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Effects of housing environment on social isolation response, weaning stress, and immune reaction in goat kids

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Abstract: This study investigated how environmental structuring in the growing units of goat kids influences their ability to cope with social isolation and weaning-induced stress. Two experimental groups were arranged in an enriched (E) or barren (B) environment and each of the two groups had 10 female and 10 male kids. During the social isolation, the goat kids in group E started bleating earlier (P = 0.1612) and more frequently (P = 0.0125). Goat kids also bleated earlier (P = 0.0005) and more often after the weaning period (P = 0.0006). Only the rearing behavior of the groups exhibited significant differences in social isolation. However, after the weaning, kids showed more bipedal stance behavior (P = 0.0204) and less running behavior (P = 0.0394) than before the weaning period. Cortisol, triiodothyronine (T3), and thyroxine (T4) hormone concentrations were lower at weaning than in the other periods with social isolation (P < 0.05). The T3 hormone concentrations of the groups at weaning were significantly different (P = 0.0201). The immune response to *Brucella* vaccination did not differ between the groups (P = 0.2753). Findings revealed that both social isolation tests created stress in the goat kids. Considering the bleating frequencies, it can be concluded that the goat kids in the enriched environment experienced more social isolation stress than the goat kids in the barren environment.

Key words: Environmental enrichment, bleating, rearing, bipedal stance, cortisol, thyroid hormones

1. Introduction

Intensive production has been implemented in goat husbandry for about two decades. Environmental structuring has significant impacts on animal behavior and welfare (1,2). Thus, various production techniques considering the animals' welfare and aiming to raise healthy animals have been developed (3).

Environmental structuring arrangements not only allow animals to exhibit basic behaviors such as resting and feeding but also let them exhibit species-specific behaviors. Environmental enrichment improves the performance of the behavioral repertoires of animals and makes a significant contribution to animal welfare and animal health (4–6).

Animals may exhibit behavioral and physiological responses under acute stress conditions (7,8). Such behavioral characteristics are the initial and observable parameters. As well as behavioral characteristics, serum hormone concentrations are also used to assess animal welfare and stress levels (9,10). Throughout a period of stress, cortisol concentrations increase, thyroid hormone secretion is suppressed, and growth and development are interrupted (11). It is known that thyroid hormones increase oxygen use in the body and result in an increase in body temperature. Thyroxine (T4) is the hormone most released from the thyroid. In the somatic cells, T4 is transformed into triiodothyronine (T3); therefore, the T4 concentration indicates the biochemical transformation potential of T3 (12).

Cortisol, T3, and T4 hormones are monitored to assess the stress levels of animals (13,14). It is also known that the immune system is suppressed under stress conditions (15,16). Antibody titer generated by vaccinations is also a reliable parameter to assess immune reactions to psychological, environmental, and physiological stress (17–19).

Farm animals are social species. The social environment is an important source of support and its absence is an acute stressor for animals (13,20). Isolation of animals from the social environment may result in fear and mental pain (21). For instance, separation from the herd and/or separation of kids from their mothers may create significant stress (22). The stress level can vary based on weaning age and solid feed consumption ability (23).

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It has been reported that environmental enrichment may positively affect the animal's ability to cope with stress factors behaviorally and physiologically (4). Significant variations were observed in the behavior of Turkish Saanen goat kids with different environmental arrangements in their housing units (24). The same authors reported that in a rich environment the frequency of social interaction and bipedal stance increased.

The present study was conducted to investigate how spatial environmental arrangements provided in raising units affected the ability of goat kids to cope with social isolation and weaning-induced stress and also their immune response to the *Brucella* vaccine.

2. Materials and methods

The research protocol of the current study was approved by the Animal Ethics Committee of Çanakkale Onsekiz Mart University (approval number: HADYEK 2009/13/8-2).

The present study was conducted at the Small Ruminant Unit in the Technological and Agricultural Research Center of Çanakkale Onsekiz Mart University, Turkey. The two experimental groups were housed in either an enriched environment (E) structured with different objects or a barren environment (B) without any structuring objects. In the enriched environment were placed a roughage feeder (0.55 m width × 2.30 m length × 0.95 m height), a semiautomatic concentrate feeder (0.93 m width × 2.30 m length × 0.70 m height), a laddered double-level "bunk" (Figure 1, Group E, left), a ladder walking "bridge" (Figure 1, Group E, right), a wood block (0.28 m diameter, 0.21 m height), and a plastic water bowl (30 L). No equipment was placed in the barren environment and the paddock sides were enclosed with smooth and slippery sheet iron to provide environmental isolation and prevent bipedal stance behavior. One side of group B was separated with a fence, into which animals could insert their heads, and roughage and food were placed in this separated space (Figure 1).

2.1. Animals and management

Forty Turkish Saanen goat kids born in the same week were used in the study. Following birth, the kids stayed continuously with their mothers until the age of 7 days and were then divided into the two experimental groups on the 8th day. Each group included 10 females and 10 males with 6 single and 14 twins kids. The average initial

Figure 1. Barren (B) and enriched (E) environments of experiment.

weight of kids in E was 6.13 ± 0.80 kg and in B was 6.16 ± 0.88 kg. Mothers were housed in enclosures adjacent to the experimental paddocks. The kids stayed with their mothers at night until weaning in both paddocks, even that without any installations. Kids were separated from their mothers at 0900 hours in the morning and reunited at 1800 hours. Following weaning, they remained continuously in the experimental paddocks. In both experimental paddocks the size was 5.50 × 5.20 m (1.4 m^2 of floor space/kid). Roughage and concentrate feed, water, and a mineral licking block were supplied ad libitum. The daily forage and concentrate feed consumption of the groups was determined at weekly intervals.

Alfalfa hay (91.20% DM, 18.90% CP, 42.11% NDF, 31.10% ADF, 2200 kcal ME/kg) and pelleted concentrate mixture (92.85% DM, 17.34% CP, 36.43% NDF, 13.50% ADF, 2600 kcal ME/kg) were provided daily throughout the experimental period. Goat kids were weaned at an average age of 61 ± 2.8 days. The experiments were terminated at an average age of 128 ± 2.8 days.

2.2. Social isolation tests

Kids were subjected to social isolation tests before weaning (average 54 ± 2.8 days) and after weaning (average 89 ± 2.8 days). Social isolation tests were conducted after the separation of kids and mothers in the mornings. For the social isolation tests, a 2 × 3 × 5 m fully enclosed chamber supplied only with wheat straw bedding was used. Kids were left alone in this chamber for 5 min. Goat kids were taken into the chamber through a 0.90 m width × 1.80 m length door (gray in color) and the door was closed instantly. The behavior and sounds of the goat kids were then recorded with a camera system (GL1263PL D/N Camera 1/4″ Sony Super Had CCD, 0.1 lx, AGC, BLC, F1.6 (3.9–85.8 mm) 22× optic, 10× digital zoom). Data collected included the time that elapsed from the initiation of isolation to the commencement of bleating (seconds), bleating frequency, bipedal stance (wall, door), walking, walking to the back, rearing (jumping or standing up to support the body with the hind limbs and a vertical surface), sniffing (wall, door, floor), turning around, and running behaviors (13).

For hormone analyses, blood samples were taken from each goat kid following the social isolation tests (within 2 min) before weaning (BW), the 2nd day of weaning (W), and after weaning (AW). Blood collection from a kid was completed within 5 min. Blood samples were taken in 10-mL vacuum blood tubes from the jugular vein. Samples were then centrifuged at 3500 rpm at 15 °C for 10 min and blood serums were separated. The separated blood serum was transferred to sterile storage tubes and preserved at –20 °C in a deep freezer until the day of analysis. Hormone and antibody titer analyses were carried out in accordance with the enzyme-linked immunosorbent assay (ELISA) method (25). A Thermo Scientific Multiskan FC microplate reader was used in the analyses. DiaMetra brand kits were used for cortisol, T3, and T4. To determine the serum antibody titer generated by the *Brucella melitensis* Rev 1 vaccine, kids were vaccinated at the age of 103 ± 2.8 days and blood samples were taken before vaccination and at the 21st day after vaccination. Svanovir (Brucella-Ab C-ELISA) commercial kits were used for the *Brucella melitensis* Rev 1 serum antibody titer. Analyses were performed in accordance with the procedures specified for relevant kits.

2.3. Statistical analysis

SAS statistical software was used for the data analysis (26). Logarithmic transformation (log 10 (y + 10)) was applied to behavioral data, which were saved by the continuous method, to meet the precondition for variance analysis. Transformed data were then subjected to variance analysis for repeated measurements in a fixed model including group (E, B), sex (♂, ♀), period (BW, AW), and their interactions. The Tukey test was used in post hoc analyses.

Square root (√y) transformation was applied to the hormone analysis data to meet the prerequisites of variance analysis. Because of significant group and period interactions, analyses were performed separately for each period ($P < 0.05$). Variance analyses with a fixed model including group (E, B), sex (♂, ♀), and their interactions were conducted. A variance analysis including group (E, B), sex (♂, ♀), and interaction was made for pre- and postvaccination antibody titers. However, the statistical model of the postvaccination antibody titer included in addition the prevaccination antibody titer as a covariant.

3. Results

Social isolation tests revealed significant differences in the time that elapsed from the initiation of isolation to the commencement of bleating and bleating frequencies between the groups and periods (Table 1; Figure 2). Although commencement of bleating was earlier for the goat kids of group E following inclusion in the isolation chamber, differences with the other group were not significant ($P = 0.1612$). However, the goat kids of group E had higher bleating frequencies than group B ($P = 0.0125$). Female kids of group E had significantly higher bleating frequencies than the female and male kids of group B ($P < 0.05$; Figure 2). After weaning, the bleating frequencies of the sexes were close to each other in both the groups. The time that elapsed between the initiation of isolation and commencement of bleating significantly varied based on period and the goat kids began to bleat earlier in the AW time span ($P = 0.0005$). Kids bleated less in the BW period than in AW ($P = 0.0058$); in other words, bleating frequencies significantly increased with age. Sex did not significantly affect the bleating frequency for the AW period ($P = 0.9968$). The interactions between experimental groups and sex were not statistically significant ($P > 0.05$).

Table 1. Raw means, standard errors (SEs), and P-values[1] for commencement of bleating (seconds) and bleating frequency (times/5 min) according to groups and periods in isolation test.

Factor		Commencement of bleating		Bleating frequency	
		Mean	SE	Mean	SE
Group	Barren	10.00	2.63	68.21	6.04
	Enriched	5.32	0.91	94.32	4.50
	P	0.1612		0.0125	
Period	Before weaning	10.92	2.58	75.92	6.04
	After weaning	4.28	0.69	87.28	5.17
	P	0.0005		0.0058	

[1] Based on statistical analyses performed with transformed ($\log_{10}$ (y + 10)) data.

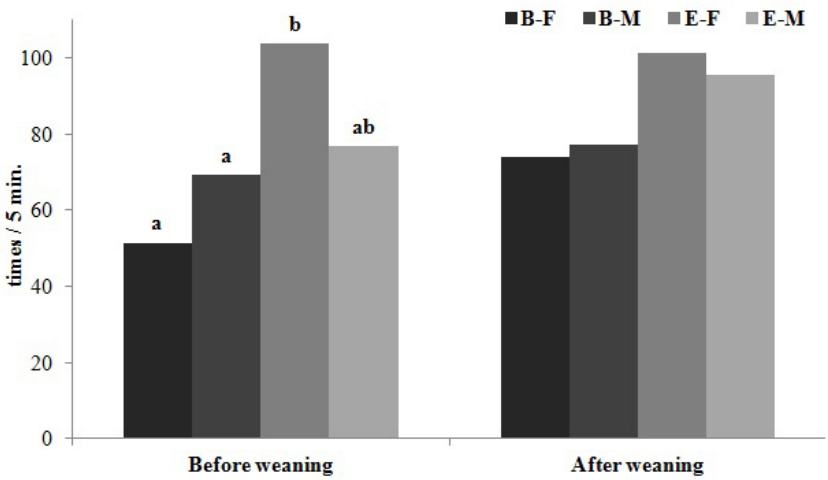

Figure 2. Variation in kid bleating frequency based on group and sex throughout the isolation tests.
B-F: Female kids of barren group; B-M: Male kids of barren group; E-F: Female kids of enriched group; E-M: Male kids of enriched group. Bleating frequencies indicated with different letters are significantly different according to the Tukey test, P < 0.05.

As seen in Table 2, the rearing behavior of groups showed significant differences while other behaviors did not significantly change between groups. While rearing was not observed in the kids of group E, a rearing frequency of 1.44 times/5 min was recorded in group B during the isolation test. Kids exhibited more bipedal stance behavior in AW than in BW (P = 0.0204) and exhibited less frequent running behavior in AW (Table 2; P = 0.0394). Significant differences were not observed between the other behaviors of the experimental groups (P > 0.05).

Hormone concentrations in blood samples taken during BW, AW, and the 2nd day of weaning exhibited significant variations based on treatment period (Table 3). The hormone concentrations of weaning were lower than those after isolation tests (P < 0.05).

Only the T3 concentrations of groups B and E were significantly different in the W period (Table 3; P = 0.0201). The E group had a higher T3 concentration than the B group in the W period. After weaning, cortisol concentrations were found to be 56.21 ± 6.03 ng/mL in group B and 42.09 ± 6.61 ng/mL in group E (P = 0.0778). Among the model parameters, sex had a significant effect on T3 hormone in the BW period (P = 0.0134) and on T4 hormone in the BW (P = 0.0169) and W (P = 0.0506) periods. The concentrations were higher in females than in males. The relationship between housing

Table 2. Raw means, standard errors (SEs), and P-values[1] for observational behaviors according to group and period in isolation test.

Behavior	Barren		Enriched		P
	Mean	SE	Mean	SE	
Bipedal stance	3.63	0.44	2.65	0.39	0.1638
Sniffing	4.92	0.49	3.92	0.46	0.1941
Running	2.28	0.38	1.37	0.32	0.0685
Walking to back	2.00	0.42	2.05	0.41	0.9212
Rearing	1.44	0.42	-	-	0.0001[2]
Turning around	1.34	0.22	1.75	0.21	0.2112
	Before weaning		After weaning		P
Bipedal stance	2.56	0.36	3.69	0.46	0.0204
Sniffing	4.79	0.45	4.02	0.50	0.1066
Running	2.38	0.43	1.25	0.24	0.0394
Walking to back	1.77	0.37	2.28	0.45	0.2856
Rearing	0.46	0.17	0.97	0.40	0.1462
Turning around	1.51	0.22	1.59	0.22	0.7888

[1] Based on statistical analyses performed with transformed ($\log_{10}$ (y + 10)) data.
[2] Significance from zero.

environment and sex was not significant in BW, AW, or W (P > 0.05).

Antibody titers generated by the *Brucella melitensis* Rev 1 vaccine were observed as being close to each other in the groups (Table 3). Prevaccination antibody titer was identified as 17.15% in group B and 15.66% in group E (P = 0.5079), and postvaccination values were 71.04% and 63.32%, respectively (P = 0.2753).

4. Discussion

Siebert et al. (20) reported that bleating is a good indicator of stress experienced during an isolation test. The goat kids of group E bleated more frequently than the kids of group B (Table 1; P = 0.0125). As was explained in Section 2, the environmental conditions of the isolation chamber are closer to the environmental conditions of the B group paddock than the E group paddock. Therefore, it could be speculated that goat kids from the E group showed more reaction with bleating behavior during the social isolation test to such structural differences between the environmental conditions.

The goat kids began to bleat later (P = 0.0005) and bleated less (P = 0.0058) in the BW compared to the AW period. The goat kids also had more frequent bipedal stance and running behavior in BW than AW. This could be explained by more intensive social ties of the kids with their group mates in AW. At a younger age, organisms can adapt to changing environments faster. Boivin et al. (27) indicated that calves isolated from their mothers and fellows at an early age were not scared of humans and accepted them as friends.

Rearing behavior was observed at a frequency of 1.44 times/5 min in group B, while it was never observed in group E (Table 2). It is known that social isolation causes hyperactivity in animals (13,20). Since the smooth and slippery structure of the paddock walls of group B were similar to the walls of the isolation chamber, the goat kids of group B might have been in contact with the walls more through rearing behavior.

The cortisol concentrations in isolation tests carried out in BW and AW were 2 and 3 times higher as in the W period, respectively, suggesting an acute stress effect due to isolation (Table 3). However, according to the hormone levels, the stress conditions established in the experiment (social isolation and weaning) did not reflect the different housing environments. That cortisol concentration increased in lambs during social isolation and weaning conditions has also been reported by Mears and Brown (28). The authors further determined that the cortisol was higher in social isolation than in weaning. However, in different goat breeds, significant changes were not observed in T3, T4, and epinephrine hormones of pre-

Table 3. Raw means and standard errors (SEs) of primary data and P-values[1] for hormone concentrations and antibody titers by group and period.

Hormone / antibody titer	Barren		Enriched		P
	Before weaning				
	Mean	SE	Mean	SE	
Cortisol (ng/mL)	66.09	8.04	85.03	10.96	0.1703
T3 (ng/mL)	2.56	0.15	2.79	0.16	0.2915
T4 (ng/mL)	43.54	1.81	41.54	3.35	0.3895
	After weaning				
Cortisol (ng/mL)	56.21	6.03	42.09	6.61	0.0778
T3 (ng/mL)	2.07	0.13	2.05	0.13	0.9268
T4 (ng/mL)	50.71	4.61	43.75	3.54	0.2566
	Weaning				
Cortisol (ng/mL)	26.88	8.08	25.41	6.94	0.9605
T3 (ng/mL)	1.82	0.13	1.40	0.11	0.0201
T4 (ng/mL)	27.43	2.22	28.44	2.72	0.8641
	Brucella melitensis (%)				
Prevaccination	17.15	5.08	15.66	1.10	0.5079
Postvaccination	71.04	4.62	63.32	5.08	0.2753

[1] Based on statistical analyses performed with transformed ($\sqrt{y}$) data.

and postisolation samples, but significant changes were observed in postisolation neuro-epinephrine hormone concentrations (13).

The higher cortisol hormone value obtained from the isolation tests in BW in comparison with the tests in AW may have resulted from the age of the goat kids. Cortisol hormone level may decrease with age (29). Similar to the cortisol hormone, T3 hormone concentrations were higher in isolation tests in the BW and AW periods than in weaning ($P < 0.05$). The T3 hormone may also respond to social isolation-induced acute stress conditions. T3 hormone concentrations were lower during weaning and were significantly different in both groups ($P = 0.0201$). The T3 hormone increases basal metabolism, heat generation, and oxygen consumption in all tissues. Therefore, the kids might have responded differently because of the feeder arrangements, feed provided to groups, late access of group B to concentrated feed, or variations in feed consumption. Average feed consumption of groups E and B before weaning (25–60 days of age) was identified as 0.202 kg/head and 0.262 kg/head forage and as 0.219 kg/head and 0.203 kg/head concentrate feed, respectively. In a study carried out with lactating Angora goats and their kids, thyroid hormone concentrations were reported to be higher in the group fed energy and protein supplements than in the control group (12). Similarly, it was also reported that differences in feed consumption of weaned Saanen goat kids might result in greater changes in hormone concentrations instead of stress factors (30).

In this study, it was found that the hormone concentrations of females were higher than those of males. The values for T3 and T4 hormones were significantly different in BW and T4 in the W period. Furthermore, female kids in group E bleated more often compared to group B kids in BW (Figure 2). It may be speculated that the female kids of group E showed higher isolation stress. However, the relationship between housing environment and sex for the hormone concentrations was not significant ($P > 0.05$). The result of the hormone concentration between the sexes differing significantly had no practical outcomes under the conditions of this experiment.

The antibody titer levels, determined to find out the effects of environmental structuring on immune response generated by vaccination (*Brucella melitensis* Rev 1), were

close between the groups (Table 3). On the other hand, Bolhuis et al. (6) reported improved immunity (total Ig titers and titers of IgM and IgG isotypes) in pigs in an enriched environment. Based on our results, it is difficult to say what the antibody titer levels signified since there was no control group in terms of kids not weaned and not in social isolation. İmik et al. (18) reported for Angora goat kids that the *Brucella* antibody titer of the control group without any stress was significantly lower than the titer levels of groups under stress. However, in the present experiment the immune responses of the structured or unstructured environment groups were not different.

Findings revealed that both social isolation tests created stress in the goat kids. It is to be expected that environmental structuring increased the animals' behavioral and physiological ability to cope with human interactions, experimental manipulations, and environmental variations. However, in our study, bleating frequency data revealed that the goat kids from group E might have been exposed to higher social isolation stress levels than the goat kids of group B. Similarities between the housing units of group B and the isolation chamber might have caused such findings. However, considering all parameters, it was observed that the goat kids raised in groups B and E did not show significantly differing social isolation and weaning-induced stress. On the other hand, differences in the housing units may, in the long run, improve the ability of kids to cope with stressors. Furthermore, it can be questioned whether or not harsh environments would increase their adaptive capability.

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