

## Factors influencing the welfare of dairy goats

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### Abstract

A variety of stressful situations commonly occur on dairy farms which can impair the well-being of the animals. The aim of this study was to analyse the concentration of cortisol in the saliva of dairy goats and on the basis thereof to determine the degree of stress experienced by them in relation to selected situations on farms. The following situations were selected as stressful: first visit to the milking parlour; weaning off; loading and transport; deworming; and the disruption of social hierarchy. We examined 344 samples from 100 animals using cotton swabs for the saliva collection. Commercially available ELISA kits (Cortisol EIA Kit, BosterBio, California, USA) which can detect cortisol in the saliva of all animal species, were used for the analysis. During the first visit to the milking parlour, weaning off, deworming and disruption of social hierarchy there was a significant ( $P < 0.05$ ) increase in cortisol concentrations compared to the basal values. For loading and transport there was a highly significant ( $P < 0.01$ ) increase in cortisol concentrations compared to the basal values. Although these situations are inevitable on farms, efforts should be made to eliminate them as much as possible due to the stress the animals experience in them.

*Cortisol, saliva, non-invasive method, stress*

Dairy goats on farms have to deal with various stressful situations that occur on a daily basis and affect their welfare. Defining animal welfare is complicated. Some define it as the ability of an animal to successfully adapt to environmental conditions without having its mental and physical health harmed (Broom 1986; Gračner et al. 2018). According to Hofer and East (1998), a potential indicator of animal welfare is the absence of stress. Stress has negative impact not only on the animals' growth but also on their milk production, reproduction, and resistance to disease (Kumar et al. 2012; Renčínová et al. 2021). As a result of these differing definitions, several methods have been developed to assess the level of stress in animals.

Measurements of hypothalamic-pituitary-adrenal (HPA) axis activity are standardly used to study stress and welfare in livestock. The HPA axis regulates the secretion of glucocorticoid hormones – cortisol and corticosterone, which are produced in the adrenal cortex (Mormède et al. 2007). Activation of the HPA axis and the subsequent secretion of glucocorticoids occurs due to stressful situations. The determination of the concentration of these hormones and their metabolites is therefore used as an indicator of animal welfare (Broom and Johnson 1993). However, glucocorticoid concentrations can be affected by other factors that should be taken into account (environment, sex, age, etc.), as well as the circadian rhythm of cortisol, which in most livestock peaks in the morning (Mormède et al. 2007).

Plasma is traditionally used to measure glucocorticoid hormones. However, its use is limited because the blood collection itself is stressful for the animals. Several alternative methods have therefore been developed. These methods involve measuring corticosteroids in saliva, milk, urine, faeces, fur, or eggs (Palme 2012). It is within this context that we chose a non-invasive method for determining salivary cortisol.

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## Materials and Methods

### Samples

We took a total of 344 saliva samples from 100 White Shorthaired goats from the DoRA organic farm in Ratibořice near Třebíč. The farm runs an extensive goat breeding programme based on the principles of organic farming. The following stressful situations were selected: first visit to the milking parlour (fear of the new); weaning off; loading and transport; human interventions (deworming); and change of groups (disruption of social hierarchy). For the first stressful situation, 10 goats (first-graders) were selected and sampled before and after their first visit to the milking parlour. The same thing was repeated one and two weeks later. For weaning off, 8 kids were selected from which saliva samples were taken before and after weaning off. For loading and transport, 15 kids were selected. Basal samples were taken, with subsequent samples immediately after loading/transport and then 30 min and 60 min later. For deworming, samples were taken from 30 goats: 10 goats were sampled before and 20 min after deworming; 10 goats were sampled before and 30 min after deworming; and the last group of 10 goats were sampled before and 40 min after deworming. For disruption of social hierarchy, 22 goats, unknown to each other, were selected. Basal samples were taken from them one hour after grouping, and then again one and two weeks later.

For the saliva collection, cotton swabs were used to wipe the oral cavity of the goats. The samples were then centrifuged at 800 g for 3 min and stored at  $-80^{\circ}\text{C}$  until analysis.

### ELISA

Commercially available ELISA kits (Cortisol EIA Kit, BosterBio, California, USA) which can detect salivary cortisol in all animal species, were used for the analysis. The detection limits are 100–3200 pg/ml and the sensitivity is 17.3 pg/ml. The analysis of the samples was performed according to the enclosed instructions. Quantitative calorimetric detection of cortisol was performed by a reader at a wavelength of 450 nm. The results were determined by subtraction from a standard curve designed in ELISA Software free (Elisaanalysis 2012). The obtained results were statistically processed using the statistical package Unistat 6.5 (Unistat Ltd., London, England). If the data corresponded with the Gaussian distribution, parametric Student's paired *t*-test was applied to compare two paired data sets. If this was not the case, a non-parametric Wilcoxon test was applied. Friedman's analysis of variance with repeated measures was applied to compare multiple data sets.

Differences were considered significant when the probability of a null hypothesis was less than 0.05 (i.e.  $P < 0.05$ ) and highly significant when the probability of a null hypothesis was less than 0.01 (i.e.  $P < 0.01$ ). If the probability of a null hypothesis was greater than 0.05 (i.e.  $P \geq 0.05$ ), it was considered as a non-significant result.

## Results

The results show that the first visit to the milking parlour is stressful for the goats (Table 1). There was a significant increase in cortisol concentrations ( $P < 0.05$ ) after the stressor. However, in the samples taken one and two weeks later, the cortisol concentrations after visiting the milking parlour were not significantly increased ( $P > 0.05$ ), which can be attributed to the fact that the goats had got used to the situation and were no longer stressed.

Table 1. Average cortisol concentrations around the first visit to the milking parlour (pg/ml).

	First visit to the milking parlour		After 1 week		After 2 weeks	
	Before	After	Before	After	Before	After
Mean	434.11	722.11	416.41	279.32	187.36	234.80
SD	188.85	262.42	255.48	160.38	95.90	91.96
SEM	54.52	75.75	73.75	46.30	27.68	26.55
Statistics	Paired <i>t</i> -test		Wilcoxon		Paired <i>t</i> -test	
		0.0158		0.1934		0.2089

SD – standard deviation; SEM – standard error of the mean

Weaning is stressful for goat kids. This is confirmed by the cortisol concentrations found, which increased significantly after weaning ( $P < 0.05$ ; Table 2). It is therefore necessary to wean kids in a gradual way, so that they can adapt to the new situation.

The loading and transport of the animals also proved to be significant stressors. Cortisol concentrations immediately after loading and then 30 min after loading were significantly increased ( $P < 0.01$ ) compared to the basal values (Table 3). Samples taken 60 min

Table 2. Average cortisol concentrations at weaning (pg/ml).

	Weaning	
	Before	After
Mean	237.30	350.95
SD	130.45	85.17
SEM	41.25	26.93
Statistics	Wilcoxon	0.0391

SD – standard deviation; SEM – standard error of the mean

after loading were only significantly increased ( $P < 0.05$ ), which means that cortisol concentrations began to decrease again and return to the baseline. For transport, cortisol concentrations were significantly increased ( $P < 0.01$ ) immediately after transport, but also 30 min and 60 min after transport.

Table 3. Average cortisol concentrations at loading and transport (pg/ml).

	Loading				Transport			
	Before	0 min	30 min	60 min	Before	0 min	30 min	60 min
Mean	223.38	691.07	862.20	573.86	501.58	889.22	863.74	938.91
SD	112.05	512.04	833.64	654.64	162.26	277.39	338.47	470.07
SEM	28.93	132.21	215.24	169.03	41.89	71.62	87.39	121.37
Statistics	F. Anova	0.0002	0.0001	0.0297	F. Anova	0.0033	0.0020	0.0033

SD – standard deviation; SEM – standard error of the mean

Deworming as a human intervention was also shown to be a stressor for the animals. We used Aldifal given to the goats with a deworming gun. However, a significant increase in cortisol concentrations ( $P < 0.05$ ) compared to the basal values was only observed in the samples taken 20 min after deworming (Table 4). There was no significant increase in cortisol concentrations in samples taken 30 min and 40 min after deworming compared to the basal values ( $P > 0.05$ ). We can conclude that the highest concentrations of cortisol in saliva were reached just 20 min after a stressful situation.

Table 4. Average cortisol concentrations at deworming (pg/ml).

	Deworming, 20 min		Deworming, 30 min		Deworming, 40 min	
	Before	After	Before	After	Before	After
Mean	641.28	815.90	321.63	337.00	332.02	306.81
SD	103.91	242.64	162.77	79.34	100.09	149.88
SEM	32.86	76.73	51.47	25.09	31.65	47.40
Statistics	Paired <i>t</i> -test	0.0492	Paired <i>t</i> -test	0.7962	Paired <i>t</i> -test	0.6413

SD – standard deviation; SEM – standard error of the mean

Changes in goat groups are stressful for the animals due to the need to establish a new social hierarchy (Table 5). This is confirmed by our results, where cortisol concentrations

Table 5. Average cortisol concentrations at disruption of social hierarchy (pg/ml).

	Disruption of social hierarchy			
	Before	1 hour	1 week	2 weeks
Mean	273.44	440.86	426.32	315.30
SD	239.48	173.73	128.77	169.87
SEM	51.06	37.04	27.45	36.22
Statistics	F. Anova	0.0142	0.0200	0.5884

SD – standard deviation; SEM – standard error of the mean

were significantly increased ( $P < 0.05$ ) in samples taken 60 min after regrouping, with the same result one week later. On the contrary, after two weeks there were no significant differences in cortisol concentrations ( $P > 0.05$ ), which again signals that the goats got used to each other and this situation was no longer stressful for them.

## Discussion

In our study, we found that the fear of unknown things – first visit to the milking parlour – is stressful for the goats. Similarly, Rushen et al. (2001) examined the effect of an unfamiliar environment (fear of the new) on cows being milked. They found that both plasma cortisol concentrations and the heart rate were higher when cows were milked in an unknown room. Cows milked alone in an unknown room showed signs of acute stress and milk production decreased.

Our results show that even gradual weaning is stressful for goat kids. There was a significant increase in cortisol concentrations after weaning. Zavy et al. (1992) found that the cortisol concentration increased dramatically in beef calves after weaning, just as it did in beef calves post-transport. In contrast, Magistrelli et al. (2012) found that weaning at 48 days of age was not stressful for goat kids and therefore had no effect on cortisol concentrations or weight gain. Tolu et al. (2017) found that social isolation for 5 min was more stressful for goat kids than weaning and also found that weaning cortisol concentrations were not affected by the environment.

The loading and transport of animals are well-known stressors. Our results confirmed this. This is in line with Al-Badwi et al. (2012), who stated that the transport of goats, regardless of the duration, causes an increase in cortisol concentrations in their blood plasma and saliva. The same has been shown to be true in other studies involving goats (Ali et al. 2006; Kadim et al. 2010), cattle (Palme et al. 2000), horses (Schmidt et al. 2010) and sheep (Al-Muffarej et al. 2008). However, the handling of animals during loading and unloading is usually the most stressful part of the whole transportation process (Nwe et al. 1996).

The way in which breeders treat livestock and the potentially increased fear of humans livestock have has a major effect on their welfare (Rushen et al. 1999). Due to mechanization, modern agricultural practices have restricted the opportunities for a frequent, kind contact between livestock and humans. Moreover, aversive livestock management tasks, such as capture and restraint for vaccination, hoof care or deworming still require breeder intervention. As a result, there is a potential for the livestock's direct negative experience with humans. Without the compensatory effect of positive everyday interactions, we may enhance the animals' natural fear of humans (Rushen et al. 1999). In our study, it was found that handling animals during deworming causes a degree of stress.

Breeding practices often involve grouping animals that are unknown to one another. With regards to the change in social hierarchy, it is clear that some new individuals can experience great stress when placed into large herds (Alley and Fordham 1993). Our study confirms that mixing mutually unknown goats causes stress and therefore affects their welfare. It appears that a new social hierarchy is established after two weeks.

In conclusion, all the examined situations proved stressful for the goats. We recorded a significant increase in salivary cortisol concentrations after exposure to the stressor compared to the basal values. Although these situations are inevitable on farms, attempts should be made to eliminate them as much as possible. In addition to the negative impact of stress on animals and their production, the stress hormone cortisol is also excreted into the milk later consumed by humans.

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