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Behavioral and physiological measures in dairy goats with and without small ruminant lentivirus infection

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ABSTRACT

Although goats exhibit no visible symptoms of discomfort in the subclinical stage of small ruminant lentivirus (SRLV) infection, we examined their welfare at the behavioral and physiological level. Twelve SRLV-seropositive (SRLV) and eight SRLV-seronegative (control) goats in their first, second, and more than second lactation were observed for pain behaviors, locomotor activity, standing, lying, eating, rumination, social interactions, and comfort behaviors. Heart rate was recorded during resting and morning and afternoon milking. Concentrations of serum amyloid A and haptoglobin in blood serum, as well as milk yield and the following milk parameters were assessed: somatic cell count, fat, casein, total protein, lactose, total solids, solids nonfat, urea, citric acid content, freezing point depression (FPD), free fatty acids, density, and titratable acidity. Age but not infection status affected the time budget of the goats. The oldest animals were the most active and spent the most time on comfort behaviors. In general, the goats spent more time on neutral interactions and on standing still in the morning than in the afternoon. The average heart rate (HR) from all recordings was higher in control than in SRLV goats, but did not differ between goats of different ages. HR was lower in the morning than in resting and afternoon recordings. HR variability (root mean square of successive interbeat interval differences [RMSSD]) was higher at low temperatures. The strong correlation between cardiac response and temperature was particularly evident in SRLV goats (HR and temperature: $r = 0.54$, $P < 0.01$, RMSSD and temperature: $r = -0.39$, $P = 0.01$). However, RMSSD and acute phase proteins (serum amyloid A and haptoglobin) were affected neither by infection nor age of the animal. Milk yield and its composition did not differ between SRLV-infected and control goats, except for FPD, which was higher in controls. The lower FPD value of milk from SRLV goats than control goats may indicate a deterioration of metabolic processes in the infected mammary gland. As the goats presented no abnormal behaviors and the concentrations of the acute phase proteins were not elevated, the welfare of the infected animals was confirmed. The higher susceptibility to high temperatures should be considered in managing SRLV goats, and longer observation sessions are highly recommended to monitor any pain reactions.

Small ruminant lentivirus (SRLV) causes caprine arthritis and encephalitis (CAE) in goats and maedi-visna in sheep. SRLVs cause serious economic losses in the goat production sector worldwide, and CAE eradication programs have been implemented successfully in many EU countries (Peterhans et al., 2004). The prevalence of SRLV in Poland has risen steadily since the early 1990s, when the import of live animals from Western countries began. Between 1996 and 2007, the seroprevalence of SRLV in Polish herds increased from 30.8% to 71.9% (Czopowicz et al., 2017; Kaba et al., 2013). The main route of transmission is the lactogenic route (vertically) from dam to progeny through virus-infected milk cells (monocytes and macrophages) and horizontally via the natural close contact between dam and kid or between adult goats, as well as during interspecies contact with sheep (bidirectional transmission, Denner 2007; Jarczak et al., 2016; Nowicka et al., 2015).

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The consequences of CAE have a direct effect on the quality of life and welfare of the affected animal. In the subclinical stage of the illness, no visible symptoms of discomfort in the animal can be observed. However, when clinical symptoms occur, the goats suffer from severe arthritic pain while standing and walking, signaling the beginning of welfare problems. The main clinical symptoms are arthritis, pneumonia, mastitis, weight loss, and in the final stage of the disease, emaciation of the animal, which leads to premature euthanasia and economic waste (De la Concha-Bermejillo, 2003; de Andrés et al., 2005; Deubelbeiss et al., 2014; Kaba et al., 2011).

Although the clinical signs of locomotion impairment and joint pain related to arthritis in late stages of the illness have been addressed, no studies have been done to examine the welfare of goats in the initial stages of the illness, who do not show clinical symptoms of CAE. In the present study, we examined the welfare of SRLV-seropositive dairy goats compared to SRLV-seronegative (control) goats. Since the behavior, and particularly pain behaviors like kneeling or oblivion, is an indication of welfare (AWIN Welfare Assessment Protocol for Goats, 2015; Molony and Kent, 1997), we analyzed the incidence of pain behaviors, time budget, and social interactions. To assess the physiological effects of welfare problems, heart rate (HR) and heart rate variability (HRV), especially the root mean square of successive interbeat interval differences (RMSSD) as an indicator of stress in different farm animals (von Borell, 2007), were studied. Cardiac parameters are often related to the psycho-physiological state of an animal, indicating the environment's positive or negative effect on that animal. To assess health status, infection markers (acute phase proteins [APPs]), as direct indicators of the health of animals (González et al. 2008; Reczyńska et al., 2018), were used. Finally, as related to the SRLV infection (Kaba et al., 2012; Nord and Adnoy, 1997; Nowicka et al., 2015; Peterhans et al., 2004), milk production and its composition, as well as technological parameters, were measured.

Materials and methods

The study was accepted by the II Local Commission for Ethics in Animal Experimentation, Warsaw, Poland.

Material

Twelve SRLV-seropositive (SRLV) and eight SRLV-seronegative (control) lactating female Polish White Improved dairy goats were used in the study. Hornless SRLV-seropositive and control animals were selected from one stock, in which a subpopulation of the animals had already been infected via the lactogenic route of transmission. For the last 20 years, goats from this herd have been tested serologically for SRLV twice a year (in November and June). The goats were in their first (N = 8: SRLV, N = 4; control, N = 4), second (N = 8: SRLV, N = 4; control, N = 4), and more than second lactation (N = 4: SRLV, N = 2; control, N = 2). All goats were in the mid-lactation stage (120-150 days postpartum). All goats were milked twice a day, kept on straw bedding in groups of 5-10 animals of the same age in four pens (12-21 m^2 in size), and fed oat grain during milking. The basic diet consisted of corn silage, wilted grass silage, and concentrates. Hay and corn silage with concentrates and a mineral-vitamin mixture was provided twice a day; water and salt blocks were available *ad libitum*. During the study, the goats were kept inside a barn in their usual pens and were handled by familiar caretakers using regular procedures. The studied goats had visual, auditory, and tactile contact with other goats kept in neighboring pens. Their temperature was checked before morning and afternoon milking, and resting HR was recorded at 7:00, 12:00, and 16:00. The average diurnal temperature was 19.7 ± 4.2°C.

Two days before the study, the goats were examined by a veterinarian, and blood samples were taken from the jugular vein. The mean body temperature was 38.8 ± 0.65°C, the mean respiratory rate was 26.1 ± 9.49 breaths/minute, and the manually measured HR was 104.8 ± 11.5 beats/minute. The goats presented no pain symptoms during udder and body palpation. All animals were free from clinical signs of infection, and no locomotor problems were observed.

Methods

Behavioral observations

The protocol for the recording and sampling procedures is presented in Table 1. To habituate the goat to human touch and presence, two days and one day before the start of the observations, the experimenter entered the goat's pen for 30 minutes and let the animal habituate and interact. All animals allowed touching by the experimenter.

Each goat was observed over the course of one day. The behavior of each goat was registered for 30 minutes twice a day: one session two hours after morning milking and the other session two hours after afternoon milking. The experimenter (camera operator) was positioned 1 m from the pen and remained silent while registering the goats. When needed, the experimenter slowly followed a goat if it changed position or was hidden by a pen mate.

Behavior was registered using all-occurrence sampling. The analyzed behavioral measures (duration in seconds, s) were as follows: pain behaviors (nonfeeding kneeling and oblivion), locomotor activity, standing still, eating (standing, lying, total), lying (total), rumination (standing, lying, total), social interactions (neutral, negative-received, negative-initiated, defensive), comfort behaviors, and yawn. For detailed definitions, please refer to Table 2.

HR recordings

HR was recorded 10 minutes before, during, and 10 minutes after morning and afternoon milking. Resting HR was recorded for 30 minutes in between milking times (Table 1). HR was recorded in R-R mode using a Polar s810i monitor (Polar, Finland). The experimenter entered the pen, gently held the goat, and affixed the electrodes. The skin in the cardiac area was shaved and moisturized with ECG jelly. The electrodes were additionally secured with an elastic belt. The experimenter started and stopped the monitor in the goat's pen.

Acute phase proteins (APPs)

The concentrations (ng/mL) of serum amyloid A (SAA) and haptoglobin (Hp) in the serum of SRLV-infected and uninfected goats were analyzed. Blood samples were taken into 9 mL S-Monovette® tubes with clot-activator (Sarstedt AG & Co., Germany) three days before behavioral observations. They were centrifuged at 2000 × *g* for 20 min at 20°C. Blood samples were stored at −20°C until further analysis. All serological examinations were performed with an ELISA kit (ID Screen MVV/CAEV Indirect—Screening test, IDvet Innovative Diagnostics, Grabels, France, Nowicka et al., 2014).

Table 1
Recording protocol for each goat

Recording protocol	Time of day	Behavior	HR
Morning milking: 10 minutes before, during, 10 minutes after	7:00-8:00		√
Two hours after morning milking	10:00-10:30	√	
Between milkings (rest)	12:00-12:30		√
Afternoon milking: 10 minutes before, during, 10 minutes after	16:00-17:00		√
Two hours after morning milking	19:00-19:30	√	

Table 2
Definitions of behavioral measures

Behavioral measure	Definition
Kneeling	Kneeling is a posture that consists of goats standing on their hind limbs and on their carpal joints (AWIN, 2015). Only nonfeeding kneeling was recorded.
Oblivion	Isolating from the group, standing, lying, or kneeling for long time, frequently facing the wall or other parts of the housing structure, sometimes with ears down (AWIN, 2015)
Locomotor activity	Displacing within the pen area
Standing still	Remaining motionless without any purposeful activity
Eating standing	Remaining in a standing position during feeding
Eating lying	Remaining recumbent during feeding
Eating total	Total time of feeding
Ruminating standing	Chewing the cud from the stomach in a standing position
Ruminating lying	Chewing the cud from the stomach in a laying position
Ruminating total	Total time of chewing the cud
Lying total	Total time of lying
Neutral social interaction	Direct contact with a pen mate followed by no evident aggression or avoidance, e.g., sniffing, rubbing against, leg leaning on lying goat during self-scratching
Negative-received interaction	Direct contact with pen mate followed by evident avoidance, e.g., receiving leg leaning followed by avoidance, being threatened with head, butted, pushed to move away
Negative-initiated interaction	Direct contact with pen mate followed by her evident avoidance, e.g., leg leaning by lying goat followed by her avoidance, threating with head, butting, pushing another goat to move away
Defensive interaction	Response to direct contact of a pen mate after her negative interaction, opposite butting, pressing with head
Comfort behaviors	Self-scratching with hoof, against the wall or pen walls, rolling, shaking ("wet dog shake")

ELISA plates were read by spectrophotometry using a Sunrise reader (Tecan Austria GmbH, Austria).

Milk parameters and performance

Milk yield was recorded, and milk samples were taken into 30 mL tubes with preservative (Microtabs, Bentley, USA) during the evening milking, one day before behavior observation. The day after the following milk parameters were assessed, somatic cell count (SCC) was assessed using IBCm (Bentley, USA), whereas the percentage of fat, casein, total protein, lactose, total solids, solids nonfat (SNF), urea, and citric acid content, freezing point depression (FPD), free fatty acids (FFA), density, and titratable acidity (°T, Turner degrees) were assessed using a MilkoScan FT2 (Foss, Denmark).

Statistical analysis

To assess the effect of the time point of the behavioral observation, behavioral data were analyzed separately (morning vs. afternoon sampling). To assess the effects of infection status and age, behavioral data from two observations were totaled, amounting to one hour of observation per animal. Behavioral variables did not present normal distributions, so nonparametric Wilcoxon and Kruskal-Wallis tests were used to compare SRLV and control goats as well as to examine the age effect. A sign test was used to assess the differences in behaviors in the morning and afternoon. The results are presented as medians, Q1 and Q2.

The HR files contained artifacts, despite several adjustments made to attain better quality recordings. Because of technical problems in recording the HR of goats older than three years, only the HR of one- and two-year-old females was analyzed. Thirty second (30-s), artifact-free samples from resting, premilking, milking, and postmilking (pooled) recordings were randomly selected for analyses. These fragments were analyzed for mean HR and RMSSD (HRV) in SRLV and control goats of different age groups. To assess the effect of time point of recording on cardiac response during milking, the HR and HRV were compared between resting and morning and afternoon milking recordings.

APPs, milk yield, milk parameters, and cardiac data, as well as ambient temperatures measured during morning, afternoon, and during rest, the HR recordings followed normal distributions and were analyzed with two-factorial analyses of variance (General Linear Model). To consider repeated recordings of HR during rest and during morning and afternoon milkings, a mixed model with random animal effect was applied. To analyze SCC, the data were transformed into natural logarithm of somatic cell count (lnSCC). The results are presented as LSM ± SE. The relationship between ambient temperature and cardiac parameters was assessed using Pearson correlation. The SAS statistical package (SAS/STAT version 9.4) was used for all calculations.

Results

Behavior

Pain-related behaviors were observed in one goat in second lactation, who spent 26.4% of the one-hour observation time (15 min 49 s) kneeling and 27% (16 min 12 s) hiding its head between the rack's boards (thus showing signs of oblivion). This goat was not infected with SRLV. In the other goats, no kneeling was observed other than feed-related kneeling (reaching for food at the feeding rack), so these (very low) incidences/durations were not considered pain behaviors (AWIN, 2015). Descriptive statistics of the behavioral variables observed in time budget and the significance of the differences between the groups are presented in Table 3. There were no differences in the durations of behaviors observed in SRLV-infected versus control goats. However, the goats of different ages differed significantly in terms of total durations of locomotor activity ($P = 0.0472$) and comfort behaviors ($P = 0.0238$), as well as in defensive interaction durations at tendency level ($P = 0.0590$). The most active were the oldest goats as compared to one- ($P = 0.0472$) and two-year-old goats ($P = 0.0538$). Two-year-old goats spent less time on comfort behaviors as compared to the oldest group ($P = 0.0238$). The goats remained in a standing position longer during the morning observations than during the afternoon observations ($P = 0.0395$), whereas the opposite was observed for lying ($P = 0.0181$). Also, they were involved in neutral interactions longer in the mornings than in the afternoons ($P = 0.004$).

Heart rate, HRV, and ambient temperature

Average HRs from all recordings were higher in control than in SRLV goats (HR: $P < 0.0001$, Table 4) but did not differ between goats of different ages. Neither infection nor age of the animals affected RMSSD.

The HR was lower in the morning than in the afternoon milking ($P < 0.001$) and in the resting recording ($P < 0.001$, Table 4). RMSSD was higher in the morning milking compared to the resting ($P = 0.0273$) and afternoon ($P = 0.0153$) recordings, whereas the resting and afternoon recordings did not differ. This result was probably due to the cardiac response to ambient temperature in the afternoon (HR and temperature: $r = 0.29$, $P = 0.009$) as it was lower

Table 3
Descriptive statistics of behavioral variables (duration in seconds) in SRLV-infected and control goats of different ages during morning and afternoon observations (median, [Q1, Q3])

Behavioral variable (s)	Infection status		Age			Time of day	
	SRLV[c]	Control[c]	Primiparous[c]	Two-year-olds[c]	Three-year-olds and older[c]	Morning	Afternoon
	N = 12	N = 8	N = 8	N = 8	N = 4	N = 20	N = 20
Locomotor activity	114 [105; 172]	94.5 [52.5; 153]	120 [105; 172]	89 a [86; 114]	292 b [179; 405]	59 [36; 97]	58 [22; 102]
Standing	1248 [776; 1928]	1845 [1569; 2190]	1842 [1248; 2486]	1382 [776; 1678]	1908.5 [1089; 2728]	896 a [577; 1356]	399 b [289; 1033]
Eating standing	914 [486; 1066]	541 [135.5; 1154]	826.5 [560; 1437]	914 [486; 1066]	334 [202; 467]	399 [89; 757]	309 [120; 616]
Eating lying	0 [0; 37]	0 [0; 297]	0 [0; 250]	0 [0; 50]	0 [0; 0]	0 [0; 0]	0 [0; 0]
Eatin total	987 [560; 1228]	763 [433; 1154]	826 [560; 1437]	995 [871; 1228]	334.5 [201; 667]	399 [180; 871]	377 [122; 639]
Ruminating standing	61 [0; 182]	18 [0; 248]	56.5 [0; 461]	36 [0; 104]	91 [0; 181]	0 [0; 221]	0 [0; 0]
Ruminating lying	288 [0; 482]	182.5 [11.5; 123]	279 [23; 720]	342 [0; 441]	81.5 [0; 163]	0 [0; 90]	91 [0; 389]
Ruminating total	288 [96; 613]	413 [171; 1212]	503.5 [96; 804]	342 [96; 613]	172.5 [163; 182]	182 [0; 461]	91 [0; 358]
Lying total	901 [453; 1644]	865.5 [392.5; 255.5]	570.5 [30; 901]	1260 [800; 1511]	1065 [0; 2130]	0 a [0; 328]	800 b [82; 1251]
Neutral social interaction	8 a [5; 11]	4 b [2.5; 5]	4.5 [3; 8]	6 [5; 9]	12 [5; 19]	4 a [2; 7]	2 b [1; 3]
Negative-received interaction	15 [6; 17]	6.5 [2.5; 14]	10 [3.17]	10 [8; 17]	10.5 [3; 18]	5 [2; 12]	4 [1; 5]
Negative-initiated interaction	7 [5; 9]	8.5 [2.5; 16]	10 [5; 15]	6 [3; 9]	7 [5; 9]	3 [1; 5]	6 [2; 11]
Defensive interaction	4 [1; 6]	0 [0; 1.5]	4 [1; 6]	0 a [0; 3]	6 b [6; 6]	1 [0; 5]	0 [0; 1]
Comfort behaviors	38 [26; 46]	19 [16; 33]	41.5 [34; 54]	27 a [17; 28]	49 b [46; 52]	14 [7; 17]	15 [11; 28]

a, b—values within infection status, age, and time of the day differ at $P < 0.05$.
[c] Groups where durations of behavioral variable were totaled for morning and afternoon observations.

in the morning (16.3 ± 0.54°C) than in the resting (21.8 ± 0.86 °C, $P < 0.01$) and afternoon (22.2 ± 0.53°C, $P < 0.01$) HR recordings. Also, RMSSD was higher when the temperature was low ($r = -0.35$, $P = 0.0016$). The correlation between cardiac response and temperature was particularly strong in SRLV goats (HR and temperature: $r = 0.54$, $P < 0.001$, RMSSD and temperature: $r = -0.39$, $P = 0.0039$) when analyzed separately from the control animals, whose HR and RMSSD were not significantly correlated with temperature.

Acute phase proteins

The levels of the studied APPs SAA and Hp in blood serum did not differ between SRLV-infected goats (SAA: 0.10 ± 0.00 ng/mL; Hp: 0.09 ± 0.00 ng/mL) and uninfected goats (SAA: 0.09 ± 0.00 ng/mL; Hp: 0.09 ± 0.01). Furthermore, the concentrations of SAA and Hp did not depend on the age of the examined animals (SAA for one-year-olds: 0.10 ± 0.00 ng/mL, for two-year-olds: 0.10 ± 0.00 ng/mL, and for more than two-year-olds: 0.09 ± 0.00 ng/mL; Hp for one-year-olds: 0.10 ± 0.01 ng/mL, for two-year-olds: 0.09 ± 0.01 ng/mL, for more than two-year-olds: 0.08 ± 0.01 ng/mL).

Table 4
Effects of SRLV infection, age, and milking on cardiac measures in observed goats (LSM ± SE)

Studied factor	Cardiac measure	
	HR (beats/min)	RMSSD (ms)
Infection status		
SRLV, N = 10	113.8 ± 1.72 c	6.57 ± 0.71
Control, N = 4	122.4 ± 2.48 d	7.63 ± 1.04
Age (years)		
1, N = 8	116.8 ± 2.01	6.02 ± 0.83
2, N = 8	119.7 ± 2.07	7.44 ± 0.91
Milking		
Morning, N = 14	109.8 ± 2.09 c	8.03 ± 0.68 a
Afternoon, N = 14	117.0 ± 2.84 d	5.54 ± 0.93 b
Rest, N = 14	122.1 ± 2.10 d	6.27 ± 0.70 b

HR, heart rate; RMSSD, root mean square of successive interbeat interval differences.
a, b—values within infection status and age differ at $P < 0.05$.
c, d—values within infection status and age differ at $P < 0.01$.

Milk yield and composition

Milk yield and milk composition did not differ between the SRLV-seropositive and control goats (Table 5). Only the FPD differed between these groups ($P = 0.0443$). LnSCC was lower ($P < 0.001$) in the oldest goats than in the younger groups. Moreover, the concentrations (%) of proteins and caseins, as well as the titratable acidity was also highest in the milk of the oldest goats ($P < 0.001$), whereas lactose content was the lowest in this group ($P < 0.001$). The content of urea in milk was lower in primiparous goats than in older goats ($P = 0.0235$).

Discussion

SRLV infection did not impair the behaviors of goats during veterinary examinations or during time budget observations. This is an important welfare observation because infected animals can normally live in social groups with no obvious signs of pain or discomfort. Periodic clinical examinations are generally efficient ways of detecting discomfort related to the progression of pain, enabling the owner to make a decision as to what to do with a sick animal. However, one of studied goats presented some behaviors defined as pain-related not during veterinary examination but during time budget observations when humans were not present in the pen. This finding confirms the importance of daily monitoring for the first signs of abnormal behaviors or pain symptoms in animals in their social groups.

Our results showed that in contrast to what was expected, the oldest goats were the most engaged in locomotor behaviors. Although initiated and received negative interactions were evenly distributed across the age groups, the higher locomotor activity observed in the oldest goats could be partly related to their active response to threats or to aggressive interactions initiated by pen mates (defensive interactions), which was preceded and followed by the animals' movement. The less time spent on comfort behaviors by the two-year-old goats may be related to their lower activity in general, or, hypothetically, to lower chronic social stress in this age group because excessive self-grooming may be interpreted as dearousal behavior (Spruijt et al., 1992). The goats in this

Table 5
Milk yield and component contents in milk of SRLV-infected and control goats of different ages (LSM ± SE)

Parameter (unit)	Infection status		Age		
	SRLV N = 12	Control N = 8	One-year-olds, N = 8	Two-year-olds, N = 8	More than two-year-olds, N = 4
Yield[e] (l)	1.25 ± 0.21	0.97 ± 0.28	0.79 ± 0.26	0.83 ± 0.27	1.55 ± 0.37
LnSCC	7.10 ± 0.41	6.85 ± 0.55	6.19 ± 0.37 c	6.25 ± 0.39 c	8.48 ± 1.06 d
Fat (%)	3.77 ± 0.73	3.42 ± 0.30	3.87 ± 0.20	3.81 ± 0.23	3.11 ± 0.58
Casein (%)	2.14 ± 0.08	2.11 ± 0.10	1.90 ± 0.07 c	1.90 ± 0.08 c	2.57 ± 0.19 d
Total protein (%)	3.14 ± 0.07	3.07 ± 0.10	2.78 ± 0.07 c	2.78 ± 0.08 c	3.77 ± 0.20 d
Lactose (%)	4.40 ± 0.07	4.48 ± 0.09	4.67 ± 0.06 c	4.61 ± 0.07 c	4.05 ± 0.18 d
TS (%)	11.9 ± 0.27	11.5 ± 0.35	11.9 ± 0.24	11.7 ± 0.27	11.5 ± 0.68
SNF (%)	8.14 ± 0.12	8.08 ± 0.16	8.08 ± 0.10	7.94 ± 0.11	8.31 ± 0.30
Urea (%)	266.0 ± 42.3	315.6 ± 55.9	170.1 ± 37.3 a	343.7 ± 42.3 b	358.8 ± 107.3 b
Citric acid (%)	0.10 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.07 ± 0.02
FPD (°C)	−598.0 ± 6.46 a	−617.5 ± 8.51 b	−613.6 ± 5.66	−596.0 ± 6.42	−613.7 ± 16.3
FFA (%)	0.92 ± 0.23	1.01 ± 0.17	1.10 ± 0.15	1.00 ± 0.17	0.80 ± 0.44
Density (g/cm^3)	1023.8 ± 0.73	1023.1 ± 0.56	1022.9 ± 0.49	1022.4 ± 0.55	1025.0 ± 1.40
Titratable acidity (°T)	16.5 ± 0.44	15.3 ± 0.58	13.6 ± 0.39 c	13.5 ± 0.44 c	20.6 ± 1.12 d

FPD, freezing point depression; FFA, free fatty acids; lnSCC, natural logarithm of somatic cell count; SNF, solids not-fat; TS, total solids; °T = Turner degrees.
SRLV—SRLV-seropositive goats; control—SRLV-seronegative goats.
a, b—values within infection status and age differ at $P < 0.05$.
c, d—values within infection status and age differ at $P < 0.01$
[e] Afternoon milking.

study were dehorned, thus they may have experienced more social stress; according to Aschwanden et al. (2008a,b) and Waiblinger (2018), hornless goats show more pronounced aggressive behaviors due to a longer period of social hierarchy establishment and a less transparent social hierarchy in their groups than horned animals. Although goats are considered to be the best adapted to high temperatures among farm animal species (Silanikove, 2000), our study confirmed that goats are sensitive to thermal stress, which can cause decreased welfare and milk production (Al-Tamimi, 2006; Hamzaoui et al., 2013; Salama et al., 2014). Our results show a positive correlation between heart rate and increasing temperature in infected animals, where their ability to cope with high temperatures may be impaired. As suggested by Al-Tamimi (2006), higher HR may be caused by increased frequency of chest movements due to a higher respiration rate or to an increase in the cutaneous perfusion and maximization of heat dissipation from the skin by means of radiation and conduction.

The decreased RMSSD at higher temperatures found in our study indicates a decrease in the influence of the parasympathetic branch of the autonomic nervous system, which is interpreted as related to discomfort, arousal, and stress in different farm animal species (horses: e.g., Górecka-Bruzda et al., 2017; Werhahn et al., 2012; cattle: e.g., Mohr et al., 2002; Kovács et al. 2015; pigs: e.g., de Jong et al., 2000) including goats (e.g., Aschwaden et al,. 2008a,b; Patt et al., 2016). Thus, the change in cardiac parameters related to high temperatures may be informative for both physical and psychological discomfort in goats and should be considered as a potential confounding factor in different behavioral studies carried out in changing thermal conditions.

Considering that no differences in APP concentrations were found between SRLV-seropositive and SRLV-seronegative goats, it could be stated that the homeostasis of SRLV-seropositive animals was not disrupted by the presence of the virus, as in the case of behavior. This phenomenon of a virus's ability to evade the immune system of the host may increase infection capacity (Blacklaws et al., 1995; Crespo et al., 2013; Larruskain and Jugo, 2013; Reczyńska et al., 2018). Reczyńska et al. (2018) also found no differences in the studied APPs between goats of various ages, which confirm that age has no influence on the concentrations of SAA and Hp in the blood serum of infected animals.

Attempts at relating SRLV infection, milk yield, and milk component contents have produced conflicting results for different goat populations. Although de Azevedo et al. (2017) found that productivity and milk quality was lower in SRLV-seropositive goats, no effect of SRLV infection on milk yield has been shown (Kaba et al., 2012, Nowicka et al., 2015; Turin et al., 2005). Similarly, divergent results regarding the number of milk somatic cells have been reported. Kaba et al. (2012) did not confirm the effect of SRLV infection on SCC, whereas the opposite was reported by Turin et al. (2005). The SCC of the milk of SRLV-seropositive goats in the present study did not differ from that of controls but, as expected, the lowest SCC and the highest lactose content in milk were found in the primiparous goats; these results are consistent with previous studies in the Polish goat population (Bagnicka et al., 2015, 2016). However, other studies, in contrast to our results, have reported the highest protein content in the first lactation (Bagnicka et al., 2015, 2016; Olechnowicz and Sobek, 2008). This discrepancy may be partly explained by differences in feeding regimes and the quality of the feed. In the present study, only the FPD was influenced by the presence of the virus, with the value close to zero in the milk from SRLV-seropositive goats. According to Janštová et al. (2007), the value of FPD is associated with SNF content in milk—the lower the SNF, the closer the FPD value is to zero. The FPD depends on many factors, including milk components (mainly lactose and chlorides, but also Ca, K, Mg, and phosphates), the number and stage of lactation, the breed, the feeding and water intake, the health of the mammary gland, and weather conditions (Janštová et al., 2007). Lower lactose content indicates an inflammatory reaction in the mammary gland. The animals maintain an osmotic balance between milk and blood, thanks to lactose and saline solutions; in goats with mastitis, the lower lactose content is compensated by secreting salt into the milk (Harding, 1995). Therefore, the lower FPD value of milk from SRLV-seropositive than from SRLV-seronegative goats may indicate a deterioration of metabolic processes in the infected organ. However, the obtained values for both seropositive and seronegative animals were much lower than reference values for either raw cow milk (−0.512 to −0.580; Polish Norm PN-A-86002, 1999) or for different dairy cattle breeds maintained in Poland (−0.544 to −0.536) (Kędzierska et al., 2011). The FPD for goat milk seems to be much lower anyway, with average values of −0.5527°C obtained in Canada (Szijarto and van de Voort, 1983) and −0.544°C obtained for Czech goats (Hanuš et al., 2009).

Conclusions

As the goats presented no abnormal behaviors or pain symptoms and because the concentrations of the main acute phase proteins were not found to be elevated, the welfare of the infected individuals was confirmed. However, longer observation periods of animals in their social groups, undisturbed by human contact, are highly recommended to monitor pain reactions. Also, to provide optimal welfare for goats, the higher susceptibility to high temperatures should be considered in the management of SRLV-infected animals. Moreover, the lower value of FPD of milk from SRLV-seropositive versus SRLV-seronegative goats may indicate a deterioration of metabolic processes in the infected mammary gland.

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References

Al-Tamimi, H.J., 2007. Thermoregulatory response of goat kids subjected to heat stress. Small Rum. Res. 71, 280–285.

Aschwanden, J., Gygax, L., Wechsler, B., Keil, N.M., 2008a. Cardiac activity in dairy goats whilst feeding side-by-side at two different distances and during social separation. Physiol. Behav. 95, 641–648.

Aschwanden, J., Gygax, L., Wechsler, B., Keil, N.M., 2008b. Social distances of goats at the feeding rack: influence of the quality of social bonds, rank differences, grouping age and presence of horns. Appl. Anim. Behav. Sci. 114, 116–131.

AWIN, 2015. AWIN Welfare Assessment Protocol for Goats. https://doi.org/10.13130/AWIN_GOATS_2015.

Bagnicka, E., Hamann, H., Distl, O., 2015. Structure and the non-genetic and genetic effects on milk traits in Polish dairy goat population. Anim. Sci. Pap. Rep. 33, 59–69.

Bagnicka, E., Łukaszewicz, M., Ådnøy, T., 2016. Genetic parameters of somatic cell score and lactose content in goat's milk. J. Anim. Feed Sci. 25, 210–215.

Blacklaws, B.A., Bird, P., Allen, D., 1995. Initial lentivirus-host interactions within lymph nodes: a study of maedi-visna virus infection in sheep. J. Virol. 69, 1400–1407.

Crespo, H., Bertolotti, L., Jaganaru, M., Glaria, I., de Andrés, D., Amorena, B., Rosati, S., Reina, R., 2013. Small ruminant macrophage polarization may play a pivotal role on lentiviral infection. Vet. Res. 44, 1–13.

Czopowicz, M., Szaluś-Jordanow, O., Mickiewicz, M., Moroz, A., Witkowski, L., Markowska-Daniela, I., Bagnicka, E., Kaba, J., 2017. Influence of true within-herd prevalence of small ruminant lentivirus infection in goats on agreement between serological immunoenzymatic tests. Prev. Vet. Med. 144, 75–80.

de Andrés, D., Klein, D., Watt, N.J., Berriatua, E., Torsteinsdottir, S., Blacklaws, B.A., Harkiss, G.D., 2005. Diagnostic tests for small ruminant lentiviruses. Vet. Microbiol. 107, 49–62.

de Azevedo, D.A.A., dos Santos, V.W.S., Sousa, A.L.M., de Peixoto, R.M., Pinheiro, R.R., Andrioli, A., da Teixeira, M.F.D.S., 2017. Small ruminant lentiviruses: economic and productive losses, consequences of the disease. Arq. Inst. Biol. 84, 1–10.

de Jong, I.C., Sgoifo, A., Lambooij, A., Korte, S.M., Blokhuis, H.J., Koolhaas, J.M., 2000. Effects of social stress on heart rate and heart rate variability in growing pigs. Can. J. Anim. Sci. 80, 273–280.

de la Concha-Bermejillo, A., 2003. Caprine/Arthritis-Encephalitis: an update. Sheep Goat Res. J. 18, 69–78.

Denner, J., 2007. Transspecies transmissions of retroviruses: new cases. Virology 369, 229–233.

Deubelbeiss, M., Blatti-Cardinaux, L., Zahno, M.-L., Zanoni, R., Vogt, H.-R., Posthaus, H., Bertoni, G., 2014. Characterization of small ruminant lentivirus A4 subtype isolates and assessment of their pathogenic potential in naturally infected goats. Virol. J. 11, 1–11.

González, F.H., Tecles, F., Martínez-Subiela, S., Tvarijonaviciute, A., Soler, L., Cerón, J.J., 2008. Acute phase protein response in goats. J. Vet. Diagn. Invest. 20, 580–584.

Górecka-Bruzda, A., Jaworski, Z., Suwała, M., Sobczyńska, M., Jastrzębska, E., Ogłuszka, M., Sankey, C., Boroń, M., Jezierski, T., 2017. Aversiveness of husbandry procedures for pre-weaned foals: A comparison using behavioural and physiological indices. Appl. Anim. Behav. Sci. 191, 31–38.

Hanuš, O., Genčurová, V., Kučera, J., Vyletělová, M., Třináctý, J., 2009. Analyse of relationships between freezing point and selected indicators of udder health state among cow, goat and sheep milk. Acta Univ. Agric. Silvic. Mendel. Brun. 5, 103–110.

Harding, F., 1995. Aduleration of milk. In: Harding, F. (Ed.), Milk Quality. Springer Science+Business Media, Dordrecht, pp. 60–74.

Hamzaoui, S., Salama, A.A.K., Albanell, E., Such, X., Caja, G., 2013. Physiological responses and lactational performances of late-lactation dairy goats under heat stress conditions. J. Dairy Sci. 96, 6355–6365.

Janštová, B., Dračková, M., Navrátilová, P., Hadra, L., Vorlová, L., 2007. Freezing point of raw and heat-treated goat milk. Czech J. Anim. Sci. 52, 394–398.

Jarczak, J., Kaba, J., Reczyńska, D., Bagnicka, E., 2016. Impaired expression of proinflammatory and anti-inflammatory cytokines as a result of viral infection. Viruses 8, 186.

Kaba, J., Czopowicz, M., Ganter, M., Nowicki, M., Witkowski, L., Nowicka, D., Szaluś-Jordanow, O., 2013. Risk factors associated with seropositivity to small ruminant lentiviruses in goat herds. Res. Vet. Sci. 94, 225–227.

Kaba, J., Nowicki, M., Sobczak-Fillpiak, M., Witkowski, L., Nowicka, D., Czopowicz, M., Szaluś-Jordanow, O., Bagnicka, E., 2011. Case-control studies on the occurrence of the neurologic form of caprine arthritis-encephalitis in Poland. Med. Weter. 67, 263–266.

Kaba, J., Strzałkowska, N., Jóźwik, A., Krzyżewski, J., Bagnicka, E., 2012. Twelve-year cohort study on the influence of caprine arthritis-encephalitis virus infection on milk yield and composition. J. Dairy Sci. 95, 1617–1622.

Kędzierska-Matysek, M., Litwińczuk, Z., Florek, M., Barłowska, J., 2011. The effect of breed and other factors on the composition and freezing point of cows' milk in Poland. Int. J. Dairy Technol. 64, 336–342.

Kovács, L., Kézér, F.L., Jurkovich, V., Kulcsár-Huszenicza, M., Tőzsér, J., 2015. Heart rate variability as an indicator of chronic stress caused by lameness in dairy Cows. PLoS One 10, e0134792.

Larruskain, A., Jugo, B.M., 2013. Retroviral infections in sheep and goats: small ruminant lentiviruses and host interaction. Viruses 5, 2043–2061.

Mohr, E., Langbein, J., Nürnberg, G., 2002. Heart rate variability. A noninvasive approach to measure stress in calves and cows. Physiol. Behav. 75, 251–259.

Molony, V., Kent, J.E., 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. J. Anim. Sci. 75, 266–272.

Nord, K., Adnøy, T., 1997. Effects of infection by caprine arthritis-encephalitis virus on milk production of goats. J. Dairy Sci. 80 (10), 2391–2397.

Nowicka, D., Czopowicz, M., Bagnicka, E., Rzewuska, M., Strzałkowska, N., Kaba, J., 2015. Influence of small ruminant lentivirus infection on cheese yield in goats. J. Dairy Res. 82, 102–106.

Nowicka, D., Czopowicz, M., Mickiewicz, M., Szaluś-Jordanow, Witkowski, L., Bagnicka, E., Kaba, J., 2014. Diagnostic performance of ID Screen® MVV-CAEV Indirect Screening ELISA in identifying small ruminant lentiviruses-infected goats. Polish J. Vet. Sci. 17, 501–506.

Olechnowicz, J., Sobek, Z., 2008. Factors of variation influencing production level, SCC and basic milk composition in dairy goats. J. Anim. Feed Sci. 17, 41–49.

Patt, A., Gygax, L., Wechsler, B., Hillmann, E., Langbein, J., Keil, N.M., 2016. Context specificity of the ANS stress response during two regrouping experiments in goats. Front. Vet. Sci. 3, 58.

Peterhans, E., Greenland, T., Badiola, J., Harkiss, G., Bertoni, G., Amorena, B., Eliaszewicz, M., Juste Kraßnig, R., Lafont, J.-P., Lenihan, P., Pétursson, G., Pritchard, G., Thorley, J., Vitu, C., Mornex, J.-F., Pépin, M., 2004. Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. Vet. Res. 35, 257–274.

Reczyńska, D., Zalewska, M., Czopowicz, M., Kaba, J., Zwierzchowski, L., Bagnicka, E., 2018. Small ruminant lentivirus infection influences expressions of some acute phase proteins and cathelicidin genes in milk somatic cells and peripheral blood leukocytes of dairy goats. Vet. Res. 49 (1), 113.

Salama, A.A.K., Caja, G., Hamzaoui, S., Badaouic, B., Castro-Costa, A., Façanha, D.A.E., Guilhermino, M.M., Bozzi, R., 2014. Different levels of response to heat stress in dairy goats. Small Rum. Res. 121, 73–79.

Silanikove, N., 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livest. Prod. Sci. 67, 1–18.

Spruijt, B.M., van Hooff, J.A.R.A.M., Grispen, W.H., 1992. Ethology and Neurobiology of Grooming Behavior. Physiobiol. Rev. 72, 825–852.

Szijarto, L., Voort van de, F.R., 1983. Determination of added water and bovine milk to caprine milk. J. Dairy Sci. 66, 620–623.

Turin, I., Pisoni, G., Giannino, M.L., Antonini, M., Rosati, S., Ruffo, G., Moroni, P., 2005. Correlation between milk parameters in CAEV seropositive and negative

primiparous goats during an eradication program in Italian farm. Small Rum. Res. 57, 73–79.
Von Borell, E., Langbein, J., Després, G., Hansen, S., Letterier, C., Marchant-Forde, J., Marchant-Forde, R., Minero, M., Mohr, E., Prunier, A., Valance, D., Veissier, I., 2007. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – A review. Physiol. Behav. 92, 293–316.
Waiblinger, S., 2018. Social stress in dairy goats – a matter of horns?. In: The Joint East and West Central Europe ISAE Regional Meeting "Animal Preferences and Animal Welfare", 11-12 June 2018, Warsaw, Poland, pp. 26–27. Book of Abstracts.
Werhahn, H., Hessel, E.F., Van den Weghe, H.F.A., 2012. Competition horses housed in single stalls (II): effects of free exercise on the behavior in the stable, the behavior during training, and the degree of stress. J. Equine Vet. Sci. 32, 22–31.