SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2022v43n5p1891

Cumulative effect of different acute stressors on physiological and hormonal responses and milk yield in lactating Saanen goats

Efeito cumulativo de diferentes estressores agudos sobre as respostas fisiológicas, hormonais e produção de leite de cabras Saanen em lactação

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Highlights

Stress affects hormonal, metabolic, and behavioral responses in goats.
Exposure to different acute stressors can cause cumulative stress in goats.
Cumulative stress causes a decrease in milk yield.

Abstract

This study addresses the hypothesis that acute stress can cumulatively cause a decrease in milk yield in Saanen goats. In fact, dairy animals are subject to several environmental and management challenges that may cause acute stress during the same lactation. However, the cumulative effect of acute stress on milk yield remains unclear. Thus, the objective of this study was to evaluate the effects of different acute stressors on milk yield and milk quality in goats. Thirty Saanen goats were either maintained on their usual routine and comfort conditions (control group) or subjected to different environmental stressors (heat stress, adrenocorticotropic hormone [ACTH] administration, hoof care, and rain). These stressful challenges were performed sequentially, one challenge per day, on four consecutive days to evaluate the influence of the challenge on milk yield and milk quality. The acute stress imposed on goats caused significant changes in respiratory rate, rectal temperature, cortisol, insulin, triiodothyronine, insulin-like growth factor 1, and glucose concentrations when compared to the control group. Although these acute-stress-triggered physiological responses are fundamental to restoring homeostasis, the cumulative effects of different imposed challenges caused a change in hormone release, an increase in somatic cell count

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Received: Mar.02, 2022 - Approved: May 19, 2022

(SCC), and a decrease in milk yield. In this context, the results of this study indicate that farmers should avoid concurrently subjecting goats to management and environmental challenges that can induce acute stress because these stressors have a negative and cumulative impact on SCC and milk yield.
Key words: Acute stressors. Goat. Lactation. Mammary gland.

Resumo

Este estudo aborda a hipótese de que o estresse agudo pode causar cumulativamente uma diminuição na produção de leite de cabras Saanen. De fato, os animais leiteiros estão sujeitos a vários desafios ambientais e de manejo que podem causar estresse agudo durante a mesma lactação. No entanto, o efeito cumulativo do estresse agudo na produção de leite ainda não está claro. Assim, o objetivo deste estudo foi avaliar os efeitos de diferentes estressores agudos na produção e qualidade de leite de cabras Saanen. Trinta cabras Saanen foram mantidas em sua rotina habitual e condições de conforto (grupo controle) ou submetidas a diferentes estressores ambientais (estresse por calor, administração de hormônio adrenocorticotrófico [ACTH], cuidados com os cascos e chuva). Esses desafios estressantes foram realizados sequencialmente, um desafio por dia, em quatro dias consecutivos para avaliar a influência do desafio na produção e qualidade do leite. O estresse agudo imposto as cabras causou alterações significativas na frequência respiratória, temperatura retal, cortisol, insulina, triiodotironina, fator de crescimento semelhante à insulina 1 e concentrações de glicose quando comparado ao grupo controle. Embora essas respostas fisiológicas desencadeadas pelo estresse agudo sejam fundamentais para restaurar a homeostase, os efeitos cumulativos de diferentes desafios impostos causaram uma mudança na liberação de hormônios, um aumento na contagem de células somáticas (CCS) e uma diminuição na produção de leite. Neste contexto, os resultados deste estudo indicam que os produtores devem evitar submeter as cabras concomitantemente a desafios de manejo e ambientais que possam induzir estresse agudo, pois esses estressores têm impacto negativo e cumulativo na CCS e na produção de leite.
Palavras-chave: Estressores agudos. Cabra. Lactação. Glândula mamária.

Introduction

The interaction between an animal and its environment triggers physiological and behavioral responses aimed at maintaining the animal's homeostasis (Gupta et al., 2018; Hooper et al., 2020). However, global warming is negatively associated with milk quality and milk yield (Hamzaoui et al., 2013; Baumgard et al., 2017; Hooper et al., 2020). Indeed, under adverse environmental conditions, even goats described as tolerant to heat stress show physiological, hormonal, and biochemical responses aimed at restoring homeostasis that may be detrimental to milk yield (Fonseca et al., 2016; Contreras-Jodar et al., 2019; Stachowicz et al., 2019). However, the biological mechanisms of this process on milk yield have not yet been fully elucidated.

Routinely, dairy goats face a set of stressful management and heat stress challenges caused by global warming, which can be an additional challenge to lactation. In fact, several management practices, such as batch changes, weaning, first milking, vaccination, hoof trimming, and transport, that are associated with acute stress occur frequently during lactation (Hertem et al., 2014; Hulbert & Moisá, 2016; Polycarp et al., 2016; Bomfim et al., 2018). Indeed, stress-related physiological responses also stimulate changes in body tissue mobilization and metabolic processes associated with extra energy required to restore homeostasis; consequently, a lower flux of nutrients to mammary glands during stressful events is associated with a decrease in milk yield (Bernabucci et al., 2009; Baumgard et al., 2017). For these reasons, environmental and management factors negatively influence dairy goats, allowing adjustments to be implemented on farms to improve goat welfare and milk yield (Patt et al., 2013).

Although several studies have demonstrated that different stressors can cause a decrease in milk yield (Hertem et al., 2014; Gupta et al., 2018; Hooper et al., 2020), the effects of cumulative acute stress on milk yield remain unclear. For these reasons, we hypothesized that different acute stresses can cumulatively lead to a decrease in milk yield in Saanen goats. In this context, the respiratory rate (RR), heart rate (HR), rectal temperature (RT), cortisol, insulin, triiodothyronine (T3), insulin-like growth factor 1 (IGF1) and glucose concentrations after the imposition of different acute stressors could be important tools to assess the negative effects of stress on milk yield. Furthermore, the aim of this study was to evaluate the influence of cumulative acute stressors on the milk quality and milk yield of Saanen goats exposed to environmental and management challenges that can routinely occur on a dairy farm.

Materials and Methods

Experiments and analyses were performed in Pirassununga, São Paulo, Brazil (21°58′ S, 47°26′ W, and 625 m altitude). The climate of the region is classified as humid subtropical (Köppen-Geiger classification). The experimental week occurred from December 10-13, a time frame that corresponds to a hot and rainy period. All animal procedures were approved by the Ethics Committee on Animal Use of the Faculty of Animal Science and Food Engineering of the University of Sao Paulo (protocol 9546150719), which follows federal guidelines.

Animals, Housing, Diets, and Milking Routine

This study used 30 healthy, nonpregnant, lactating (150 ± 10 days) Saanen goats with a mean age of 2.7 ± 1.2 years (24 primiparous and 6 multiparous), a live weight of 63.3 ± 11.4 kg, and an average body score of 3.0 ± 0.5. The goats were fed twice a day (08h00 and 15h00) and had free access to total diet with corn silage and concentrate (corn and soybean meal). The diet's dry matter proportion was 50% silage and 50% concentrate to provide the goats' dietary requirements according to the National Research Council [NRC] (2007). This total diet was adjusted weekly to ensure 10% leftovers after considering body weight, milk yield, and lactation stage to ensure 10%. During the day, goats had free access to paddocks of *Cynodon* spp. (Tifton) At night, all goats were kept in partially covered collective pens (3 m^2 $goat^{-1}$). At all times, all goats had free access to shade-covered pens, a feed trough, fresh water, and a vitamin and mineral mix.

The goats were milked once a day at 07h00 with equipment set at a vacuum level of 48 kPa and a pulsator rate of 120 cycles per minute. All goats were gently guided to the milking parlor and subjected to predipping of the teats with antiseptic iodine solution. Afterward, the teats were dried, the dark-bottomed mug test was performed, and teat cups were attached to each goat. At the end of the milking session, the teat cups were removed, and postdipping of the teats was again performed with antiseptic iodine solution. The milk yield was measured daily throughout lactation using individual collector cups, and milk quality was analyzed daily during the experimental period.

Experimental Design

The different challenges imposed on goats (heat stress, ACTH administration, hoof care, and rain) were considered acute stressors. The 30 Saanen goats were randomly divided into two homogeneous groups. The 15 goats in the control group were maintained in their habitual routine and comfort conditions (control group). The other 15 goats in the stress group were subjected to different challenges (heat stress, ACTH administration, hoof care, and rain) that were sequentially imposed, one challenge per day, over four consecutive days to evaluate the influence of cumulative stress on milk yield and milk quality. During these four experimental days, HR, RR, RT, and blood samples were sampled at 08h00, 12h00, and 16h00. Blood samples were collected just after HR, RR, and RT sampling for analyses of cortisol, insulin, T3, IGF-1, and glucose levels.

In the present study, the stress-related challenges imposed on goats were planned after consideration of maintenance of the general routine of the herd because daily lactating goats are milked between 07h00 and 09h00, have free access to pasture from 09h00 to 17h00, and pass the night closed in partially covered collective pens. After milking, experimental goats were calmly guided to the waiting room in the milking barn for measurements of HR, RR, RT, and blood sampling. At 10h00, the 15 goats from the stress group were subjected to daily challenges (heat stress, ACTH administration, hoof care, and rain). At the same time, the 15 goats from the control group were maintained in their habitual routines and environmental comforts and were not exposed to the different stressors. At 12h00, the same physiological parameters and blood samples were obtained after the goats received the respective treatments (control or stressors challenges). At 15h00, at the end of exposure to stressor-induced challenges, all goats returned to their habitual pens and were kept in the same environment with identical housing conditions and environmental comforts (with free access to shade, total diet, water, and vitamin and mineral mix). At 16h00, one hour after treatment cessation (control or stress) and in the same environment, physiological parameters and blood samples were again obtained for both experimental groups.

During the experimental period, the air temperature and relative humidity of the experimental environment were recorded daily at intervals of 10 min using a data logger (HOBO U12-001, Cape Cod, MA, USA). Indeed, the black globe temperature was taken at 08h00, 12h00, and 16h00. The temperature-humidity index (THI) was calculated as described by Buffington et al. (1981). Experimental samplings were also performed at 08h00, 12h00, and 16h00 from goats subjected to control or stress treatments. For this reason, air temperature, relative humidity, black globe temperature, and THI were presented at 08h00, 12h00, and 16h00 during the imposition of the four challenges on consecutive experimental days (Table 1).

Table 1
Air temperature (AT), relative humidity (RH), and black globe temperature (BGT) at 8h00, 12h00, and 16h00 after stress treatment during the four consecutive experimental days

Date	Challenge	Hour (h)	AT (°C)	RH (%)	BGT (°C)	THI[†]
Dec, 10	1: Heat stress	08h00	22.8	54	27.1	69
		12h00	32.6	47	38.7	81
		16h00	34.0	40	40.3	81
Dec, 11	2: ACTH	08h00	24.2	68	28.8	72
		12h00	30.8	55	36.6	80
		16h00	32.9	39	39.0	80
Dec, 12	3: Hoof care	08h00	24.1	68	28.7	72
		12h00	34.4	45	40.8	83
		16h00	36.3	34	43.1	83
Dec, 13	4: Rain	08h00	25.8	66	30.7	75
		12h00	28.0	72	33.3	79
		16h00	31.8	70	37.7	84

[†]In the present study, the environmental conditions were calculated by THI as suggested by Buffington et al. (1981). THI was class as normal condition (when < than 80), alert (when ≤ 80 and < 85), danger (when 85 ≤ and < 90), and extreme condition (when ≥ 90) for goats (Silanikove & Koluman 2015).

Challenges imposed on goats

Challenge 1 (heat stress)

On the first day of our study (December 12), goats from the heat stress group were subjected to sun exposure for 5 h (10h00 to 15h00) in Tifton pasture without shade and water access to mitigate the heat stress challenge. At the same time, control goats were kept in Tifton pasture with free access to shade and water. At 15h00, all goats were taken to their respective pens, in which they had access to total diet, water, and a vitamin and mineral mix.

Challenge 2 (ACTH)

The second challenge was ACTH administration (December 11), during which ACTH goats received a physiological dose of ACTH (0.6 mg of ACTH kg^{-1} body weight intravenously). Control goats received an intravenous placebo. The ACTH dose used was based on previous studies to compare the impact of different acute stressors on cortisol release (Verkerk et al., 1994; Bomfim et al., 2018). The low ACTH dose ensured that cortisol returns to baseline levels within 6 h after ACTH administration. Placebo and ACTH were administered at 10h00 in their habitual pens. Afterward, the ACTH and control animals were kept in Tifton pasture, where they had access to shade, total diet, and water. At 15h00, all goats were taken to their respective pens, in which they had access to total diet, water, and a vitamin and mineral mix.

Challenge 3 (hoof care)

The third challenge was the hoof care procedure (December 12). This challenge was similar to routine management performed on experimental farms aimed at maintaining the health of the animals' hooves. During this challenge, goats from the hoof care group were guided to the habitual hoof pen, in which they were kept in the shade. The hoof care started at 10h00, and during this challenge, goats were kept in the pen used for hoof care. These goats were taken individually and calmly one by one to the containment box where the hoof manipulation was performed. The hoof trimming in the contained box lasted for 5 to 8 min per animal and was performed using a hoof knife and scissors to remove accumulated organic matter and to trim any excess hoof. At the end of the hoof care procedure, which lasted 2h10 min, goats were then taken to Tifton pasture, in which they had access to shade, total diet, and water. During this time, the control goats were kept in Tifton pasture, in which they had access to shade, total diet, and water. At 15h00, all goats were returned to their respective pens, in which they had access to total diet, water, and a vitamin and mineral mix.

Challenge 4 (rain)

The fourth challenge was rain (December 13). Although rain was forecasted by the National Institute of Meteorology on this date, the goats' habitual pens were equipped with sprinklers and covered with a shading screen to reduce luminosity to guarantee the application of this treatment. In fact, December 13 was a warm day with heavy clouds and without natural rain (just at 10h00), and during the challenge (from 10h00 to 15h00) some rapid drizzles were observed. For this reason, the sprinklers assured a precipitation of approximately 30 mm over a period of 5h00, which corresponds to moderate-strong rainfall in our subtropical region. The amount of water used in the sprinklers ranged from 5 to 10 mm per hour, with intervals of 30 min with rain and 30 min without to simulate periods of more and less intense rainfall, which are also characteristic of our subtropical region. As on rainy days, our goats remained in their pens, and the control goats remained in their habitual pens covered with a shading screen and without exposure to drizzles and artificial rain as done in their habitual routine.

Data Collection, sampling, and Laboratory Analyses

Experimental samplings were taken before (at 08h00), during (at 12h00), and after (at 18h00) the imposition of stress (heat stress, ACTH administration, hoof care, and rain challenges) or control treatment (habitual routine and confront conditions/ environment). Heart rate (HR) was measured at 08h00, 12h00 and 18h00 by auscultation with a flexible stethoscope in the thoracic region and presented as heart beats per minute. Afterward, respiratory rate (RR) was measured by counting the flank movements for 1 min. RT was measured by a digital clinical thermometer with an accuracy of ± 0.10 °C. Just after these measurements (at 8h00, 12h00, and 18h00), blood samples from the jugular vein of goats were collected in tubes containing heparin, and tubes were kept on ice until centrifugation at 3000 rpm for 17 min at 4 °C. The resulting plasma was transferred to tubes and stored at -20 °C until further laboratory analyses.

The plasma concentrations of cortisol, insulin, T3, IGF-1, and glucose were determined using an ELISA reader (Multiscan MS Labsystem, Tiilitie, Vantaa, Finland). All samples were analyzed in duplicate. Glucose was measured using an enzyme kit (Glicose GOD-PAP, Laborlab, Guarulhos, SP, Brazil), and hormone concentrations were analyzed using commercial enzyme immunoassay (EIA) kits for IGF-1 (MyBioSource, San Diego, CA, USA), total T3 (Monobind Inc., Lake Forest, CA, USA), insulin (Monobind Inc., Lake Forest, CA, USA), and cortisol (Monobind Inc., Lake Forest, CA, USA).

Milk yield and milk quality were measured daily before, during, and after the challenges. For microbiological analysis, the milk samples were collected aseptically directly from the teats into sterile tubes after discarding the first three streams. The samples consisted of 50% milk from each teat. Physicochemical analysis and somatic cell counts (SCC) were performed as a representative portion of the milk of each animal sampled from the collector cups after milking. The percentages of fat, protein, and lactose in the milk were determined by ultrasound equipment (MilkScope, Razgrad, Bulgaria). For SCC, duplicate milk smears (10 µL) were prepared and stained with pyronin Y and methyl green for direct microscopic analysis (Raynal-Ljutovac et al., 2007). The SCC was obtained as the average of two fields after counting the cells and multiplying by the microscope factor. The results were converted into a logarithmic scale.

Microbiological analysis of the raw milk was performed using the methods and procedures described in Standard Methods for the Examination of Dairy Products (Wehr & Frank, 2004). Mesophilic bacteria were counted on agar plates prepared with three different culture media and inoculated with 0.1 mL of pure milk for the determination of the number of bacterial colonies. The result was expressed in colony-forming units per milliliter of milk (CFU mL^{-1}). The samples were spread onto a Baird-Parker agar plate supplemented with egg yolk tellurite emulsion for the identification of *Staphylococcus* sp. in addition to plates with MacConkey agar for the identification of total coliforms (gram-negative bacteria originating from the gastrointestinal tract). The plates were incubated for 48 h at 37 °C.

Statistical Analysis

The data were analyzed with the Statistical Analysis System (SAS Institute Inc., Cary, NC). Initially, descriptive analysis was performed using the FREQ and MEANS procedures, and the normality of the data was tested using the Shapiro-Wilk procedure. Afterward, RT, RR, HR, cortisol, insulin, T3, IGF-1, and glucose data were subjected to an analysis of variance by MIXED procedure, which separated the treatment (heat stress, ACTH, hoof care, rain, or control), hour of sampling, the interaction treatment-hour of sampling, and animal as causes of variation. In the model, the treatment and hour of sampling were considered fixed effects, and goats were considered random effects. Milk yield, milk composition, and quality were subjected to an analysis of variance by the MIXED procedure, which separated the treatment (heat stress, ACTH, hoof care, rain, or control), day of lactation, and animal as causes of variation. In the model, the treatment and day of lactation were considered fixed effects, and goats were considered random effects. Several covariance matrices were tested, and the one that had the best structure according to Bayesian information and criteria was selected. When a significant effect was observed, the means were tested using Fisher's test with the threshold for significance defined as P ≤ 0.05. The results are presented as the mean ± the standard error of the mean (SEM).

Results and Discussion

All challenges imposed on goats in the present study were considered acute stressors that can normally occur on a dairy farm. Indeed, acute stressors imposed on experimental goats caused significant effects on RR, RT, HR, cortisol, insulin, T3, IGF-1, and glucose (Table 2). Compared to the control group, the RR was significantly higher at 12h00 during heat stress (72.05 ± 5.18 versus 60.40 ± 4.20 number min-1) and hoof care challenges (55.92 ± 3.50 versus 44.14 ± 4.06 number min-1). In contrast, the RR of goats subjected to ACTH and rain challenges was significantly lower at 12h00 than those measured in the control group (65.87 ± 4.08 versus 83.02 ± 6.54 and 74.47 ± 5.80 versus 84.00 ± 6.00 number min-1, respectively). Indeed, RT at 12h00 was higher for goats exposed to heat stress (38.60 ± 0.10 versus 39.10 ± 0.08 oC) and hoof care (38.85 ± 0.07 versus 39.06 ± 0.08 oC) than control goats. However, at 16h00, RT was significantly higher in goats that received ACTH (39.06 ± 0.08 versus 38.74 ± 0.05 oC) and were subjected to rain challenges (39.55 ± 0.06 versus 39.04 ± 0.03 oC) than in the control goats. In the present study, the environmental conditions calculated by THI were classified as normal or alert conditions for goats (Silanikove & Koluman, 2015). Except during the rain challenge, AT was higher than the limit recommended for goats, and RH was lower than the recommended limit (Maia et al., 2015, 2016).

Consequently, these acute stresses were characterized by short durations ranging from a few minutes to hours and induced physiological responses aimed at restoring the goat's homeostasis (Trevisi & Bertoni, 2009; Chen et al., 2015). In fact, goats subjected to heat stress and hoof care challenges showed an increase in RR as a mechanism of responding to heat loss by breathing with the goal of maintaining thermal equilibrium. Although during these two challenges, RR increased significantly above the physiological range (Maia et al., 2015, 2016; Gómez et al., 2018), goats exposed to heat stress and hoof care showed significantly higher RT than control goats. Consequently, during these challenges, the increase in RR was not effective enough to maintain the normal RT. In contrast, goats subjected to the ACTH and rain challenges showed a reduction in RR as a physiological response to maintain thermal balance and RT because the reduction in RR caused a reduction in the heat exchange with the environment and maintained the internal temperature in the normal range. Similar results have been reported by other studies with goats during rainy periods (Bernabucci et al., 2009; Stachowicz et al., 2019).

Table 2

Effect of treatment (T), sampling hour (H), and their interaction (T H-1) on target responses of goats subjected to heat stress, ACTH administration, hoof care, and rain or maintained in the control conditions/environment. Data are presented as the mean ± standard error of mean.

Trait†	Treatment				
	Heat stress	Control	P-value		
	(n = 15)	(n = 15)	T	H	T H^{-1}
RT (°C)	38.88 ± 0.07	38.89 ± 0.06	0.68	0.01	0.01
RR (number min^{-1})	76.62 ± 6.15	67.29 ± 6.53	0.08	0.01	0.01
HR (number min^{-1})	103.73 ± 3.12	100.53 ± 3.44	0.25	0.11	0.23
CORT (ng mL^{-1})	9.86 ± 2.09	7.56 ± 2.04	0.19	0.35	0.46
INS (ng mL^{-1})	0.21 ± 0.06	0.78 ± 0.22	0.04	0.72	0.44
T3 (ng mL^{-1})	1.69 ± 0.13	1.70 ± 0.15	0.93	0.45	0.69
IGF-1 (pg mL^{-1})	9.62 ± 1.02	10.55 ± 0.70	0.19	0.91	0.86
GLUC (mg mL^{-1})	72.67 ± 12.02	72.56 ± 11.50	0.96	0.01	0.01
Trait	ACTH	Control	P-value		
	(n = 15)	(n = 15)	T	H	T H^{-1}
RT (°C)	39.03 ± 0.10	38.92 ± 0.06	0.10	0.01	0.01
RR (number min^{-1})	63.02 ± 4.50	66.84 ± 6.00	0.40	0.01	0.01
HR (number min^{-1})	100.62 ± 2.57	103.29 ± 2.76	0.23	0.01	0.09
CORT (ng mL^{-1})	27.54 ± 2.93	6.88 ± 1.41	0.01	0.01	0.01
INS (ng mL^{-1})	0.46 ± 0.05	0.53 ± 0.13	0.44	0.17	0.25
T3 (ng mL^{-1})	1.48 ± 0.09	1.56 ± 0.14	0.48	0.82	0.47
IGF-1 (pg mL^{-1})	6.60 ± 0.41	6.49 ± 0.41	0.73	0.14	0.54
GLUC (mg mL^{-1})	75.39 ± 15.20	64.75 ± 6.12	0.01	0.01	0.01
Trait	Hoof care	Control	P-value		
	(n = 15)	(n = 15)	T	H	T H^{-1}
RT (°C)	38.88 ± 0.09	38.82 ± 0.07	0.38	0.01	0.01
RR (number min^{-1})	71.82 ± 5.47	68.18 ± 5.60	0.45	0.01	0.01
HR (number min^{-1})	104.80 ± 2.51	102.66 ± 2.26	0.28	0.01	0.10
CORT (ng mL^{-1})	7.12 ± 2.25	6.72 ± 1.19	0.80	0.01	0.12
INS (ng mL^{-1})	0.46 ± 0.06	0.59 ± 0.16	0.27	0.36	0.14
T3 (ng mL^{-1})	1.60 ± 0.13	1.48 ± 0.16	0.29	0.04	0.19
IGF-1 (pg mL^{-1})	5.94 ± 0.48	5.23 ± 0.31	0.10	0.01	0.09
GLUC (mg mL^{-1})	62.83 ± 1.61	66.70 ± 3.39	0.10	0.07	0.08

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Trait	Rain	Control	P-value		
	(n = 15)	(n = 15)	T	H	T H^{-1}
RT (°C)	39.00 ± 0.06	38.9 ± 0.08	0.26	0.01	0.01
RR (number min^{-1})	53.24 ± 5.83	59.29 ± 5.61	0.33	0.01	0.01
HR (number min^{-1})	104.09 ± 3.07	100.18 ± 2.49	0.21	0.06	0.12
CORT (ng mL^{-1})	7.41 ± 1.75	6.48 ± 1.00	0.50	0.01	0.01
INS (ng mL^{-1})	0.28 ± 0.05	0.51 ± 0.15	0.03	0.01	0.01
T3 (ng mL^{-1})	1.84 ± 0.10	1.62 ± 0.11	0.02	0.01	0.01
IGF-1 (pg mL^{-1})	5.37 ± 0.84	4.52 ± 0.23	0.09	0.58	0.57
GLUC (mg mL^{-1})	82.27 ± 5.27	81.60 ± 4.69	0.87	0.01	0.01

†RT: rectal temperature; RR: respiratory rate; HR: heart rate; CORT: cortisol; INS: insulin; T3: triiodothyronine; IGF-1: insulin-like growth factor 1.

In our study, cortisol concentrations were significantly higher in goats subject to the ACTH challenge than in the control goats that received placebo (Table 2). Indeed, goats subjected to rain had significantly higher cortisol concentrations at 16h00 when compared to the control group (6.05 ± 1.15 versus 3.20 ± 0.41 ng mL^{-1}). In the same way, other authors have shown similar cortisol responses during different farm management practices that are considered stressful to dairy goats (Romero et al., 2015; Bomfim et al., 2018). In fact, acute stress is also characterized by a rapid increase in cortisol levels followed by a return to baseline levels (Trevisi & Bertoni, 2009; Chen et al., 2015). In our study, the cortisol concentrations were significantly higher for goats that were subjected to ACTH and rain challenges than in control goats. Moreover, adrenocorticotropic hormone (ACTH) challenge was used in the present study to test adrenal gland responsivity to acute stressors (Verkerk et al., 1994; Bomfim et al., 2018). As other authors have demonstrated, ACTH administration caused a rapid cortisol peak (between 20 and 60 min), and cortisol concentration returned to baseline between 4 and 6 h after the stress ended (Trevisi & Bertoni, 2009; Chen et al., 2015). Taking into account RR, RT and cortisol release, all challenges imposed on experimental goats were considered acute stressors that can normally occur on a dairy farm.

Moreover, heat stress and rain challenges caused a significant decrease in insulin concentrations when compared to the control treatment (Table 2). Furthermore, the T3 concentration was significantly higher in goats subjected to rain than in the control goats (Table 2). However, the different challenges did not affect IGF-1 levels in experimental goats (Table 2). Finally, goats subjected to heat stress had significantly higher glucose concentrations at 16h00 than the control group (97.80 ± 5.11 versus 73.06 ± 4.13 mg mL^{-1}). Indeed, in our study, goats subjected to rain showed significantly higher cortisol, T3, and glucose levels and significantly lower insulin levels than control goats. Previous studies have demonstrated that these hormones play roles in metabolism, hyperglycemia, energy redirection, and thermogenesis by inducing physiological responses to promote the maintenance of homeostasis (Trevisi & Bertoni, 2009; Al-Samawi et al., 2014; Hough et al., 2015). Contrary to our expectations in lactating animals (Ollier et al., 2016; Ponchon et al., 2017; Bomfim et al., 2018), any challenges caused changes in the IGF-1 concentration. For this reason, we cannot speculate about the relationship between IGF-1 and challenges imposed on experimental goats. Finally, the different stressful challenges mobilized hormonal and adaptive physiological responses, which together re-established the goat's homeostasis.

During our study, there was a significant effect of treatment on milk yield, and goats subjected to stressor challenges produced less milk than control goats (Table 3). Indeed, goats subjected to stressor challenges showed significantly higher SCC than control goats (Table 3). Furthermore, the cumulative effect of challenges led to a significant decrease in milk yield during the last challenge (on day 154 of lactation) and on subsequent days of lactation (on days 155, 160, and 161 of lactation) when compared to control goats (Figure 1). Other studies have associated cortisol and SCC increases with an increase in the apoptosis rate in mammary glands and a consequent increase in the number of mammary epithelial cells in the milk of stressed animals (Romero et al., 2015; Mehdid et al., 2019). However, as cortisol is considered an immunosuppressor, the higher SCC observed in the present study cannot be explained by an increase in defense cells in milk (Gonçalves et al., 2017).

In our study, no differences in milk quality or microbiological status were observed after the imposition of different stressors. Indeed, our results also showed that cumulative stress did not trigger mastitis (Table 3). Moreover, the total bacterial count and *Enterobacteriaceae* and *Staphylococcus* sp. counts for stressed and control goats were within the normal ranges, as reported previously by other authors (Muehlherr et al., 2003; Toquet et al., 2021). In our study, the adequate microbiological quality of goat milk can be explained by the hygiene of the experimental milking and the low mastitis rate on the experimental farm. In fact, inadequate hygiene conditions during milking cause an increase in the bacterial count in milk, with a high prevalence of *Staphylococcus* sp., because it is the main bacteria related to clinical mastitis in goats (Muehlherr et al., 2003; Toquet et al., 2021). These data are important and reinforce the argument that cumulative stress caused by different acute stressors increased cellular apoptosis in the mammary gland, increased SCC and compromised milk production without changing the mastitis rate in experimental goats.

Table 3
Effect of T, H, and their interaction (T H^{-1}) on milk yield and milk quality. Milk yield and milk quality presented as the average produced from days 152 to 154 of lactation during the challenges (heat stress, ACTH, hoof care, rain) imposed to Saanen goats. Data are presented as the mean ± standard error of mean. Treatment (T), day (D) and interaction treatment day^{-1} (TD).

Trait	Challenged	Control	P-value		
	(n = 15)	(n = 15)	T	D	T D^{-1}
Milk yield (kg)	1.85 ± 0.25	2.33 ± 0.20	0.01	0.48	0.35
Fat (%)	3.79 ± 0.33	3.55 ± 0.34	0.45	0.01	0.10
Protein (%)	2.94 ± 0.04	3.06 ± 0.13	0.29	0.80	0.52
Lactose (%)	4.37 ± 0.44	4.00 ± 0.04	0.36	0.28	0.34
Somatic cell count (cells mL^{-1})[1]	2 209 ± 225	1 557 ± 140	0.01	0.01	0.01
Total bacterial count (CFU mL^{-1})[2]	5.01 ± 4.85	2.24 ± 1.19	0.33	0.51	0.88
Enterobacteriaceae (CFU mL^{-1})	1.34 ± 0.30	1.22 ± 0.22	0.32	0.37	0.42
Staphylococcus sp. (CFU mL^{-1})	573.41 ± 150.52	234.50 ± 54.71	0.32	0.402	0.58

[1]Values × 10^3 mL^{-1}; [2]CFU: colony-forming units, values × 10^2 mL^{-1}.

Comparing the effect of each challenge on milk yield, the first challenges imposed on the goats were considered acute stressors that did not cause changes in the milk yield. However, after considering all of the stressful challenges, cumulative stress caused a significant decrease in the milk yield during the last challenge (on day 154 of lactation) and on subsequent lactation days (on days 159, 160, and 161 of lactation) when compared with the control goats (Figure 1). Other studies have also described a persistent effect of long-term stressors on the milk production of goats (Canaes et al., 2009; Hooper et al., 2020). However, to our knowledge, this is the first study evaluating the effect of cumulative stress in dairy animals. Indeed, there was a significant effect of cumulative stress on SCC because goats subjected to stressor challenges showed significantly higher SCC than control goats. In the literature, minimal information concerning the impact of cumulative stress on animal production can be found, and further studies are necessary to elucidate the action of cumulative stress on cellular apoptosis in the mammary gland. In this context, the results of this study indicate that farmers should avoid concurrently subjecting goats to management and environmental challenges that can induce acute stress because these stressors have negative and cumulative impacts on SCC and milk yield.

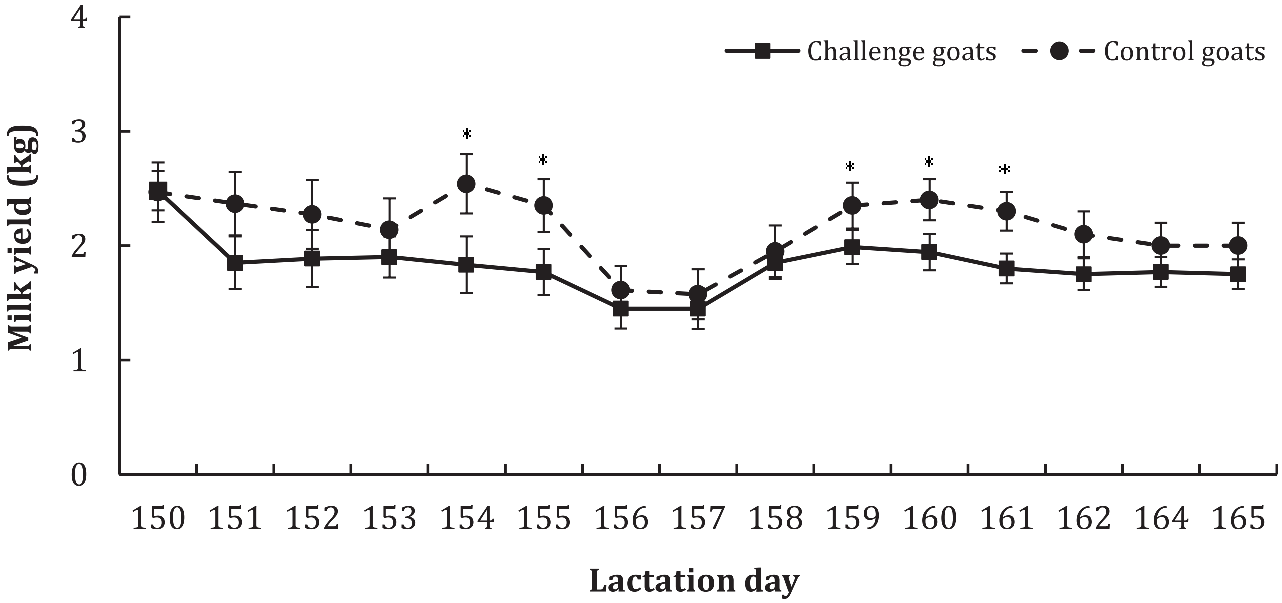

Figure 1. Milk yield (kg) of Saanen goats subjected to stressful challenges (heat stress, ACTH administration, hoof care, and rain challenges) or control treatment (habitual routine and comfort conditions/environment). Before (on day 150) during (on days 151-154) and after (on days 155 to 165) imposition of challenges or control treatments. The stressful challenges were performed sequentially, one challenge by day, from day 151 to 154. Data are presented as the means ± standard error of mean. Mean with * differ at P ≤ 0.05.

Conclusion

Our study shows that acute stress-triggered physiological responses are fundamental for restoring homeostasis; however, the cumulative effects of different imposed challenges caused an increase in SCC and a decrease in milk yield in Saanen goats. Indeed, our results highlight the need to avoid stressful management when weather conditions are unfavorable, such as on very hot and rainy days.

Acknowledgments

This work was financed by the São Paulo Research Foundation (FAPESP Sao Paulo, SP, Brazil, grants 2018/09093/5); postgraduate students were funded by Coordination for the Improvement of Greater Education Personnel (CAPES, Brasilia, DF, Brazil) Finance Code 001, and the National Counsel of Technological and Scientific Development (CNPq, Brasilia, DF, Brazil). J. A. Negrao was supported by the CNPq. The authors declare no conflict of interest.

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