Hair cortisol concentrations in different breeds of cows: Comparison of hair from unshorn and previously shorn areas and from various regions of the body

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Haarcortisolkonzentrationen bei Kühen verschiedener Rinderrassen, in natürlich gewachsenem und in nachgewachsenem Haar sowie in Haar verschiedener Körperstellen

Das Ziel war es, die Haarcortisolkonzentrationen (HCC) bei 7 Rinderrassen zu untersuchen, Referenzwerte für Braunviehkühe zu bestimmen und 4 Entnahmestellen in Bezug auf die HCC zu vergleichen. Die Untersuchungen wurden an 3 Gruppen von Kühen durchgeführt. In der Gruppe 1 wurden die HCC bei 70 Kühen von 4 Milchvieh- (Brown Swiss, Swiss Fleckvieh, Holstein Friesian, Wasserbüffel) und 3 Mutterkuhrassen (Rätisches Grauvieh, Limousin, Schottisches Hochlandrind) untersucht. In der Gruppe 2 wurden bei 60 gesunden Brown-Swiss-Kühen A- und B-Proben zur Ermittlung von Referenzwerten am Thorax entnommen. Die A-Proben bestanden aus nachgewachsenen Haaren aus einer vor einem Monat geschorenen Fläche und die B-Proben aus nicht vorgeschorenem Haar. Die Gruppe 3 bestand aus 21 gesunden Brown-Swiss-Kühen, bei denen die HCC in A- und B-Proben von 4 verschiedenen Körperstellen (Hals, Schulter, Thorax, Kruppe) untersucht und miteinander verglichen wurden. Die HCC-Bestimmung erfolgte mittels LC-MS/MS (Flüssigkeitschromatographie, gekoppelt mit Massenspektrometrie).

In der Gruppe 1 waren die HCC der Holstein-Friesian-Kühe mit 1,75 pg/mg signifikant höher als diejenigen der Brown Swiss-, Swiss Fleckvieh- und Wasserbüffelkühe. Zwischen den HCC und der Tagesmilchleistung der 40 Milchkühe bestand eine signifikante Korrelation ($r = 0,57$, $P < 0,01$). Bei der Gruppe 2 waren die HCC in 77 % der A- und 85 % der B-Proben unterhalb der Bestimmungsgrenze (limit of quantification, LOQ) von 0,50 pg/mg und wurden semiquantitativ als < LOQ angegeben. In den restlichen Proben lagen die HCC zwischen 0,50 und 1,20 pg/mg. Die Werte der A- und B-Proben unterschieden sich nicht signifikant. Bei der Gruppe 3

Summary

The goals of this study were to investigate hair cortisol concentration (HCC) in seven different breeds of cows, to establish reference intervals for HCC in Brown Swiss cows and to compare cortisol concentrations of hair collected from four different areas of the body. Three groups of cows were used. Group 1 comprised 70 healthy cows representing four dairy breeds (Brown Swiss, Swiss Fleckvieh, Holstein Friesian, Water Buffalo) and three beef breeds (Raetian Grey, Limousin, Highland). Group 2 consisted of 60 healthy Brown Swiss cows in which two different hair samples were collected from the thoracic region to establish reference intervals; A samples consisted of hair that had grown for one month in a pre-clipped area, and B samples consisted of hair from a previously unshorn area. Group 3 comprised 21 healthy Brown Swiss cows, in which HCCs were measured in A and B samples from four different body regions (neck, shoulder, thorax, rump). Liquid chromatography tandem mass-spectrometry was used for cortisol measurement.

In group 1, the highest HCCs were measured in Holstein Friesian cows at 1,75 pg/mg, which was significantly higher than those of the Brown Swiss, the Swiss Fleckvieh and the Water Buffalo cows. Hair cortisol concentration and daily milk yield of the 40 dairy cows were highly correlated ($r = 0,57$, $P < 0,01$). In group 2, the HCCs of 77 % of the A samples and 85 % of the B samples were below the laboratory's limit of quantification (LOQ) of 0,50 pg/mg and the results were expressed semiquantitatively as <LOQ. The concentrations of the remaining samples ranged from 0,50 to 1,20 pg/mg, and the A and B samples did not differ. In group 3, the median quantifiable HCCs of the four body regions ranged from 0,50 to 1,00 pg/mg. The concentrations did not differ significantly among the body regions and between A and B samples. These findings suggest that Holstein Friesian cows have significantly higher HCC than Brown Swiss, Swiss Fleckvieh and Water Buffalo

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lagen die HCC-Medianwerte der messbaren Proben für die 4 Lokalisationen zwischen 0,50 und 1,00 pg/mg Haar. Die HCC unterschieden sich weder zwischen den verschiedenen Körperstellen noch zwischen den A- und Proben signifikant. Die Untersuchungen lassen die Vermutung zu, dass Holstein-Friesian-Kühe signifikant höhere HCC als Brown Swiss-, Swiss Fleckvieh- und Wasserbüffelkühe aufweisen und dass dies mindestens teilweise durch die hohe Milchleistung bedingt ist.

Schlüsselwörter: Stress, Rind, Haarcortisolkonzentration

cows, and we assume that this is due in part to the milk yield in this breed.

Keywords: Stress, cattle, hair cortisol concentration

Introduction

Hair cortisol concentration (HCC) has been investigated in healthy and diseased cattle.[2,3,6,7,9,10,11,15,28] The most important aspects of using HCC as a measure of stress (experimental stimulation, living, housing and management conditions, social behaviour, body condition and nutritional status, diseases and disorders) in dairy cattle were recently described.[18,35] With respect to the relationship between welfare and HCC, two studies concluded that the protocols might not yield valid indices for cow welfare.[34,35] HCCs vary with different analytical techniques; the majority of studies used radioimmunoassays (RIAs)[9,10,11,15,28] and enzyme immunoassays (EIAs).[6,14,19,20,34] Only a few studies measured HCC using a combination of liquid chromatography and mass spectrometry (LC-MS/MS),[2,3] which has a high specificity and sensitivity and is considered the gold standard for the detection of cortisol in hair.[1,16,29] Cortisol concentrations measured using immunoassay methods are 2,5 to 10 times those measured using LC-MS/MS because of cross-reaction with other steroid hormones.[29] The degree of cross-reactivity varies greatly depending on the type of immunoassay and has been illustrated in a table.[29] In addition to the analytical technique, other factors including breed, anatomical site of hair collection and hair pigmentation affect the HCC in cattle. Most studies involved Holstein-Friesian cows[6,7,10,11,12,13,15,17,19,20,27,31,32,34] but the HCC was also measured in a few other breeds such as Angus,[25] Simmental,[9] F1 Swedish Red × Montbéliard,[28] Brown Swiss[2,3] and Buša[26] cattle.

There are only few studies comparing HCCs of different cattle breeds.[12,26] The anatomical site of hair collection for HCC measurement has not been standardised in cattle. Collection sites have included the head,[9,10,11,28,32] neck,[2,31] shoulder,[3] thorax,[15] flank,[30,34] back[20,27] and the tip of the tail.[6,7,12,13,14] Some authors examined hair from multiple anatomical sites, for instance from the head, neck, shoulder and hip,[25] shoulder, topline, hip and tail,[6] shoulder and hip[17] and shoulder, back and tail.[19] The maximum HCCs were measured in tail hair.[6,19,25] Therefore, it was concluded that the tail switch is the optimal location for hair sampling[6] and the most suitable way for analysis of cortisol concentration of hair.[25] Direct comparison of those findings was not possible because of differences in analytical techniques. However, the HCC of tail hair was higher than that of the shoulder, top line and hip regions.[6] The reasons for the higher cortisol concentration in tail hair could be linked to the fact that tail hair grows ten times faster than hair at the hip and shoulder.[6] It has been speculated that growing tail hair requires a high blood supply[35] resulting in more exposure to cortisol, which is incorporated into the hair at higher levels than other regions.

The stage of hair growth must be considered when interpreting HCC. Two studies investigated the cortisol concentration of regrown hair (one month after clipping) and normal hair from a previously unshorn area.[3,20] Regrown hair is limited to hair in the anagen phase whereas hair from previously unshorn areas includes hair from the anagen, catagen and telogen phases. The difference between regrown hair and hair from a previously unshorn area with respect to HCC most likely relates to the fact that the actively growing anagen-phase hair is capable of capturing much more circulating cortisol than the predominantly catagen and telogen-phase hair samples from an unshorn area.[3] Therefore, the cortisol concentration of regrown hair is a more accurate reflection of the circulating cortisol concentration of the past 30 days than the concentration of the relatively static samples of unshorn hair.[3] An earlier study concluded that the effect of parturition on the HCC was more pronounced in regrown hair than in hair that had not been previously shorn,[3] Likewise, the HCCs 4 and 8 weeks after treatment with ACTH were higher in regrown hair than in hair that had not been previously shorn.[20]

The studies to date show that HCCs of different cattle breeds have not been compared and only limited information is available for Brown Swiss cattle. The latter represent an important cattle breed in Switzerland. Furthermore, the site for collection of hair for cortisol measurements has not been standardised; to the authors' knowledge, only one study compared the cortisol concentrations of regrown hair and hair from a previously unshorn body region.[3] Therefore, the goals of the present study were a) to investigate the HCCs in cattle of different breeds, b) to establish reference intervals for HCCs in Brown Swiss cattle, c) to compare HCCs of hair from different regions of the body and d) to compare HCCs of regrown hair and hair from previously unshorn areas.

Materials and Methods

Overview

We used three groups of cattle: Group 1 comprised 70 cows of different breeds in which hair was collected from the neck; group 2 consisted of 60 Brown Swiss × Braunvieh cross cows, referred to as Brown Swiss cows in this text, in which hair was collected from the thorax; group 2 consisted of 60 Brown Swiss × Braunvieh cross cows, referred to as Brown Swiss cows in the text, in which hair was collected from the thorax. Sampling at the neck (group 2) was found to be impractical when the cows were restrained in a head gate. Group 3 consisted of 21 Brown Swiss cows in which hair was collected from different regions of the body. Cows of group 1 were the subject of a Master's thesis,[22] and the cows of groups 2 and 3 were part of a dissertation.[36] All hair samples were from pigmented areas. Within each breed, hair of the same colour were used.

Group 1 (70 cows of different breeds, sampled at the neck)

Group 1 was used to investigate differences in HCC of different cattle breeds, which included healthy cows from four dairy breeds (Brown Swiss, Swiss Fleckvieh, Holstein Friesian, Water Buffalo) and three beef breeds (Raetian Grey, Limousin, Highland). Each breed was represented by ten cows that were recruited from seven different herds. The following inclusion criteria were used: clinically healthy, had not have received any medication during the two months before enrolment in the study, and the time since the last calving was at least 30 days. The Highland cows were pastured year-round, and the other breeds were housed in free-stall barns with straw-bedded cubicles. The age, milk yield (preceding lactation), herd size and feed management varied among cows of the different breeds (Table 1). The average yearly milk production was highest in the Holstein Friesian cows (13,329 kg), lowest in the Water Buffaloes (2,050 kg) and intermediate in the Brown Swiss (6,330 kg) and the Swiss Fleckvieh (5,780 kg) cows. The basic ration of all cows consisted of grass and first- and second-cut hay, and all cows except the Water Buffaloes received grass and/or corn silage. The Brown Swiss, Swiss Fleckvieh and Holstein Friesian cows also received concentrate according to production. All hair samples were collected from April to June from a 5 by 5 cm pigmented area in the neck area with the exception of the Highland cows, in which hair was collected from the forehead to avoid horn injuries to the operator. The hair was removed as close to the skin as possible using electric clippers. Only the 1,5 cm of hair closest to the body was used in Water Buffaloes and Highland cows. The hair samples were protected from light and dried at room temperature for seven days, after which time they were wrapped in tin foil and stored in airtight plastic bags until analysis.

Group 2 (60 Brown Swiss cows, samples collected from the thoracic region)

Group 2 comprised 60 healthy Brown Swiss cows from different farms and was used to establish reference intervals for HCC in this breed and to investigate differences in HCC between hair that had been previously shorn (A sample) and hair that had not been previously shorn (B sample).[3] The cows were housed in a tie-stall and had daily access to an exercise yard but were not pastured during this trial. All samples were collected in the winter when the cows were stabled. The cows were 2,0 to 3,9 years (mean ± sd = 2,7 ± 0,5 years) of age and

Table 1: Breed, age, milk yield, herd size and feeding management in group 1, which consisted of 70 cows (10 cows per breed).

Breed	Age (years, mean ± sd)	305-day milk yield (kg)[1]	Daily milk yield (kg)	Herd size	Feeding
Brown Swiss	3.8 ± 1.75	6,330 ± 1,319	23.9 ± 6.98	75	D E F I
Swiss Fleckvieh	6.2 ± 2.91	5,780 ± 634	19.6 ± 2.97	69	D E G I
Holstein Friesian	5.5 ± 2.47	13,329 ± 1,550	47.6 ± 9.94	60	D E I
Raetian Grey	6.3 ± 3.78	NA	NA	14	D E
Limousin	10.0 ± 3.65	NA	NA	70	D E F
Highland	6.6 ± 2.08	NA	NA	25	D E
Water Buffalo	11.4 ± 4.17	2,050 ± 321	4.2 ± 2.96	45	D H

[1]305-day milk yield (kg) in the preceding lactation; D basic ration: grass, first- and second-cut hay; E grass silage; F corn silage; G sugar beet silage; H pea/cereal mixture; I concentrate; NA Not applicable

had a mean daily milk yield of 25,3 ± 5,95 kg (measured 30 and 60 days after calving). The following criteria were used for the inclusion of cows in the study: normal parturition and puerperal period; clinically healthy; and except for dry cow treatment, received no medications during the month before enrolment. Furthermore, to exclude cows with clinical ketosis, the blood β-hydroxybutyrate (BHB) concentration was measured (BHB-Check Keton Test-System, WDT, Garbsen, Germany) 30 and 60 days after calving, and cows with concentrations exceeding 1,20 mmol/l, as well as cows with rectal temperatures outside the range of 38,3 to 39,0 °C, were excluded.

The collection of hair samples was done as described for group 1. All samples were procured from areas that had been shorn one month previously.[3] To achieve this, a 10 cm × 10 cm area of skin was clipped with electric clippers on the left thoracic wall between intercostal spaces 8 and 10, 20 cm ventral to the dorsal midline, 30 days after calving. Thirty days later, the A sample, consisting of 3 to 5 g of hair, was collected from the centre of the previously shorn area. The B sample was collected immediately caudal to this area. Processing and storage of hair samples was the same as in group 1.

Group 3 (21 Brown Swiss cows, A and B hair samples from four body regions)

In group 3, which comprised 21 healthy Brown Swiss cows, A and B hair samples were collected from the neck, shoulder, thorax and rump areas. The hair samples were used to investigate differences in cortisol concentrations between hair from different regions of the body. The cows were 2,0 to 3,8 years (2,8 ± 0,6 years) of age, and at 60 days in milk had a daily production of 27,6 ± 5,92 kg (range, 18 to 38 kg). The inclusion criteria were the same as those described for group 2.

Thirty days after calving, 10 cm × 10 cm areas of skin were clipped in the neck region in the same manner as described for group 2 (20 cm cranial to the scapula and 15 cm dorsal to the jugular groove), shoulder (centre of the scapula), left thoracic wall (as in group 2) and rump (halfway between the coxal and ischial tuberosities). As in group 1, cows of group 2 were sampled in the winter when they were stabled. The text has been amended accordingly. Thirty days later, A and B samples were collected from all four regions as described for group 2.

Hair cortisol measurement

The hair cortisol concentration was measured by means of liquid chromatography tandem mass spectrometry (LC-MS/MS) as described.[1] The same technique was previously used to measure cortisol concentration in hair of adult cattle[2,3] and calves.[4,5] Hair cortisol concentrations between 0,10 and 0,49 pg/mg were above the limit of detection (LOD) but below the laboratory's limit of quantification (LOQ) of 0,50 pg/mg, and therefore concentrations from 0,11 to 0,49 pg/mg were expressed semiquantitatively as <LOQ and were not included in the statistical calculations.

Statistical analysis

The program SPSS Version 27 (IBM SPSS Statistics 27,0, Switzerland) was used. Normality of the data was tested using the Shapiro-Wilk test. Means ± standard deviations were calculated for normal data and medians and 25th and 75th percentiles for non-normal data. The HCCs of group 1 were presented as box-and-whisker plots. Differences between medians in group 1 were analysed using the Kruskal-Wallis test, and Pearson's correlation coefficients between HCCs, daily milk yield, 305-day milk yield of the preceding lactation, age, number of calvings, time of the last calving and duration of pregnancy were calculated. Scatter plots were used to show the relationship between HCCs and milk yield in group 1. Frequency distributions among the 4 body regions in group 3 were compared using the chi-square test.

Ethics approval

The study was approved by the ethical committees of the canton of Zurich, St Gallen and Appenzell Outer Rhodes, Switzerland, and all experiments were per-

Table 2: Hair cortisol concentrations in 70 cows representing 7 different breeds.

Breed	Median (pg/mg hair)	Mean ± sd (pg/mg hair)	Percentiles (25th to 75th (pg/mg hair)
Holstein Friesian1	1.75	1.86 ± 0.62	1.30 – 2.35
Brown Swiss2	0.55	0.70 ± 0.37	0.50 – 0.73
Swiss Fleckvieh	0.60	0.68 ± 0.16	0.58 – 0.80
Raetian Grey	0.90	0.93 ± 0.19	0.78 – 1.10
Limousin	0.90	1.08 ± 0.53	0.80 – 1.15
Highland	1.05	1.30 ± 0.71	0.80 – 1.43
Water Buffalo	0.70	0.92 ± 0.44	0.58 – 1.43

[1]Different from Brown Swiss ($P < 0.01$), Swiss Fleckvieh ($P < 0.01$) and Water Buffaloe ($P < 0.05$) (Kruskal-Wallis test);
[2]Different from Highland ($P < 0.05$) (Kruskal-Wallis test)

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formed in accordance with relevant institutional, national, and international guidelines and regulations.

Results

Hair cortisol concentration of 70 cows of different breeds (group 1)

The median HCCs ranged from 0,55 (Brown Swiss) to 1,75 pg/mg (Holstein Friesian) (Table 2, Figure 1). The HCCs of the Holstein Friesian cows were significantly higher than those of the Brown Swiss ($P < 0{,}01$), the Swiss Fleckvieh ($P < 0{,}01$) and the Water Buffalo cows ($P < 0{,}05$). The HCCs of the Brown Swiss cows were significantly lower than those of the Highland cows ($P < 0{,}05$). There were significant correlations between HCC and daily milk yield and between HCC and 305-day yields of the 40 dairy cows (including 10 Water Buffaloes) ($r = 0{,}57$ and $r = 0{,}58$, respectively, $P < 0{,}01$) (Figure 2). Hair cortisol concentration was not significantly correlated with the age of the cows, lactation number, days in milk and stage of pregnancy in the 43 cows that were pregnant.

Hair cortisol concentration of 60 Brown Swiss cows (group 2)

In 77% (n=46) of the A samples and 85% (n=51) of the B samples, the HCCs were <LOQ (Figure 3). The HCCs of the remaining 14 A samples and 9 B samples ranged from 0,50 to 1,20 pg/mg (median, 0,55 pg/mg) and from 0,50 to 1,00 pg/mg (median, 0,60 pg/mg), respectively (Table 3). The BHB concentrations in blood were 0,89 ± 0,18 mmol/l on day 30 and 0,90 ± 0,16 mmol/l on day 60.

Cortisol concentration in hair collected from four body sites in 21 Brown Swiss cows (group 3)

In 62 to 67% of the A samples and 71 to 95% of the B samples, the HCCs were <LOQ (Table 3). In the samples collected from the rump, but not in those from other regions, the percentage of B samples with semiquantitative results (95%) was significantly greater than the percentage of A samples with the same results (62%; $chi^2 = 5{,}1$; $P < 0{,}05$). The median quantifiable HCCs (≥0,50 pg/mg) in all four locations ranged from 0,50 to 0,75 for the A samples and from 0,55 to 1,00 pg/mg for the B samples. The concentrations did not differ significantly between A and B samples and among the body regions.

Discussion

For methodological reasons related to the analytical technique, the findings of the present study cannot be directly compared with those of most other authors. Group 1, which was used for breed comparisons, includ-

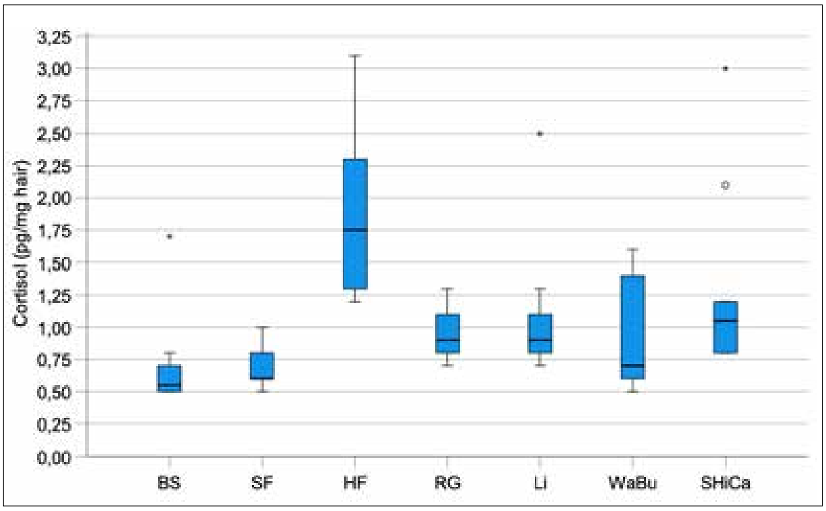

Figure 1: Box-and-whisker plots of hair cortisol concentrations in 70 cows representing 7 different breeds. BS Brown Swiss, SF Swiss Fleckvieh, HF Holstein Friesian, RG Raetian Grey, Li Limousin, WaBu Water Buffalo, SHiCa Highland. Circle = outlier, asterix = extreme score.

Table 3: Hair cortisol concentrations in A and B samples from 60 cows of group 2 (thorax) and 21 cows of group 3 (neck, shoulder, thorax, rump) (all concentrations in pg/mg hair).

Group	Location	A sample[1]				B sample[1]			
		HCC <LOQ	HCC 0.50 – 1.70	Mean ± sd[2]	Median (range)[2]	HCC <LOQ	HCC 0.50 – 2.40	Mean ± sd[2]	Median (range)[2]
2 (n=60)	Thorax	77% (n=46)	23% (n=14)	0.62±0.19	0.55 (0.50–1.20)	85% (n=51)	15% (n=9)	0.63±0.17	0.60 (0.50–1.00)
3 (n=21)	Neck	67% (n=14)	33% (n=7)	0.57±0.11	0.50 (0.50–0.80	71% (n=15)	29% (n=6)	0.70±0.29	0.55 (0.50–1.20)
	Shoulder	62% (n=13)	38% (=8)	0.60±0.11	0.60 (0.50–0.80)	86% (n=18)	14% (n=3)	1.33±0.95	1.00 (0.60–2.40)
	Thorax	62% (n=13)	38% (n=8)	0.63±0.14	0.60 (0.50–0.90)	86% (n=18)	14% (n=3)	0.83±0.15	0.80 (0.70–1.00)
	Rump	62% (n=13)	38% (=8)	0.83±0.38	0.75 (0.50–1.70)	95% (n=20)[3]	5% (n=1)[3]	NA[4]	NA[4]

[1]A samples: hair that had grown for one month in a pre-clipped area; B samples: hair from a previously unshorn area

[2]Only HCCs ≥ 0.50 pg/mg were used for calculation of means ± standard deviations, medians and ranges

[3]Frequency distributions among the 4 body regions differ between A and B samples; $P < 0.05$, $Chi2 = 5.1$

[4]NA Not applicable

ed dairy cows with very high (Holstein Friesian), medium (Brown Swiss, Swiss Fleckvieh) and relatively low milk yields (Water Buffalo), as well as beef breeds (Raetian Grey, Limousin, Highland). Holstein Friesian cows had significantly higher HCCs than the other dairy breeds. We assumed that this difference was not related to the Holstein Friesian breed *per see* but rather to the greater milk yield in these cows and the associated metabolic stress. This was supported by the significant correlations between HCC and daily as well as 305-day milk yields. Although husbandry factors were unlikely, they cannot be completely ruled out. In support of our assumption, the HCCs of dry Holstein and Brown Swiss cross-bred cows of another study did not differ significantly, whereas lactating Holstein cows, which had significantly higher production, had significantly higher HCCs 60 to 90 days in milk compared with Brown Swiss cross-bred cows. Furthermore, Holstein cows, but not Brown Swiss cross-bred cows, had significantly higher HCCs 60 to 90 days in milk compared with the dry period,[12] Likewise, a study of 412 Holstein Friesian cows showed that an increase in the plasma cortisol concentration by 1 µg/dl was associated with an increase of 580 kg in expected 305-day milk yield.[21] Of note, we determined the lowest HCCs in the Brown Swiss cows, which was in agreement with other studies that described lower HCCs in this breed compared with Holstein Friesian cows.[2,8] In addition to a lower production level, it is likely that the calm disposition of Brown Swiss cows is a factor in the lower HCCs in this breed. To the authors' knowledge, there are no studies on the effects of caretakers, herd size or social structure on HCC in cattle. Similarly, quiet Angus cows had lower blood cortisol concentrations than excitable cows.[23] However, the comparison of HCCs of cows of different breeds was compromised by the heterogenous husbandry conditions on the different farms in the present study. Ideally, cows should be from one herd and kept under the same husbandry conditions similar to another study.[12] Interestingly, the HCCs of Highland cattle were significantly higher than those of Brown Swiss cows. Beef cows generally undergo less exogenous (fewer human interactions) and endogenous (lower milk yield) stress. We therefore expected lower HCCs compared with dairy cows, but this did not occur. On the other hand, it could be argued that increased physical activity related to different housing and pasture conditions results in higher cortisol levels in beef cows. This notion is supported by the observation that Highland cows, which were pastured year-round, had the highest HCCs of the three beef breeds. In addition, all beef cows were nursing a calf, which may have been an added stress resulting in higher HCCs. Breastfeeding mothers (humans) have been shown to have higher HCCs than non-breastfeeding mothers.[33] The HCCs of a breastfeeding mother (breastfeeding was not mentioned in the article but was confirmed in writing on request by the first author), who was tested 11 times from week 6 of gestation to week 9 after birth, were considerably higher after birth than during gestation.[24] The second experiment involved 60 Brown Swiss cows and served to establish reference intervals for HCC in this breed. Sixty days in milk was chosen as the sampling time because an effect of pregnancy and parturition on the HCC could be reasonably excluded.[3,7,9] The HCCs were relatively low and confirmed earlier findings in 25 Braunvieh cows.[3] However, we did not expect the majority of samples to be below the LOQ (0,50 pg/mg). We assume that the remarkably quiet disposition of Brown Swiss cows contributed to the low HCCs. We are convinced that procurement, storage, processing and analysis of the samples did not adversely affect the measured concentrations. The

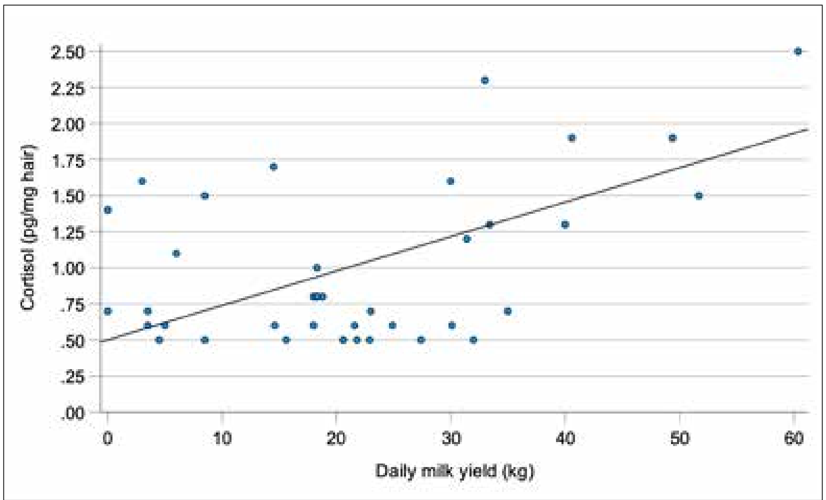

Figure 2: Correlation between hair cortisol concentration and daily milk yield in 40 Brown Swiss, Swiss Fleckvieh, Holstein Friesian and Water Buffalo cows. An outlier (3.10 pg/mg) in a Holstein Friesian cow is not shown.

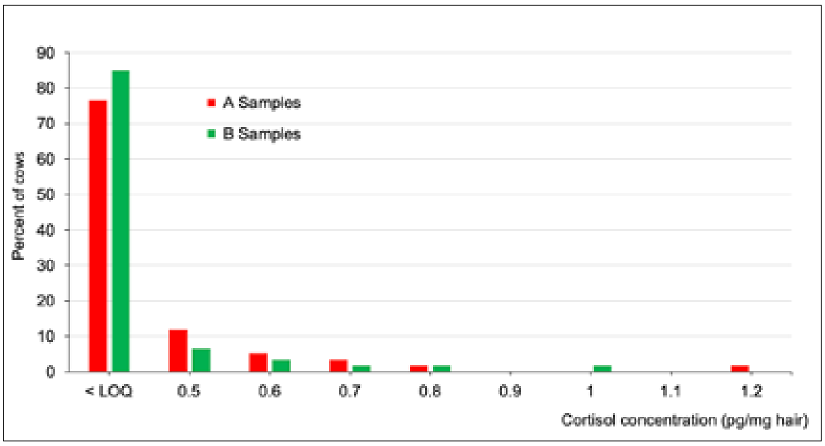

Figure 3: Frequency distribution of the hair cortisol concentration in A (hair that had grown for one month in a pre-clipped area) and B samples (hair from a previously unshorn area) from 60 Brown Swiss cows.

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HCCs of the A and B samples did not differ significantly, which, with one exception, was also in agreement with earlier findings.[3] This exception concerned HCC measurements in 25 Braunvieh cows during the month of calving; A samples had a cortisol peak, which was significantly higher than all other measured concentrations before and after calving, whereas B samples did not peak.[3]

The findings of group 3 showed no difference in HCCs of hair from four different body regions, and that A and B samples did not differ significantly. In other studies of cortisol concentration in hair from different body regions in cattle, hair from the shoulder region was found to have the lowest concentrations.[6,19,25] The strength of evidence of the results from Groups 2 and 3 is limited and the findings must be interpreted cautiously because only 15 to 23 % of the data in Group 2, and 33 to 38 % of the A samples and 5 to 29 % of the B samples could be analysed statistically.

Conclusions

The findings of this study showed that Holstein Friesian cows have significantly higher HCCs than those of the Brown Swiss, the Swiss Fleckvieh and the Water Buffalo cows. Holstein Friesian cows had the highest milk yield and we suspect that this was related to the higher HCCs. In 77 % of the A samples and 85 % of the B samples of Brown Swiss cows, the HCCs were <LOQ. The HCCs of the four body regions (neck, shoulder, thorax, rump) and between areas of unshorn or regrown hair did not differ significantly. The establishment of the HCCs reference interval in Brown Swiss cattle must be carefully interpreted because of the high number of animals with HCC <LOQ.

Concentrations de cortisol dans les poils de différentes races de vaches : comparaison entre les poils provenant de zones non tondues et celles précédemment tondues et de diverses régions du corps.

Les objectifs de cette étude étaient d'étudier la concentration de cortisol dans les poils (*hair cortisol concentration* (HCC)) chez sept races de vaches différentes, d'établir des intervalles de référence pour le HCC chez les vaches de race Suisse Brune et de comparer les concentrations de cortisol dans les poils prélevés sur quatre zones différentes du corps. Trois groupes de vaches ont été utilisés. Le groupe 1 comprenait 70 vaches saines représentant quatre races laitières (Brown Swiss, Swiss Fleckvieh, Holstein Friesian, Buffle d'eau) et trois races à viande (Grise rhétique, Limousin, Highland). Le groupe 2 était composé de 60 vaches Brown Swiss en bonne santé, pour lesquelles deux échantillons de poils différents ont été prélevés dans la région thoracique afin d'établir des intervalles de référence ; les échantillons A étaient constitués de poils ayant poussé pendant un mois sur une zone précédemment tondue et les échantillons B étaient constitués de poils provenant d'une zone non tondue auparavant. Le groupe 3 comprenait 21 vaches suisses brunes en bonne santé, chez lesquelles les HCC ont été mesurés dans des échantillons A et B provenant de quatre régions corporelles différentes (cou, épaule, thorax, croupe). La chromatographie liquide en tandem avec spectrométrie de masse a été utilisée pour la mesure du cortisol.

Concentrazioni di cortisolo nel pelo in diverse razze bovine: confronto tra i peli provenienti da aree non tosate, precedentemente tosate e da varie regioni del corpo

L'obiettivo di questo studio è stato quello di analizzare la concentrazione di cortisolo (HCC) nel pelo in sette diverse razze bovine, di stabilire gli intervalli di riferimento per l'HCC nelle vacche di razza Bruna e di confrontare le concentrazioni di cortisolo nei peli raccolti da quattro diverse aree del corpo. Sono stati utilizzati tre gruppi di vacche. Il gruppo 1 comprendeva 70 vacche sane che rappresentavano quattro razze da latte (Bruna svizzera, Swiss Fleckvieh, Frisona, Bufalo d'acqua) e tre razze da carne (Grigia alpina, Limousine, Highlander). Il gruppo 2 era composto da 60 vacche Bruna svizzera sane in cui sono stati raccolti due diversi campioni di pelo dalla regione toracica per stabilire gli intervalli di riferimento. I campioni A erano costituiti da peli cresciuti per un mese in un'area tosata, mentre i campioni B erano costituiti da peli provenienti da un'area precedentemente non tosata. Il gruppo 3 comprendeva 21 vacche Bruna svizzera sane, in cui le HCC sono stati misurate in campioni A e B provenienti da quattro diverse regioni del corpo (collo, spalla, torace, groppa). Per la misurazione del cortisolo è stata utilizzata la cromatografia liquida accoppiata con spettrometria di massa (LC-MS/MS).

Nel gruppo 1, le HCC più elevate sono state misurate nelle vacche di razza Frisona con 1,75 pg/mg, significativamente più alte di quelle delle vacche di razza Bruna

Dans le groupe 1, les HCC les plus élevés ont été mesurés chez les vaches Holstein Friesian à 1,75 pg/mg, ce qui était significativement plus élevé que ceux des vaches Brown Swiss, Swiss Fleckvieh et Buffle d'eau. La concentration de cortisol dans les poils et le rendement laitier quotidien des 40 vaches laitières étaient fortement corrélés ($r = 0,57$, $P < 0,01$). Dans le groupe 2, les HCC étaient inférieures à la limite de quantification (LOQ) de 0,50 pg/mg dans 77% des échantillons A et 85% des échantillons B et ils ont été indiquées de manière semi-quantitative comme < LOQ. Dans les échantillons restants, les HCC se situaient entre 0,50 et 1,20 pg/mg. Les valeurs des échantillons A et B n'étaient pas significativement différentes. Dans le groupe 3, les valeurs médianes des HCC des échantillons mesurables pour les 4 localisations se situaient entre 0,50 et 1,00 pg/mg de poils. Les HCC ne différaient pas significativement entre les différentes localisations corporelles ni entre les échantillons A et B. Les analyses permettent de supposer que les vaches Holstein-Friesian présentent des HCC significativement plus élevées que les vaches Brown Swiss, Swiss Fleckvieh et les bufflonnes d'eau et que cela est dû au moins en partie à leur production laitière élevée.

Mots clés: Stress, bovins, concentration de cortisol dans les poils

svizzera, Swiss Fleckvieh e Bufalo d'acqua. La concentrazione di cortisolo nel pelo e la produzione giornaliera di latte delle 40 vacche da latte erano altamente correlate ($r = 0,57$, $P < 0,01$). Nel gruppo 2, le HCC del 77% dei campioni A e dell'85% dei campioni B erano inferiori al limite di quantificazione (LOQ) del laboratorio di 0,50 pg/mg e i risultati sono stati espressi semi-quantitativamente come <LOQ. Le concentrazioni dei restanti campioni variavano da 0,50 a 1,20 pg/mg e i campioni A e B non differivano significativamente. Nel gruppo 3, la mediana delle HCC quantificabili delle quattro regioni corporee variava da 0,50 a 1,00 pg/mg. Le HCC non differivano significativamente tra le regioni corporee e tra i campioni A e B. Questi risultati suggeriscono che le vacche di razza Frisona hanno una HCC significativamente più alta rispetto alle vacche di razza Bruna svizzera, Swiss Fleckvieh e Bufalo d'acqua, e presumiamo che ciò sia dovuto in parte alla produzione di latte in questa razza.

Parole chiave: stress, bovini, concentrazione di cortisolo nel pelo

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