Monitoring saliva cortisol level in dairy goats during April, May and July in a semi-intensive rearing system

Gordana Gregurić Gračner, Ž. Pavičić, S. Žužul, Alenka Dovč, Nataša Lončarić, J. Grizelj, Marija Lipar and D. Gračner*

Introduction

The concept of welfare is very complex and is mainly defined as the condition in which the animal survives as a fully physically and mentally healthy individual able to successfully cope with environmental factors. Those factors (physical environment, available resources, and management practices) affect the physical and psychological response of animals as a result of attempts to adapt to these factors (Hughes, 1976; Broom, 1986).

Adequate space allowance, careful litter management and scrupulous monitoring of micro-climatic factors are crucial aspects for goats in confined rearing (Sevi et al., 2009). Most goats in Croatia, especially in the mountainous regions are reared in a semi-intensive manner, protected from food/water deprivation, and sheltered from climatic extremes. They are housed only during the night and in the winter period in which grazing is not feasible. During the winter, their confined environment is very predictable and less motivating. One of the primary endocrinological systems involved in metabolism and adaptation of organism is the hypothalamic-pituitary-adrenal axis (HPA) and adrenocorticotropic hormone (ACTH) is often produced in response to biological stress. Furthermore, reactivity of the adrenal cortex on ACTH and circulating glucocorticoid hormones (cortisol and corticosterone) are often used as stress indicators (Mormede at al., 2007; Gröschl, 2008). The transfer of cortisol from plasma to saliva occurs rapidly (Kirschbaum and Hellhammer, 2000), and thus elevated cortisol levels in saliva could be a reliable indicator of stress in an adult animal (Fell et al., 1985; Greenwood and Shutt, 1992; Gröschl, 2008; Al-Badawi et al., 2012; Yates et al., 2014). With regard to the daily cortisol profile, Eriksson and

Gordana GREGURIĆ GRAČNER*, DVM, PhD, Assistant Professor (corresponding author, e-mail:ggracner@gmail.com), Željko PAVIČIĆ, DVM, PhD Full Professor, Slavko ŽUŽUL, DVM, Assistant, Faculty of Veterinary Medicine, University of Zagreb, Croatia, Alenka DOVČ, DVM, PhD, Full Professor, Faculty of Veterinary Medicine, University of Ljubljana, Slovenia, Nataša LONČARIĆ, DVM, Ministry of Agriculture, Sector for Veterinary Inspection, Zagreb, Croatia; Juraj GRIZELJ, DVM, PhD, Full Professor, Marija LIPAR, DVM, PhD, Senior Expert Associate, Damjan GRAČNER, DVM, PhD, Full Professor, Faculty of Veterinary Medicine, University of Zagreb, Croatia

Teravainen (1989) found that plasma cortisol levels in goats were associated with feeding times, while Kokkonen et al. (2001) determined that a slight increase of plasma cortisol was found at night in 6/10 animals without significant average daily variations.

On the other hand, few studies have been conducted to establish how seasons effect ACTH response in different breeds of goats. Alila-Johanson et al. (2003) concluded that serum cortisol levels in goats exhibit seasonal but not daily rhythmicity.

In a study in Canada, Howland et al. (1985) established that on some mating days, changes in testosterone levels in males were highly correlated with changes in cortisol levels, though there was no obvious seasonal pattern in serum cortisol levels. Animals were kept year round in a heated barn (temperature from 5 to 25 °C) with numerous windows to experience normal seasonal changes in photoperiod.

In Finland, Alila-Johansson et al. (2003) established that significant seasonal variation was detected in overall plasma cortisol level in Finnish landrace goats, which they concluded to be dependent on photoperiod length. Animals were kept outdoors in summer and under natural indoor lighting in winter (light through window). In winter, concentrations were higher than in any other season, and were at their lowest from early spring to summer.

A one-year study in Oman conducted by Al-Busaidi (2008) revealed that Dhofari goats had significantly lower levels of plasma cortisol during the summer. Those goats were kept in shaded and partially closed pens.

In a Saudi Arabian study conducted for 35 days in summer and 35 days in winter on Aaardi goats, Al-Samawi et al. (2014) reported an increase in serum concentrations of cortisol as a result of exposure to heat stress during the summer season. The animals were kept on a concrete floor, with cement block walls and in shaded pens.

According to Chergui et al. (2017) in study conducted on Bedouin bucks native to the Sahara Desert in Algeria, plasma concentrations of cortisol showed no diurnal cycle, though high variation was evidence across seasons, with the highest levels in summer and winter when the environmental conditions are at their extreme. In contrary, the previously mentioned study on Angora goats in Turkey, housed in gender separate groups in shaded outdoor pens and kept free year round (except for the mating period), plasma cortisol concentrations increased during the winter and decreased during summer (Pehlivan and Dellal, 2017).

The previous study conducted by Fazio et al. (2006) on Alpi goats in Italy showed that different housing systems during spring affected the adrenocortical responses of domestic goats. They suggested that the lower cortisol levels observed in half-stabled goats in spring reflected their better ability to cope with environmental strategies compared to stabled goats.

The goal of the current study was to monitor the adrenocorticortical response by measuring levels of saliva cortisol and heart rate in goats depending on different housing conditions, from early spring to the peak of summer in a mountainous region in Croatia, in the warm-moderate rainy climate zone (winter from –2°C to –4°C and summer less than 20°C).

Materials and methods

The study was carried out on a commercial farm, located in Lokve (45° 21′ 28.8″ N, 14° 44′ 52.8″ E) in Croatia, at an altitude of 727 m, with an average air temperature in April of 9.8°C, May 14.8°C and July 19.9°C. The study was conducted within standard ethical norms.

Ten lactating female goats (6 Saanen and 4 Alpine) kept occasionally tethered in an insulated wooden barn in five identically equipped pens divided by wooden partitions into compartments of equal size (4 m^2) with sufficient space for every animal (1.5-2 m^2) (Sevi et al., 2009). Two goats were kept in each pen. Each pen had deep-bedded hay and an elevated (0.75 m) feeding area (Fig. 1). Goats were individually observed in:

- April - goats were still occasionally tethered in closed environment (air temperature 13°C, relative humidity 70%, air velocity 0.1 m/s)
- May - goats were released to pasture daily for several hours, after morning feeding and milking (air temperature 16.6°C, relative humidity 61%, air velocity 0.3 m/s)
- July - goats were exclusively at pasture during the day and brought into the enclosure only for milking and during the night (air temperature 21.3°C, relative humidity 63%, air velocity 0.2 m/s).

Saliva sampling:

Saliva samples were collected in the morning (11 a.m.) with special devices (Salivettes®, Sarstedt AG, Nümbrecht, Germany) and immediately frozen. After thawing and centrifugation, they were diluted (1:10) with enzyme immunoassay (EIA) buffer. An aliquot was analysed directly with a cortisol EIA as previously described (Palme and Möstl, 1997; Wagner et al., 2013).

Fig. 1. Goats in wooden barn

Statistical data was analysed using the software IBM SPSS Statistics v21, MS Excel 2007, using the statistical methods: 1. Descriptive statistics; 2. Shapiro-Wilk test; 3. t-test of paired samples. p value was set at 0.05 ($p<0.05$).

Results and Discussion

Three specific stressors for goats are: heat, handling and food/water deprivation, each individually or cumulatively. These may be the most common stressors that occur during routine handling procedures. Secondary and temporary stressors include disruption of the social hierarchy, which is more of a psychological stressor, change of habitat and overcrowding (Kruger, 2016). One of the most important challenges in barns is to maintain the appropriate microclimate. This includes air temperature, humidity, air flow velocity, low air pollution (with dust particles and microorganisms) and low content of gases and must provide sufficient protection from outside conditions (Herbut and Angrecka, 2012). The present study conducted on a small family goat farm in which animals were kept in wooden, insulated barn, on deep hay bedding with a space allowance of nearly 2 square meters per animal and sufficient daylight through windows. The microclimatic conditions in barn in this study were sufficient to ensure a healthy and productive herd. Furthermore, regardless of the fact that goats were occasionally tethered, each goat has its own name and stockman are very attentive to them during routine handling, as goats are treated gently and with care.

Blood sampling may be stressful for animals. For that reason, other sample

matrices, such as saliva, milk, excreta, hair or eggs should be taken (Palme, 2012). The speed at which hormones can be transferred from blood to saliva is controlled by passage through the lipophilic layers of the capillaries and glandular epithelial cells, so lipophilic molecules (as steroids) are transferred through these barriers more rapidly than hydrophilic molecules (as peptides) (Gröschl, 2008). The transfer of cortisol from plasma to saliva occurs rapidly. Within less than a minute, cortisol injected intravenously appears in saliva, and the peak concentration in saliva lags by less than 2-3 minutes compared to levels measured in blood (Kirschbaum and Hellhammer, 2000). The fact that the time lag between levels of plasma cortisol and saliva cortisol is very short and in accordance with animal welfare guidelines, only goat saliva was sampled and heart rate measured. All possible stressor as listed by Kruger (2016) were excluded, and the only event from early spring to summer that could possibly jeopardise animal welfare and reflect on saliva cortisol level as an indicator of stress was habitat changing during different seasons. Fazio et al. (2006) established that stabled goats showed higher cortisol levels compared to half-stabled subjects. However, in this study, no statistically significant differences were observed between the levels of saliva cortisol in dairy goats during April, May and July, regardless of housing conditions (Table 1).

This result is supported by other European studies (Howland et al., 1985; Alila-Johanson et al., 2003) which also showed no evident seasonal pattern in serum cortisol levels during the same months sampled here. In the desert, in hot climate regions characterised by extreme ambient temperatures in different seasons, an increase of cortisol level was observed during the summer (Al-Samawi et al., 2014; Chergui et al., 2017). However, contrary results were reported by Al-Busaidi (2008) and Pehlivan and Dellal (2017). In the current study, heart

Table 1. Saliva cortisol levels and heart rate in goats in April, May and July.

Goats (n=10)	Month	M	SD	Pairs (N=10)	t-test of paired samples				
Age		4.40	3.408		M	SD	t	df	p
Cortisol 1	April	0.7870	0.3811	Cortisol 3 - Cortisol 2	-0.08800	0.65649	-0.424	9	0.682
Heart rate 1		87.90	10.049	Cortisol 3 - Cortisol 1	0.09100	0.82572	0.349	9	0.735
Cortisol 2	May	0.9660	0,.6805	Cortisol 2 - Cortisol 1	0.17900	0.60695	0.933	9	0.375
Heart rate 2		89.20	20.896	Heart rate 3 - Heart rate 2	7.200	15.950	1.427	9	0.187
Cortisol 3	July	0.8780	0.6708	Heart rate 3 - Heart rate 1	8.500	12.972	2.072	9	0.068
Heart rate 3		96.40	13.525	Heart rate 2 - Heart rate 1	1.300	16.262	0.253	9	0.806

rate showed no statistically significant differences between the monitored periods.

Conclusions

In the present study, due to the comfortable ambient temperatures without extreme values, only a change in housing conditions might have an influence on cortisol levels, though this was not observed here. In the absence of other stressors (Kruger et al., 2016), the difference in housing conditions seems to be insufficient reason to caused significant cortisol level increase. Since the HPA axis is extremely sensitive to environmental conditions and its activity may be a good index of the intensity of adaptive adjustments (Chergui et al., 2017), it can be concluded that the goats tested here are kept in an ideal climate zone, environmental conditions and are properly managed to express their optimal production performance.

Summary

The aim of the current study was to evaluate the level of saliva cortisol and heart rate in lactating goats depending on different environmental condition, from early spring to the summer heat in the mountainous region of Croatia with a moderate climate zone (average winter air temperature from -2°C to -4°C and summer below 20°C). Ten lactating goats (6 Saanen and 4 Alpine) kept confined, occasionally tethered, were included in the study. The barn was wooden, thermally insulated, divided into five equal pens divided by wooden partitions. Each pen housed two goats on deep hay bedding and ensuring sufficient space allowance per animal. Saliva sampling was carried out in early April when goats were kept exclusively in the barn and occasionally tethered, at the end of May when they spent part of the day grazing freely, and the end of July when they spent only the night in barn. Saliva samples of saliva were taken at 11 a.m. using special equipment and frozen until analysis, and heart rate was determined by palpation of *a. femoralis*. Samples were analysed by the enzyme immunoassay (EIA). Following statistical analysis of the results, no statistically significant differences in cortisol levels and heart rate were observed during the sampling period. Since the hypothalamus-pituitary-adrenal gland is extremely susceptible to environmental changes, cortisol level is considered to be a credible indicator of the ability of animals to adapt to changing environmental conditions. Based on the obtained results, it is possible to conclude that the investigated goats were kept in conditions and in a way that was not stressful to them and are almost ideal for achieving their optimal production performance, and goats adapted very well to the environmental changes during different seasons.

Key words: *goats, saliva, cortisol, goat keeping*

Acknowledgements

This experiment was conducted as part of the project "L'optimisation des paramètres de reproduction, de génétique et de santé pour l'amélioration de la production des troupeaux de chèvres" (Projets de recherche conjoints AUF-IFA 2016) granted by L'Agence Universitaire de la Francophonie AUF. The authors are grateful to the Bradičić family for the opportunity to perform the study on Bradičić family farm.

References

1. AL-BADAWI, M. A., H. E. MOHAMED, A. ALHAIDARY and M. J. AL-HASSAN (2012): Plasma and salivary cortisol levels in transportation-stressed Aardi goats. J. Anim. Plant Sci. 1, 1731-1735.
2. AL-BUSAIDI, R., E. H. JOHNSON and O. MAHGOUB (2008): Seasonal variations of phagocytic response, immunoglobulin G (IgG) and plasma cortisol levels in Dhofari goats. Small Rumin. Res. 2-3, 118-123.
3. AL-SAMAWI, K. A., M. J. AL-HASSAN and A. A. SWELUM (2014): Thermoregulation of Female Aardi Goats Exposed to Environmental Heat Stress in Saudi Arabia. Indian J. Anim. Res. 48, 344-349.
4. ALILA-JOHANSSON, A., L. ERIKSSON, T. SOVERI and M. L. LAAKSO (2003): Serum cortisol levels in goats exhibit seasonal but not daily rhythmicity. Chronobiol. Int. 1, 65-79.
5. BROOM, D. M. (1986): Indicators of poor welfare. Brit. Vet. J. 142, 524-526.

6. CHERGUI, N., P. MORMEDE, A. FOURY, F. KHAMMAR and Z. AMIRAT (2017): Seasonal effects on plasma cortisol concentrations in the Bedouin buck: circadian studies and response to ACTH. Animal 11, 445-451.
7. ERIKSSON, L. and T. L. TERÄVÄINEN (1989): Circadian rhythm of plasma cortisol and blood glucose in goats. Asian. Austral. J. Anim. 2, 202-203.
8. FAZIO, E., P. MEDICA, S. CAVALERI, C. CRAVANA and A. FERLAZZO (2006): Cortisol levels as indikator of stress in domestic goats under different housing systems. XIV Congreso Internacional de la Federación Mediránea de Sanidad y Productión de Ruminantes. Lugo-Santiago de Compostela 12-15 de julio de 2006. 147-150.
9. FELL, L. R., D. A. SHUTT and C. J. BENTLEY (1985): Development of a salivary cortisol method for detecting changes in plasma "free" cortisol arising from acute stress in sheep. Aust. Vet. J. 62, 403-406.
10. GREENWOOD, P. L. and D. A. SHUTT (1992): Salivary and plasma cortisol as an index of stress in goats. Aust. Vet. J. 69, 161-163.
11. GRÖSCHL, M. (2008): Current Status of Salivary Hormone Analysis. Clin. Chem. 54, 1759-1769.
12. HERBUT, P. and S. ANGRECKA (2012): Forming of temperature-humidity index (THI) and milk production of cows in the free-stall barn during the period of summer heat. Anim. Sci. Pap. Rep. 4, 363-372.
13. HOWLAND, B. E., L. M. SANFORD and W. MARTYN PALMER (1985): Changes in Serum Levels of LH, FSH, Prolactin, Testosterone, and Cortisol Associated with Season and Mating in Male Pygmy Goats. J. Androl. 2, 89-96.
14. HUGHES, B. O. (1976): Behaviour as an index of welfare. Proceedings of the 5th European Poultry Conference. Malta Branch: World Poultry Science Association, Sept. 5-11, 1005-1018.
15. KIRSCHBAUM, C. and D. H. HELLHAMMER (2000): Salivary cortisol. In: G. Fink (ed.), Encyclopedia of stress (Vol. 3) San Diego, CA: Academic Press.
16. KOKKONEN, U. M., P. RISKILÄ, M. T. ROIHANKORPI and T. SOVERI (2001): Circadian variation of plasma atrial natriuretic peptide, cortisol and fluid balance in the goat. Acta Physiol. Scand. 171, 1-8.
17. KRUGER, L. P., T. L. NEDAMBALE, M. M. SCHOLTZ and E. C. WEBB (2016): The effect of environmental factors and husbandry practices on stress in goats. Small Rumin. Res. 141, 1-4.
18. MORMEDE, P., S. ANDANSON, B. AUPERIN, B. BEERDA, D. GUEMENE, J. MALMKVIST, X. MANTECA, G. MANTEUFFEL, P. PRUNET, C. G. VAN REENEN, S. RICHARD and I. VEISSIER (2007): Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol. Behav. 92, 317-339.
19. PALME, R. (2012): Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. Anim. Welfare 21, 331-337.
20. PALME, R. and E. MÖSTL (1997): Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Z. Saugetierkd. – Int. J. Mammal. Biol. 62, 192-197, Suppl. 2.
21. PEHLIVAN, E. and G. DELLAL (2017): Annual Changes of Thyroid Stimulating Hormone, Thyroxine, Triodothyronine and Cortisol Hormones in Angora Goats. J. Anim. Plant Sci. 27, 819-824.
22. SEVI, A., D. CASAMASSIMA, G. PULINA and A. PAZZONA (2009): Factors of welfare reduction in dairy sheep and goats. Ital. J. Anim. Sci. 8, 81-101.
23. WAGNER, K., K. BARTH, E. HILLMANN, R. PALME, A. FUTSCHIK and S. WAIBLINGER (2013): Mother rearing of dairy calves: Reactions to isolation and to confrontation with an unfamiliar conspecific in a new environment. Appl. Anim. Behav. Sci. 147, 43-54.
24. YATES, D. T., T. T. ROSS, D. M. HALLFORD, L. J. YATES and R. L. WESLEY (2014): Technical note: Comparison of salivary and serum cortisol concentrations after adrenocorticotropic hormone challenge in ewes. J. Anim. Sci., 88, 599-603.

Praćenje razine kortizola u slini mliječnih koza u travnju, svibnju i srpnju u poluintenzivnom sustavu držanja

Dr. sc. Gordana GREGURIĆ GRAČNER, dr. med. vet., docentica, dr. sc. Željko PAVIČIĆ, dr. med. vet., redoviti profesor, Slavko ŽUŽUL, dr. med. vet., asistent, Veterinarski fakultet Sveučilišta u Zagrebu, Hrvatska; dr. sc. Alenka DOVČ, dr. med. vet., redovita profesorica, Veterinarski fakultet Univerziteta u Ljubljani, Slovenija; Nataša LONČARIĆ, dr. med. vet. Ministarstvo poljoprivrede, Veterinarska inspekcija, Zagreb, Hrvatska; dr. sc. Juraj GRIZELJ, dr. med. vet., redoviti profesor, dr. sc. Marija LIPAR, dr. med. vet., viša stručna suradnica, dr. sc. Damjan GRAČNER, dr. med. vet., redoviti profesor, Veterinarski fakultet Sveučilišta u Zagrebu, Hrvatska

Cilj ovog istraživanja bio je procijeniti razinu kortizola u slini mliječnih koza te vrijednosti b\la ovisno o različitim uvjetima držanja, od ranog proljeća do vrhunca ljetnih vrućina u unutrašnjem planinskom području Hrvatske s umjerenom kontinentalnom klimom (zimi se prosječne temperature zraka kreću od -2 °C do -4 °C, a ljeti ispod 20 °C). Istraživanjem je bilo obuhvaćeno deset mliječnih koza u laktaciji (6 sanskih i 4 alpskih) koje su cijelu zimu provele zatvorene u oboru i povremeno na vezu. Obor je bio drveni, toplinski izoliran, podijeljen drvenom pregradom u 5 jednakih odjeljaka. U svakom su odjeljku bile smještene po dvije koze na dubokoj stelji od sijena i na površini više no dostatnoj po grlu. Uzorkovanje sline i utvrđivanje vrijednosti b\la provodilo se početkom travnja kada su koze držane isključivo u zatvorenom i povremeno na vezu, krajem svibnja kada su dio dana provodile slobodne na ispaši i krajem srpnja kada su u oboru jedino provodile noć. Uzorci sline korištenjem posebnog pribora uzimani su ujutro u 11 sati, i smrznuti do izvođenja pretrage. Vrijednosti b\la utvrđivane su palpacijom *a. femoralis*. Uzorci sline analizirani su imunoenzimnim postupkom (EIA). Nakon statističke obrade rezultata utvrđeno je da nema statistički značajne razlike u razini kortizola i vrijednosti b\la tijekom različitih mjeseci uzorkovanja. S obzirom da je os hipotalamus-hipofiza-nadbubrežna žlijezda iznimno osjetljiva na promjene u okolišu, razina kortizola uzima se kao vjerodostojan pokazatelj sposobnosti prilagodbe životinje na promijenjene okolišne uvjete. Na temelju dobivenih rezultata moguće je zaključiti da su istraživane koze držane u uvjetima i na način koji za njih ne predstavljaju stres te su gotovo savršeni za postizanje njihova optimalnog proizvodnog potencijala, kao i da su se na promjene u okolišu tijekom različitih godišnjih doba vrlo dobro prilagođavale.

Ključne riječi: *koza, slina, kortizol, držanje koza*