milk, as expected. To avoid collinearity between these two variables, the dry cows were dropped from the data for evaluation. Accordingly, a total of 1544 observations, (mean \pm SD) 5.36 ± 1.89 observations/cow, were modelled.

43 To assess lameness, cows were marched on a straight route without obstacles across a distance 44 of several meters. The assessment was based on the gait pattern of the cow, the homogeneity of 45 the weight distribution on the claws, the regularity of the step lengths, and the body posture 46 (curvature of the back and head position) according to the six-level score of Ebert et al. (2019, 47 modified after Starke et al. 2007). The first author had been trained in two workshops to assess 48 lameness (and BCS) taught by veterinarians. In addition, she could discuss and compare her 49 scoring with two of the workshop teachers during the April and May assessment. For the eval-50 uation of the risk for lameness, we only differentiated whether cows reached a score of three or 51 higher rather than a score of 1-2 (non-lame or slightly "clammy"). As a check for intra-observer 52 reliability, the first author scored 33 randomly selected cows on one day and five days later 53 before the investigation started. 87 % of the cows were identically re-classified as lame or non-54 lame.

55 Whenever lameness of a cow was scored, her **body condition**, was assessed simultaneously. 56 Cows were scored when they were standing either at the feed table, in the activity area or a lying cubicle. This was done visually and by palpation following the schemes of Ferguson et 57 58 al. (1994) complemented by the procedure as visualised in Spengler Neff et al. (2015). Scores 59 went from 1 (very lean) to 5 (very fat) in steps of a quarter score. At mean scores around 2.5, 60 this procedure is inexact, and the scheme of Edmonson et al. (1989) as modified by Metzner et al. (1993) was additionally used. Eight body parts were scored according to his rule, averaged 61 and eventually rounded to the nearest quarter score. Intra-observer agreement for BCS scoring 62 63 was also checked. The same 33 randomly selected cows used for re-assessment of lameness were also scored for BCS. 94% of the cows did not differ in their assessment or differed only
by a quarter score. The remaining 6% differed by half a score. In addition, pictures of 9-14
cows at each monthly scoring were taken and re-assessed after a month. Here, the assessments
again differed by a maximum of 0.5 points.

68 These lameness and BCS scores were complemented with information on lactation stage, lac-69 tation number, medical treatment, season, and time since the last claw trimming event. Based 70 on bi-variate plots, we ensured that our predictor variables were not strongly collinear. All var-71 iables varied within-cows except for lactation number that was kept constant within cows. Each 72 observation of a cow was as assigned to a lactation stage based on the number of days since 73 her last calving: 0 to 80, 81 to 160, 161 to $300, \ge 301$ DIM as well as dry cows. Observations \ge 74 301 DIM were relatively rare such that they were pooled with the category of 161 to 300 DIM 75 (resulting in a four-level factor). Only a minority of the cows changed between lactation num-76 bers in the course of the observations. Therefore, we assigned a value of "low" for all cows 77 that were observed in their first lactation and that have moved to the second during the current 78 investigation. All cows in lactation number two or three that may have moved to the next higher 79 lactation number were assigned a value of "intermediate". All the other cows that were at least 80 in lactation 4 (and up to lactation number 9) at the beginning of the investigation were assigned 81 a value of "high" for their lactation number (resulting in a three-level factor). If a cow had a medical treatment in the interval from the 15th of the previous to the 14th of the month follow-82 83 ing the current scoring, with a diagnose other than the locomotor system, and based on the 84 recordings in the herd management software, she was considered "treated" (two-level factor). 85 To investigate a seasonal effect, the monthly scorings were summarised. February, April and 86 May were assigned to "winter-spring", June, July, and August to "summer", as well as Septem-87 ber and October to "fall" (resulting in a three-level factor). The time since the last claw trim-88 ming event was determined (as a continuous variable). Young cows that had never been 89 trimmed were assigned a value of 250 d, a value which was slightly higher than the highest 90 value observed otherwise (244 d).

We evaluated the data using a Bayesian generalised linear mixed-effects model in R Version 4.0.2 (R Core Team 2020) using the package blme (Chung et al. 2013, which is based on package lme4, Bates et al. 2015). We considered lameness as the dichotomous outcome variable and used the other variables as fixed predictors. The continuous fixed effects (BCS and time since the last claw trimming event) were normalized and sum-contrasts were used for all factor variables (lactation stage, lactation number, medical treatment, season) such that p-values of main

97 effects remain meaningful even in the presence of interactions. We aimed at a maximum model 98 including up to all three-way interactions, but this model resulted in too many cases of full 99 separation and could not be estimated even with the Bayesian approach. This indicates an over-100 specification of the model. Therefore, we modelled all main effects plus all their two-way in-101 teractions. Cow identity was used as a random effect to account for the repeated measurements 102 of each cow. We set up a full model, compared this with a minimal model including an intercept 103 only on the one hand and with models that each omitted one of the fixed effects on the other. 104 To do so, one would ideally use parametric bootstrap (package pbkrtest, Halekoh & Højsgaard 105 2014). Given the sample size and the complexity of the model here, the parametric bootstrap 106 would have needed an enormous amount of time (the calculation of the p-value for the full 107 model ran longer than 24 h). Because all (except one) fixed effects were on the lowest hierar-108 chical level of the random effect (the model error), pseudoreplication issues do not seem to be 109 of high risk and we, therefore, used a likelihood-ratio test to compare models instead. Model 110 assumptions were checked using a graphical analysis of the residuals (package DHARMa, Har-111 tig 2020). This analysis suggested that the relationship of the risk of lameness and BCS was 112 non-linear. We therefore included the square of BCS as an additional fixed effect (including all 113 interactions of this quadratic effect with the other fixed effects). We focus here on the effects 114 found that included BCS as our main focus of the study and those effects with a low p-value. 115 We are fully aware that a high p-value alone does not indicate no relationship of those effects 116 with lameness. Still, the effects with a low p-value are those that can be more strongly substan-117 tiated statistically in this data set with a given between- and within-cow variability.

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