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Animal Welfare 2022, 31: 91-98
ISSN 0962-7286
doi: 10.7120/09627286.31.1.008

Cortisol is not enough: A complex stress reaction in tethered goats

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Abstract

*The purpose of this study was to assess the diversity of physiological stress responses elicited by tethering versus loose holding in the Swedish domestic goat (*Capra hircus*). Eight goats were randomly divided into two groups and the experiment was performed in a cross-over design. Six goats had implanted transmitters that registered heart rate and arterial blood pressure telemetrically and blood samples were taken on separate days. Heart rate increased around feeding both when the goats were held loose in pairs, and when they were tied up. When the goats were tethered, the heart rates and blood pressures were higher, and the concentrations of β-endorphin and oxytocin lower, compared to when held loose in pairs. In conclusion, housing and the company of another animal affect arterial blood pressure, heart rate, and the concentrations of β-endorphin and oxytocin in goats, but the cortisol and vasopressin concentrations did not differ between the treatments. The higher arterial blood pressure and heart rate, and lower concentrations of β-endorphin and oxytocin in tethered goats indicate that being tied up may be more stressful for the goats compared to being held loose. The use of single measures, eg cortisol concentrations, was not sufficient to give an accurate picture of the animals' response to the management system. This study demonstrates the importance of using a wide variety of physiological measures when evaluating stress in animal welfare research.*

Keywords: *animal welfare, β-endorphin, blood pressure, heart rate, oxytocin, vasopressin*

Introduction

Glucocorticoid concentrations are often used as a measure of stress and as a proxy measure of animal welfare (Ralph & Tilbrook 2016). While there is a widespread recognition of the many factors that may influence the level of the glucocorticoids, such as the metabolism, circadian rhythm, and physical activity (Sjaastad *et al* 2016), there is often no consideration of alternative hormones. The ubiquity of glucocorticoids as a measure of stress can be seen in articles on animal welfare research, for example in reviews on dehorning in cattle (*Bos taurus*) (Canozzi *et al* 2019), group housing of sows (*Sus scrofa*) (Verdon *et al* 2015) and the separation of lambs from ewes (*Ovis aries*) (Mora-Medina *et al* 2015).

However, stressful situations may result in a number of different physiological changes. The hypothalamus activates not only the anterior pituitary and the adrenal cortex, which results in release of glucocorticoids, but also the sympathetic nervous system, leading to increased heart rate and blood pressure, and increased catecholamine levels (Sjaastad *et al* 2016). In addition, several other hormonal systems in the body may be affected. Since different individuals may react differently in the same stress situations, it is crucial to assess multiple physiological and behavioural variables. Whilst cortisol is a well-established stress hormone, known to be connected to stress and pain in several species (Barchas & Berger 1980; Hawkes 1992), other, less-commonly recorded hormones may be involved in the stress response, eg vasopressin (Cameron *et al* 1985; Nyman *et al* 1996) and β-endorphin (Greenwood & Shutt 1990; Hydbring 1999a). In addition, different hormones may affect each other. For example, oxytocin has been shown to influence the level of cortisol (Brown *et al* 2016). In an earlier study, we have shown that several different hormones are involved during fearfulness in dogs (*Canis familiaris*), and that dogs exposed to gun shots had different physiological responses compared to when they were exposed to a floor test (Hydbring-Sandberg *et al* 2004). A previous study, on the effect of separation of a kid from its mother, showed that despite the mother goat's (*Capra hircus*) frequent vocalisation during separation, no cortisol or cardiovascular responses were involved (Winblad von Walter *et al* 2010).

In previous research, tethering has been reported to be an important stressor with tethered animals showing a physiological stress response. In cattle, tethering resulted in short-term elevated cortisol levels (Ladewig & Smidt 1989) as well as increased heart rate (Müller *et al* 1989). Likewise,

tethering of sows has been shown to result in elevated cortisol levels (van der Staay 2010; Barnett *et al* 2011). The welfare research has mainly focused on the overall effect of the housing of animals, different management systems, and the animals' need for the company of other animals (eg Friend & Dellmeier 1987; Kapp *et al* 1997; Loijens *et al* 2002; Aschwanden *et al* 2008). The rationale for choosing tethering as a stressor in the current study was that it is a known stressor in a range of species, but for which most studies have only chosen one or few physiological measures.

The aim of the current study was to investigate the physiological responses to tethering compared to loose-holding in the same goats, by recording both heart rate and arterial blood pressure, as well as plasma concentrations of cortisol, β-endorphin, vasopressin, and oxytocin. As far as we know, there have been no papers published on the effect of housing on the combination of several physiological variables, such as arterial blood pressure, heart rate, and different stress hormones in goats.

Materials and methods

Study animals

Eight non-pregnant, non-lactating female goats of the Swedish domestic breed, aged two to six years, participated in the study. Six of the goats had unique implanted transmitters that registered heart rate and arterial blood pressure telemetrically in the unrestrained animal. For details of the surgery, see Hydbring *et al* (1997). In two of the goats, the transmitter batteries had run out and, since it is not possible to change the battery without repeating the surgical procedure, these animals did not participate in the telemetric registrations. Prior to the onset of the experiment, all goats were familiar with each other, and to different experimental procedures. The goats were raised together as a group, kept loose in groups between the experiments, but during telemetric registrations tended to be kept in separate pens (1.2 × 1.5 m; length × width), where they could move around freely, with straw as bedding material. All animals were well accustomed to handling, and the local Animal Ethics Committee of Uppsala certified their care, as well as the experimental design of this study (C120/95; C105/98).

Experimental procedure

With this telemetric method, it was not possible to register several loose-housed goats simultaneously in the same pen. Therefore, the goats were randomly divided into pairs, except for the two goats with transmitters with flat batteries which were placed in the same pair. Thereafter, all pairs were randomly divided into two groups and the experiment performed in a cross-over design where the goats changed system after seven days. The goats were either kept loose in pairs in a pen (3.6 × 3 m) or tied up individually in a metabolism cage (1.2 × 0.6 m) with net flooring. The metabolism cages were placed beside each other and all animals in the two treatments were kept in the same room and could see and hear each other during the experiment. The animals were fed hay and concentrate at 0700 and 1500h and had *ad libitum* access to salt lick and water. Before the experiment started, the animals were housed in one of the new treatments for four days for acclimatisation. Thereafter, telemetry measurements were made during two consecutive days, where half of the goats in each treatment were registered for 24 h the first day, and the other half were registered for 24 h the second day, since it is not possible to register two animals in the same pen at the same time. Thus, only transmitters in half of the goats in each treatment were turned on for registrations on each day. Each goat was measured with the telemetric technique every 30 min during a 24-h period. Since we did not want to disturb the animals by taking blood samples during the telemetric measurements, samples were taken on the third day by venepuncture at 0800, 1000, 1200, 1400, and 1600h. The day after sampling the goats switched system and stayed in their new system for four days before the second registration period started, see previously. The behaviour of the goats was recorded using time-lapse cameras. However, technical difficulties meant the video recordings were unable to be used and, thus, the behavioural results are not presented here.

Heart rate and arterial blood pressure registrations

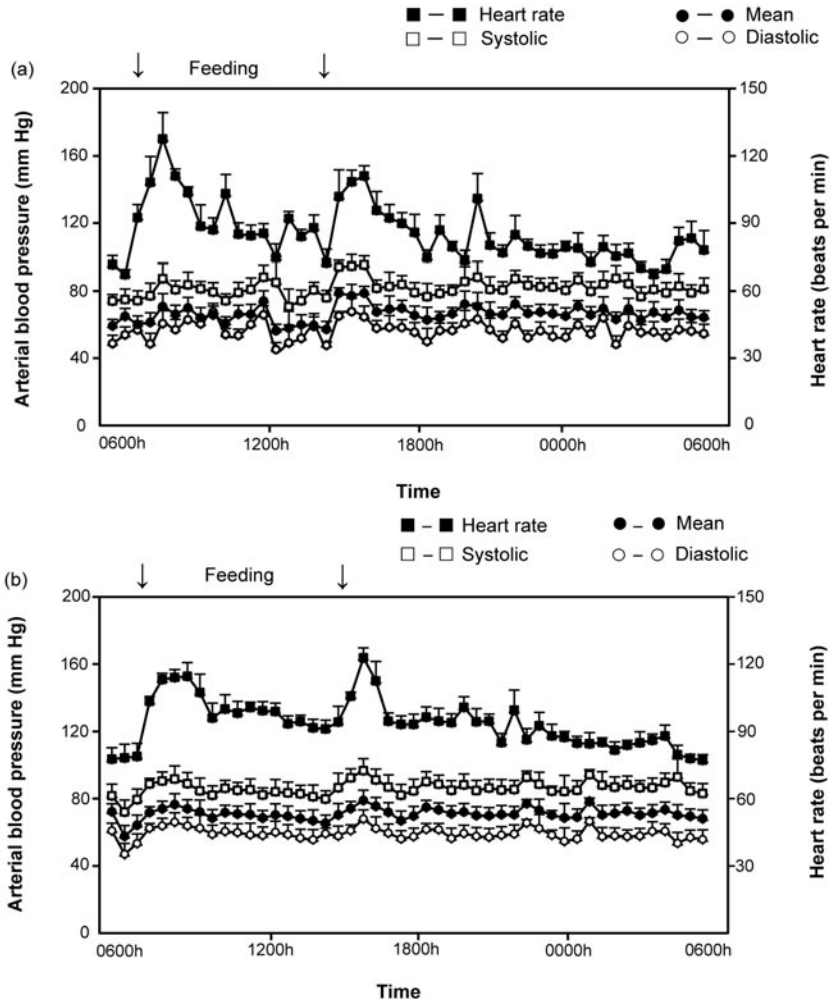
Heart rate and arterial blood pressure were measured using a unique radio telemetric technique (Hydbring *et al* 1997, 1999b). The implant (TA11PA-D70) is calibrated by the manufacturer prior to delivery (Data Sciences Inc, St Paul, Minnesota, USA) in a system also validated in other species (Brooks *et al* 1996). In addition, we also validated the technique for goats in our lab by comparing telemetric measurements of blood pressure with blood pressure measurements in exteriorised carotid artery in the same two goats (for details of this method, see Eriksson *et al* 1994). Also, the system was checked for drift. Both arterial blood pressure and heart rate were shown to be stable for at least nine months after surgery, thereafter blood pressure, but not heart rate, started to decrease with a maximum of 5 mm Hg. In this study, there was no drift in the system and since each goat participated in both treatments, they acted as their own controls. Each telemetry device consists of a sealed transmitter body and a fluid-filled catheter. The surgical procedure has been previously described (Hydbring *et al* 1997). Briefly, under anaesthesia, the transmitter body was placed subcutaneously on the lateral side of the neck, and the catheter (40 cm) was tunnelled subcutaneously to the superficial temporal artery and then into the carotid artery. During the registrations, heart rate and blood pressure signals were transmitted to a receiver, placed centrally above each box, and the signals digitised. The transmitter body contains a magnet-actuated switch so that the transmitter can be turned on and off in the conscious animal, thereby prolonging the lifetime of the battery. While the goats were not being sampled, their transmitters were turned off, in order to avoid interference with other animals' transmitters. Registrations were made every 30 min, where each value is a mean calculated over a period of 10 s in each animal. The software package used was DataQuest IV (Data Sciences Inc, St Paul, Minnesota, USA).

Blood plasma analysis

Blood was withdrawn into ice-chilled tubes containing K3-ethylenediaminetetra-acetic acid (EDTA) and aprotinin (Trasylol, Bayer, Leverkusen, Germany; 5,000 Kallikrein-Inhibitor-Units per 10 ml tube). Thereafter, the blood was

https://doi.org/10.7120/09627286.31.1.008 Published online by Cambridge University Press

Figure 1



Mean (± SEM) diurnal variations in heart rate and blood pressure values (systolic, mean and diastolic) in the same six goats when (a) held loose in pairs, and (b) tied up. Arrows denote times of feed distribution.

centrifuged at 1,500 g for 20 min at 4°C and stored at –20°C for 24 h and then at –80°C until assayed. Dilutions of goat plasma were parallel to the standard curve in all RIAs described as follows. Plasma cortisol was measured using the Coat-A-Count RIA (Diagnostic Product Corporation, Los Angeles, CA, USA). Plasma β-endorphin was extracted and analysed according to the Peninsula Laboratories RIA (Inc, San Carlos, CA, USA). Prior to vasopressin and oxytocin analysis, the plasma was extracted according to Hydbring *et al* (1999a), and the hormones were then analysed using the respective kits from Euro Diagnostica (Ideon, Malmö, Sweden). The lower detection limit was 2.9 nmol l^{-1} for cortisol, 1.7 pmol l^{-1} for β-endorphin, 0.7 pmol l^{-1} for vasopressin, and 3.0 pmol l^{-1} for oxytocin. In all four RIAs, the samples were measured in the same assay. The inter-assay coefficient of variance (CV) for cortisol was < 10% between 32–1,380 nmol l^{-1}, for β-endorphin < 10% between 4–37 pmol l^{-1}, for vasopressin < 10% between 1–60 pmol L^{-1}, and for oxytocin < 10% between 17–744 pmol l^{-1}.

Statistical analysis

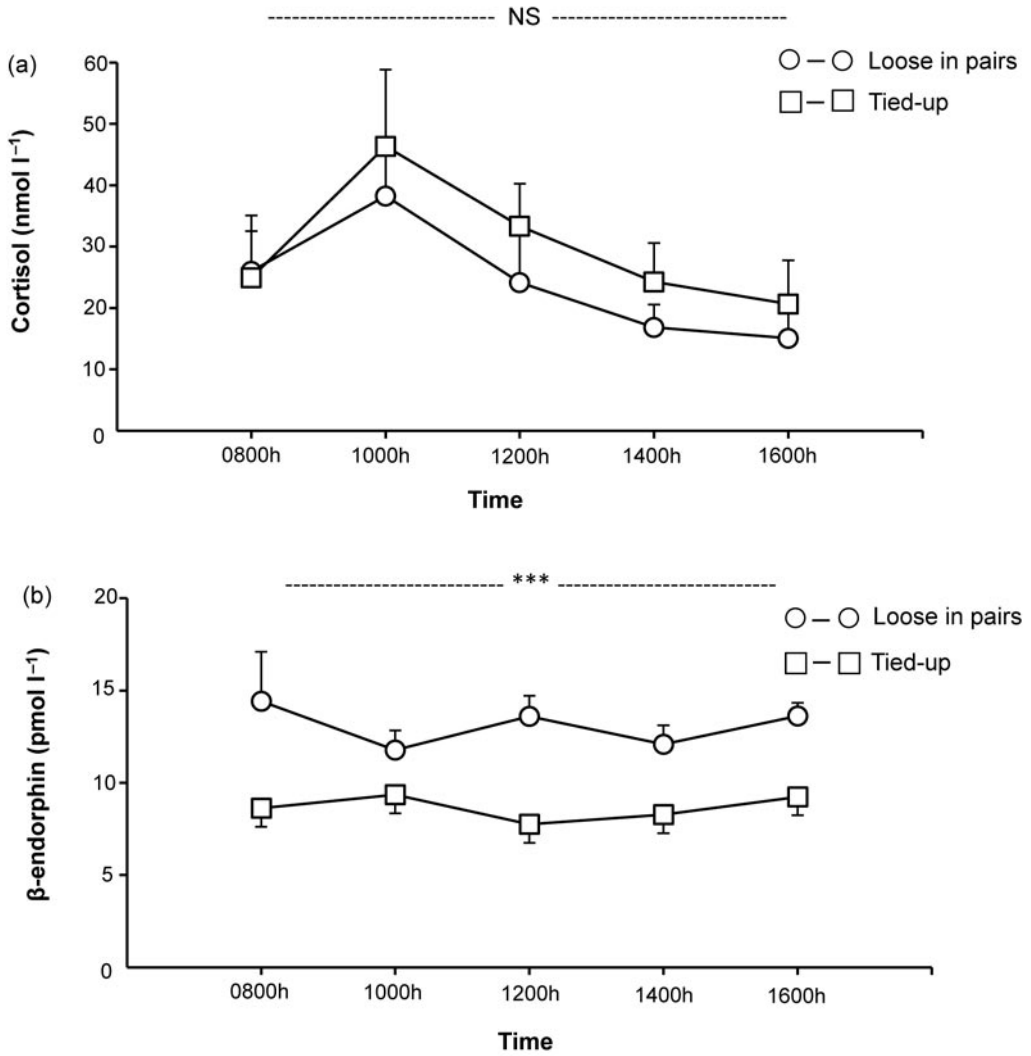
Values are presented as means (± SEM). Data were analysed using the SAS software 9.4 (SAS Institute 2002–2015). The repeated measurement analysis of variance (procedure MIXED) was applied to respective parameters. The statistical model included the fixed effect of sample and the random effect of animal. Comparison between telemetry data and treatments was carried out, including all values, during the 24-h period. In hormonal data, pair-wise comparisons against the first sample were tested for significance using differences in least square means (the DIFF option). Bonferroni adjustment was used to limit the risk for false mass significance. The significance level was set at $P \leq 0.05$.

Results

Heart rate and arterial blood pressure

Heart rate tended to increase around morning and afternoon feeding times, both when the goats were held loose in pairs, and when tethered. Arterial blood pressure values fluctuated during the day in both treatments (Figures 1[a] and [b]). Statistical comparison between the different treatments during the whole day revealed that heart rate ($P \leq 0.0001$, DF = 522), systolic ($P = 0.0002$, DF = 523), mean ($P \leq 0.0001$, DF = 521), and diastolic ($P = 0.0191$, DF = 504) blood pressure values were significantly higher when the goats were tethered (Figures 1[a] and 1[b]).

Figure 2



Mean (± SEM) plasma concentrations of (a) cortisol, (b) β-endorphin, (c) vasopressin and (d) oxytocin in the same eight goats when held loose in pairs and tied-up (filled symbols indicate significant difference compared to the first sample within treatment).
*** Indicates significant difference between treatments ($P \leq 0.001$). NS indicates no differences between treatments

Plasma concentrations of cortisol, β-endorphin, vasopressin and oxytocin

The cortisol concentration did not change during the day in either of the two treatments, and there was no significant difference between the treatments (NS; $P = 0.2653$, DF = 78) (Figure 2). The β-endorphin concentration did not change during the day in either of the treatments (NS), but the concentration was significantly lower when the goats were tethered ($P \leq 0.001$, DF = 78) (Figure 2).

The concentration of plasma vasopressin did not change during the day in either of the treatments, and there was no difference between goats held loose in pairs vs those tethered (NS; $P = 0.0714$, DF = 78) (Figure 2). When goats were held loose, the concentration of plasma oxytocin decreased in sample 2, 3 and 4, compared to the first sample ($P = 0.0384$, $P = 0.0187$, $P = 0.0095$, DF = 28), but increased to the same level as the first sample in the last blood sample ($P = 0.16$, DF = 28). The concentration was significantly lower when goats were tethered than when they were held loose ($P = 0.0009$, DF = 78) (Figure 2).

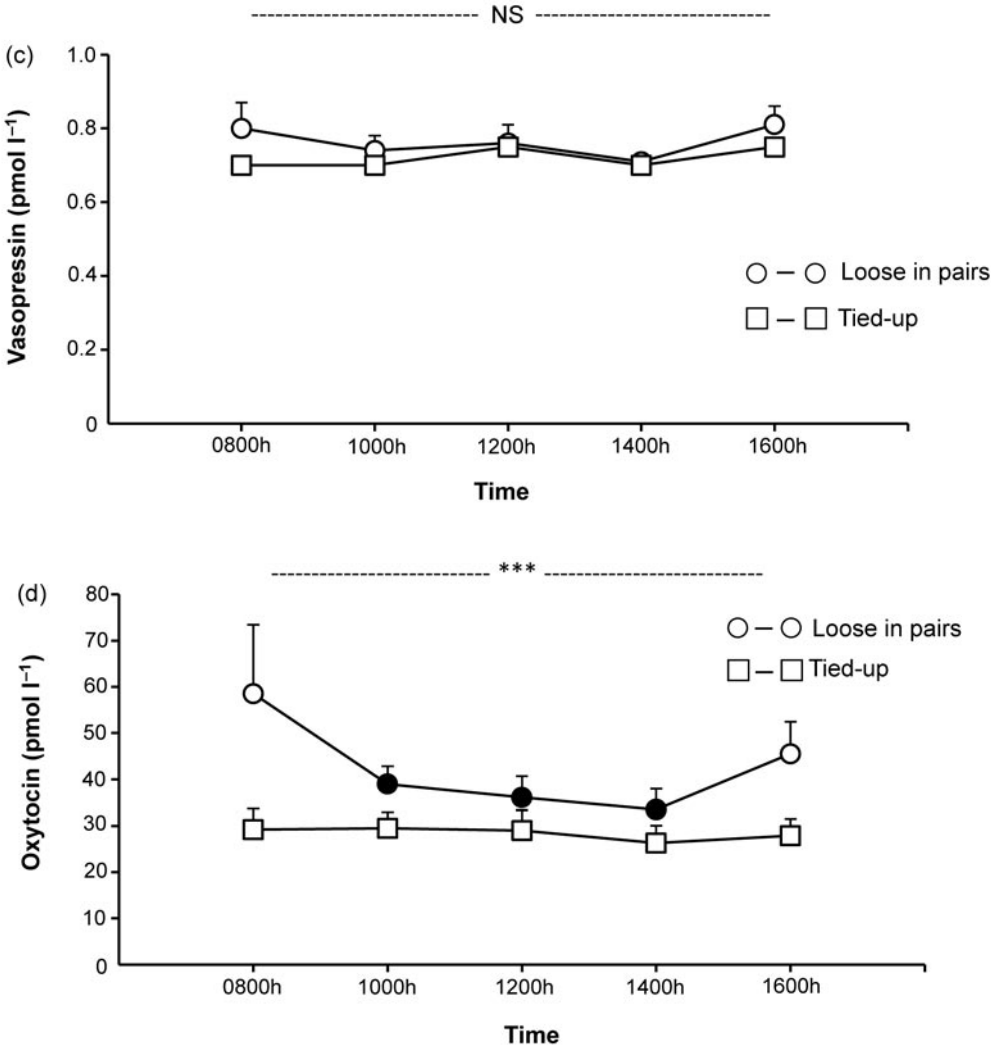
Discussion

The results of this study showed that while a number of physiological variables differed between goats free to range in a pen, and those tethered, the concentrations of cortisol and vasopressin, surprisingly, did not. Tethered goats generally had higher heart rate and arterial blood pressure measurements, compared to goats kept loose in pairs, which indicates that the sympathetic nervous system was activated to a greater extent in tethered goats. The higher heart rate and blood pressure of tethered goats should therefore be caused, for the most part, by mental stress and psychological factors, rather than physical activity.

With this telemetric method, it was not possible to register several loose-housed goats simultaneously in the same pen.

https://doi.org/10.7120/09627286.31.1.008 Published online by Cambridge University Press

Figure 2 (cont)



Therefore, we put the loose-housed goats together in pairs in different pens since we wanted to carry out the observations at the same time in both treatment groups. The different individuals in the pairs may however have affected each other differently. Nevertheless, all goats were familiar with each other before the experiment started and quickly adapted to be kept together. Our previous research has shown that goats increase both arterial blood pressure, and heart rate, in stressful situations, such as during exposure to a dog while pregnant (Olsson & Hydbring-Sandberg 2011), restraint and administration of fluid (Eriksson *et al* 1994), and parturition (Hydbring *et al* 1997), but not during separation from their kids (Winblad von Walter *et al* 2010). Heart rate and arterial blood pressure varied during the day, and heart rate, in particular, increased around feeding in both treatments. This is in accordance with our earlier studies, where heart rate increased in connection to feeding in all reproductive periods (Hydbring *et al* 1997, 1999b). Both heart rate and arterial blood pressure tended to vary more, when goats were kept loose in pairs, than when they were tied up. This could be explained by the fact that animals kept in loose-housing systems could vary their behaviours more, eg move around and interact socially with the other animal. Goats kept loose in pairs had more space, could perform additional behaviours, and thus were more physically active.

Surprisingly, there were no differences in cortisol and vasopressin concentrations between the treatments. However, this is in accordance with a previous study we performed (Winblad von Walter *et al* 2010), where plasma cortisol and vasopressin concentrations in goats did not change after separation from the goat's kid. The cortisol concentration had a greater individual variation compared to the other hormones and it could be argued that the cortisol concentration may have been significantly higher in tethered goats if we had included more animals in the study. However, from an ethical point of view, it is always important to reduce the numbers of animals, especially since the telemetric device is invasive. The experiment was performed in a cross-over design, so each goat participated in both treatments and thus operated as their own control. It is also important to notice that the cortisol levels measured in both treatments in this study were low (mean values between 20–50 nmol l^{-1}),

compared to when goats were intubated with a stomach tube (150 nmol l^{-1}; Eriksson *et al* 1994) or during parturition (250 nmol l^{-1}; Hydbring *et al* 1999a). The fact that cortisol remained at low levels in this study further strengthens the notion that the HPA-axis did not become activated to a high extent in tethered goats but that tethered goats instead activated the sympathetic nervous system and other hormonal systems. It is obvious that demanding situations do not always stimulate the HPA-axis, as shown by us recently where goat kids revealed a varied response in saliva cortisol concentrations during isolation and an arena test (Winblad von Walter *et al* 2021).

Goats that were kept loose in pairs had higher levels of β-endorphin and oxytocin, compared to goats that had been tethered. Higher β-endorphin concentrations have been reported in humans during increased pleasure and well-being (Barchas & Berger 1980; Hawkes 1992), and physical activity has been reported to increase β-endorphin concentration in humans and horses (*Equus caballus*) (Sforzo 1989; Malinowski *et al* 2006). It has been stated that oxytocin does not demonstrate a diurnal rhythm in sheep (Wathes *et al* 1992) and did not change in goats during suckling or separation from the kid (Winblad von Walter *et al* 2010). The reason for the higher oxytocin concentrations in goats kept in pairs may be increased physical activity, since this has been reported to increase oxytocin concentration in rats (*Rattus norvegicus*) (Kasting 1988). Another reason may be the social interactions between goats, since social bonding and relationships have been reported to implicate oxytocin release (Uvnäs Moberg 1998; Kendrick 2005; Lim & Young 2006).

The welfare of tethered animals is the subject of much debate (Ellis *et al* 2009; Ostojić-Andrić *et al* 2011; Battini *et al* 2014; Bernhard *et al* 2020). In this study, we sampled the animals for a shorter period when they have been in the system for four days. It would have been interesting to follow the long-term effects of tethering to investigate whether the higher levels of blood pressure and heart rate in tethered goats would persist over time. An increased sympathetic nervous system over a long period results in a serious strain on the cardiovascular system and may have a detrimental effect on welfare.

Animal welfare implications

Animal welfare is a multi-faceted concept, and several measures are typically used to assess it. While there is an acknowledgement of the need for various behavioural measures, the physiological measures used are often restricted to a much smaller number, with cortisol concentration being one of the most common, eg Veissier *et al* (2008) suggest that tethering in cattle do not lead to frustration and Hales *et al* (2016) that confinement in farrowing sows is not stressful based on cortisol results. In this article, we show that using only cortisol would lead to the conclusion that there is no difference in the goats' stress reaction between the treatments. We therefore argue that it is necessary to use a wider variety of physiological measures to capture differences in the reaction to possible welfare challenges, reactions that would otherwise go undetected.

Conclusion

Our results indicate that housing and the company of another animal affect arterial blood pressure, heart rate, and the concentrations of β-endorphin and oxytocin in goats, but that the cortisol and vasopressin concentrations did not differ between the treatments. Tethered goats had higher heart rates and arterial blood pressures, as well as lower concentrations of β-endorphin and oxytocin, indicating that tethering may be more stressful for the goat compared to when held loose in pairs. The use of single measures, eg cortisol concentrations, is insufficient to provide an accurate reflection of the animal's response to the management system. Thus, it is important that evaluations of stress situations in animals do not exclusively rely on measurements of merely one stress hormone, and that different stress variables may be appropriate for different situations and species.

Declaration of interest

None.

Acknowledgements

This work was supported by the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (project no 22.1/2001-0917). We thank Mrs Åsa Eriksson and Mrs Inger Lind for taking good care of the animals, and Mrs Gunilla Drugge for excellent help with hormonal analyses.

References

Aschwanden J, Gygax L, Wechsler B and Keil N 2008 Cardiac activity in dairy goats whilst feeding side-be-side at two different distances and during social separation. *Physiology & Behavior 95*: 641-648. https://doi.org/10.1016/j.physbeh.2008.09.016

Battini M, Vieira A, Barbieri S, Ajuda I, Stilwell G and Mattiello S 2014 Invited review: Animal-based indicators for on-farm welfare assessment for dairy goats. *Journal of Dairy Science 97*: 6625-6648. https://doi.org/10.3168/jds.2013-7493

Barchas, JD and Berger PA 1980 Endogenous opioids: Basic and clinical aspects. *Psychopharmacology Bulletin 16*: 51-52

Barnett JL, Hemsworth PH, Butler KL, Schirmer BN, Borg SS and Cronin GM 2011 Effects of stall dimensions on the welfare of pregnant sows. *Animal Production Science 51*: 471-480. https://doi.org/10.1071/EA07124

Bernhard JK, Vidondo B, Achermann RL, Rediger R, Müller KE and Steiner A 2020 Carpal, tarsal, and stifle skin lesion prevalence and potential risk factors in Swiss dairy cows kept in tie stalls: A cross-sectional study. *PLoS One* 1-18. https://doi.org/10.1371/journal.pone.0228808

Brooks D, Horner R, Kozar L, Waddell T, Render C and Phillipson E 1996 Validation of a telemetry system for long-term measurement of blood pressure. *Journal of Applied Physiology 81*: 1012-1018. https://doi.org/10.1152/jappl.1996.81.2.1012

https://doi.org/10.7120/09627286.31.1.008 Published online by Cambridge University Press

Brown CA, Cardoso C and Ellenbogen MA 2016 A meta-analytic review of the correlation between peripheral oxytocin and cortisol concentrations. *Frontiers in Neuroendocrinology 43*: 19-27. https://doi.org/10.1016/j.yfrne.2016.11.001

Cameron V, Espiner E, Nicholls M, Donald R and MacFarlane M 1985 Stress hormones in blood and cerebrospinal fluid of conscious sheep: effect of hemorrhage. *Endocrinology 116*: 1460-1465. https://doi.org/10.1210/endo-116-4-1460

Canozzi MEA, Mederos A, Turner S, Manteca X, McManus C, Menegassi SRO and Barcellos JOJ 2019 Dehorning and welfare indicators in beef cattle: A meta-analysis. *Animal Production Science 59*: 801. https://doi.org/10.1071/AN17752

Ellis KA, Billington K, McNeil B and McKeegan DEF 2009 Public opinion on UK milk marketing and dairy cow welfare. *Animal Welfare 18*: 267-282.

Eriksson L, Hydbring E, Tuomisto L, MacDonald E, Kokkonen U-M and Olsson K 1994 Intraruminal fluid administration to goats: Effects of handling and fluid temperature. *Acta Veterinaria Scandinavica 35*: 289-298. https://doi.org/10.1186/BF03548334

Friend T and Dellmeier G 1987 Effects of changing housing on physiology of calves. *Journal of Dairy Science 70*: 1595-1600. https://doi.org/10.3168/jds.S0022-0302(87)80187-X

Greenwood PL and Shutt DA 1990 Effects of management practices on cortisol, β-endorphin and behaviour in young goats. *Proceedings of the Australian Society of Animal Production 18*: 224-227

Hales J, Moustsen VA, Nielsen MBF and Hansen CF 2016 The effect of temporary confinement of hyperprolific sows in sow welfare and piglet protection pens on sow behaviour and salivary cortisol concentrations. *Applied Animal Behaviour Science 183*: 19-27. https://doi.org/10.1016/j.applanim.2016.07.008

Hawkes CH 1992 Endorphins: the basis of pleasure? *Journal of Neurology, Neurosurgery and Psychiatry 55*: 247-250. https://doi.org/10.1136/jnnp.55.4.247

Hydbring E, Cvek K and Olsson K 1999b Telemetric registration of heart rate and blood pressure in the same unrestrained goats during pregnancy, lactation and the nonpregnant nonlactating period. *Acta Physiologica Scandinavica 165*: 135-141. https://doi.org/10.1046/j.1365-201x.1999.00498.x

Hydbring E, MacDonald E and Olsson K 1997 Radiotelemetrically recorded blood pressure and heart rate in relation to plasma catecholamine levels during parturition in the conscious, unrestrained goat. *Acta Physiologica Scandinavica 161*: 295-302. https://doi.org/10.1046/j.1365-201X.1997.00223.x

Hydbring E, Madej A, MacDonald E, Drugge-Boholm G, Berglund B and Olsson K 1999a Hormonal changes during parturition in heifers and goats are related to the phases and severity of labour. *Journal of Endocrinology 159*: 75-85. https://doi.org/10.1677/joe.0.1600075

Hydbring-Sandberg E, Winblad von Walter L, Höglund K, Svartberg K, Swenson L and Forkman B 2004 Physiological reactions to fear provocation in dogs. *Journal of Endocrinology 180*: 439-448. https://doi.org/10.1677/joe.0.1800439

Kapp G, Friend T, Knabe D, Bushong D and Lay D 1997 Nutritional, physiological, and behavioral effects of metabolism crates on lambs. *Contemporary Topics in Laboratory Animal Science 36*: 61-65

Kasting N 1988 Simultaneous and independent release of vasopressin and oxytocin in the rat. *Canadian Journal of Physiology and Pharmacology 66*: 22-26. https://doi.org/10.1139/y88-004

Kendrick K 2005 The neurobiology of social bonds. *Journal of Neuroendocrinology 15*: 1007-1008. https://doi.org/10.1111/j.1365-2826.2004.01262.x

Ladewig J and Smidt D 1989 Behavior, episodic secretion of cortisol, and adrenocortical reactivity in bulls subjected to tethering. *Hormones and Behavior 23*: 344-360. https://doi.org/10.1016/0018-506X(89)90048-2

Lim M and Young L 2006 Neuropeptidergic regulation of affiliative behavior and social bonding in animals. *Hormones and Behavior 50*: 506-517. https://doi.org/10.1016/j.yhbeh.2006.06.028

Loijens L, Janssens C, Schouten W and Wiegant V 2002 Opioid activity in behavioral and heart rate responses of tethered pigs to acute stress. *Physiology & Behavior 75*: 621-626. https://doi.org/10.1016/S0031-9384(01)00650-3

Malinowski K, Shock EJ, Rochelle P, Kearns CF, Guirnalda PD and McKeever KH 2006 Plasma beta-endorphin, cortisol and immune responses to acute exercise are altered by age and exercise training in horses. *Equine Veterinary Journal 36*: 267-273. https://doi.org/10.1111/j.2042-3306.2006.tb05551.x

Mora-Medina P, Mota-Rojas D, Arch-Tirado E and Orozco-Gregorio H 2015 Animal welfare in lambs: Ewe-lamb separation. *Large Animal Review 21*: 39-44

Müller C, Ladewig J, Thielscher H and Smidt D 1989 Behavior and heart rate of heifers housed in tether stanchions without straw. *Physiology & Behavior 46*: 751-754. https://doi.org/10.1016/0031-9384(89)90362-4

Nyman S, Hydbring E and Dahlborn K 1996 Is vasopressin a stress hormone in the horse? *Pferdeheilkunde 12*: 423-427. https://doi.org/10.21836/PEM19960410

Olsson K and Hydbring-Sandberg E 2011 Exposure to a dog elicits different cardiovascular and behavioral effects in pregnant and lactating goats. *Acta Veterinaria Scandinavica 53*: 60. https://doi.org/10.1186/1751-0147-53-60

Ostojić-Andrić D, Hristov S, Novaković Ž, Pantelić V, Petrović MM, Zlatanović Z and Nikšić D 2011 Dairy cows welfare quality in loose vs tie housing system *Biotechnology in Animal Husbandry 27*: 975-984. https://doi.org/10.2298/BAH1103975O

Ralph CR and Tilbrook AJ 2016 Invited review: The usefulness of measuring glucocorticoids for assessing animal welfare. *Journal of Animal Science 94*: 457-470. https://doi.org/10.2527/jas.2015-9645

SAS Institute Inc 9.2 2002-2015 *SAS User's Guide: Statistics*. SAS: Cary, NC, USA

Sforzo G 1989 Opioids and exercise. An update. *Sports Medicine 7*: 109-124. https://doi.org/10.2165/00007256-198907020-00003

Sjaastad O, Sand O and Hove K 2016 *Physiology of Domestic Animals, Third Edition.* Scandinavian Veterinary Press: Oslo, Norway

Uvnäs Moberg K 1998 Oxytocin may mediate the benefits of positive social interaction and emotions. *Psychoneuroendocrinology 199*: 819-835. https://doi.org/10.1016/S0306-4530(98)00056-0

van der Staay FJ, Schuurman T, Hulst M, Smits M, Prickaerts J, Kenis G and Korte SM 2010 Effects of chronic stress: A comparison between tethered and loose sows. *Physiology & Behavior 100*: 154-164. https://doi.org/10.1016/j.physbeh.2010.02.020

Veissier I, Andanson S, Dubroeucq H and Pomiès D 2008 The motivation of cows to walk as thwarted by tethering1. *Journal of Animal Science 86*: 2723-2729. https://doi.org/10.2527/jas.2008-1020

Verdon M, Hansen CF, Rault JL, Jongman E, Hansen LU, Plush K and Hemsworth PH 2015 Effects of group housing on sow welfare: A review. *Journal of Animal Science 93*: 1999-2017. https://doi.org/10.2527/jas.2014-8742

Wathes D, Ayad V, Kumari M, Matthews E and Wathes C 1992 A comparison of plasma vasopressin and oxytocin concentrations during the oestrus cycle of the ewe. *Journal of Endocrinology 134*: 107-113. https://doi.org/10.1677/joe.0.1340107

Winblad von Walter L, Forkman B, Högberg, M and Hydbring-Sandberg E 2021 The effect of mother goat's presence during rearing on kid's response to isolation and to an arena test. *Animals 11*: 575. https://doi.org/10.3390/ani11020575

Winblad von Walter L, Lidfors L, Madej A, Dahlborn K and Hydbring-Sandberg E 2010 Cardiovascular, endocrine and behavioural responses to suckling and permanent separation in goats. *Acta Veterinaria Scandinavica 52*: 51. https://doi.org/10.1186/1751-0147-52-51

https://doi.org/10.7120/09627286.31.1.008 Published online by Cambridge University Press