Maternal deprivation affects goat kids’ stress coping behaviour

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ABSTRACT

Maternal deprivation early in life has been shown to disrupt neonates’ development. Nevertheless, separating the young animals from their dams soon after birth remains a common practice in dairy farm husbandry. This study investigated the effects of different rearing conditions on goat kids’ stress coping abilities. Twenty female kids were raised together with their dams (‘dam-reared’) in a herd composed of other lactating goats and kids, while twenty female kids were separated from their dams three days after birth and reared together with same-age peers (‘artificially-reared’) and visually separated from the lactating herd. All kids shared the same father and two thirds of the kids were twins allocated to each treatment. At one month of age, kids were individually submitted to a series of tests: a novel arena test, a novel goat test, and a novel object test. These tests happened consecutively in this order, and lasted 180 s each. The kids’ behaviour was video-recorded and analysed post-hoc by an observer blind to treatments. Five weeks after weaning, the kids were also subjected to human-animal relationship tests. During the three behavioural tests, artificially-reared kids vocalized more (P < 0.001), reared more (P < 0.001), ran more (P = 0.002) and jumped more (P < 0.001) than dam-reared kids, but self-groomed less (P = 0.01) and urinated less (P = 0.05) than dam-reared kids. During the novel goat test and the novel object test, artificially-reared kids gazed less at the novel goat and the novel object (P = 0.02) and initiated contact more quickly (P = 0.05) with the novel goat and the novel object than dam-reared kids. The treatments however did not differ significantly in salivary cortisol response to the tests (P = 0.96). Artificially-reared kids showed significantly less avoidance of humans than dam-reared kids during the human-animal relationship tests after weaning (P < 0.001). The higher intensity of their behavioural reaction showed that artificially-reared kids react to stressful situations more actively than dam-reared kids. The difference between the three tests were only minor, suggesting a general change in the kids’ stress coping abilities. Twenty female kids were raised together with their dams (“dam-reared”) in a herd composed of other lactating goats and kids, while twenty female kids were separated from their dams three days after birth and reared together with same-age peers (“artificially-reared”) and visually separated from the lactating herd. All kids shared the same father and two thirds of the kids were twins allocated to each treatment. At one month of age, kids were individually submitted to a series of tests: a novel arena test, a novel goat test, and a novel object test. These tests happened consecutively in this order, and lasted 180 s each. The kids’ behaviour was video-recorded and analysed post-hoc by an observer blind to treatments. Five weeks after weaning, the kids were also subjected to human-animal relationship tests. During the three behavioural tests, artificially-reared kids vocalized more (P < 0.001), reared more (P < 0.001), ran more (P = 0.002) and jumped more (P < 0.001) than dam-reared kids, but self-groomed less (P = 0.01) and urinated less (P = 0.05) than dam-reared kids. During the novel goat test and the novel object test, artificially-reared kids gazed less at the novel goat and the novel object (P = 0.02) and initiated contact more quickly (P = 0.05) with the novel goat and the novel object than dam-reared kids. The treatments however did not differ significantly in salivary cortisol response to the tests (P = 0.96). Artificially-reared kids showed significantly less avoidance of humans than dam-reared kids during the human-animal relationship tests after weaning (P < 0.001). The higher intensity of their behavioural reaction showed that artificially-reared kids react to stressful situations more actively than dam-reared kids. The difference between the three tests were only minor, suggesting a general change in the kids’ stress coping abilities rather than a specific change in their social response tested with an unfamiliar adult. Hence, artificial rearing affects goat kids’ behavioural response to challenges, probably maternal deprivation being the main factor.

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1. Introduction

In social species, the early social environment can shape the neonate’s behavioural development [1–3]. In domestic species, the young’s early social environment often relies on management practices and human decisions. In modern dairy farming, the young is usually separated from its dam in the first days of life and reared with other individuals of a similar age, preventing it from interacting with its dam [4]. Although the young’s nutritional needs might still be fulfilled, the role of a mother is not limited to suckling its young [5,6], as a ruminant dam is its neonate’s primary social bond before the young integrates into the herd [7].

In natural settings, the goat kid shows a strong attachment to its mother for more than 6 months [8,9], and dam and kid show frequent affiliative behaviours such as allogrooming, and resting in contact [10]. The presence of such social attachment enables the mere presence of the mother to reduce the stress experienced by its young in stressful situations [11–13]. In guinea pigs, rats and domestic chickens, the presence of the mother buffers the stress response of their young when they are subjected to a novel environment, electric shocks, or an air puff [14–16]. In sheep and rats, a dam also reacts to the intensity of pain cues displayed by its young by increasing its maternal behaviours [17,18], which for instance have beneficial effects in alleviating pain as assessed by thermal pain sensitivity [18,19].

The absence of such maternal care and social support can also affect the young’s stress-coping abilities when confronted with challenging situations even though the dam is not present [20,21]. In rhesus monkeys and domestic chicks, behavioural and physiological reactivity to...
stressors increases if the young is reared only with same-age peers compared with a young reared with its mother in a more socially-enriched environment [22–25], and a brooded chick shows a greater motivation to reinitate social contact with conspecifics than a non-brooded chick [26]. The opposite happens in graylag goose [27], as reactivity to stressors decreases if the chick is reared only with same-age peers compared with a chick reared with its mother or parents. In sheep, maternal deprivation does not seem to influence sensitivity to acute 
peers compared with a chick reared with its mother or parents. In sheep, with their mothers in a herd of goats and kids response to social isolation than a lamb reared with peers [28]. In cattle, maternal deprivation has been shown to have short and long-term effects on sociability, as a calf and cow that have been separated from its mother within twenty-four hours after birth is less reactive and less motivated to re-establish social contact during an isolation test than an animal reared with its mother [31–33]. A goat reared without its dam is less neophobic towards novel objects, but as neophobic toward unknown peers when compared to a goat reared with its dam [34]. Furthermore, maternal deprivation can reduce reactivity towards humans, as a relationship with a human develops more easily when lambs are reared without their mother [35]. Therefore, maternal deprivation might improve human-animal relationship, possibly due to a greater amount of positive human handling experienced at an early age [34,36,37], especially when compared to a situation where the young reared with its mother receives very little gentle contact from humans [38].

Maternal deprivation therefore seems to affect a broad range of responses to stressful and painful situations in the young, but it remains the most common practice to rear young animals in dairy farming. Therefore, this study compared the implications of rearing goat kids with their mothers in a herd of goats and kids vs. only with peers of a similar age on their response to challenging situations and a painful husbandry procedure (disbudding). We hypothesized that being reared with the dam and other adults affects the kid’s response to challenging situations, such as separation, social confrontation, novelty, presence of a human and recovery after disbudding.

2. Animals, materials and methods

All procedures were discussed and approved by the institutional ethics and animal welfare committee of the University of Veterinary Medicine, Vienna, in accordance with GSP guidelines and the national legislation (project number ETK-051/03/2019).

2.1. Animals and housing

The study was carried out at a commercial organic dairy goat farm in Austria. The farm routine practices consist in milking the lactating goats twice daily, from 04:30 h to 06:00 h and from 16:30 h to 18:00 h. The animals were fed on hay renewed every 36 h and concentrate distributed three times per day. All the animals were kept on deep litter straw in two rectangular pens (36.0 m × 4.4 m) facing each other, separated by a feeding table. These pens were subdivided into smaller pens to separate experimental groups (artificially-reared, dam-reared – see below), kids in different nutritional stages (pre-weaning, weaning), or goats in different reproductive stages (pre-parturient, postnatal, lactating). Each goat gave birth in the pre-parturient pen before being moved with its young in the postnatal pen to ensure the kids suckle enough colostrum. Three days after parturition the goat integrated the lactating herd. As the kidding season progressed, adjustable 1.1 m high and either 2.75 m or 1.85 m wide metal fences were used, enabling frequent adjustment of the size of each pen. Physical interactions through these fences were still possible.

We studied 40 female Saanen breed kids born over the course of 17 days. Kids were selected for the study based on the following inclusion criteria: female, healthy, and born on the same day than at least another healthy female kid for pair-matched allocation to the two treatments. Each focal kid was weighed and marked using long-lasting hair dye at two days of age for easy individual recognition during behavioural observations.

The focal kids were weaned on two different dates, with half of the kids of each treatment weaned on each date. A kid was considered ready to be weaned when it was at least six-weeks old and weighed at least 15 kg. Kids of the first batch were weaned at 53 days of age on average (ranging from 43 to 58 days of age) whereas kids of the second batch were weaned at 58 days of age on average (ranging from 55 to 72 days of age). At weaning, kids were retrieved from their home-pen, brought in a new pen and mixed with kids weaned on the same day, including kids of the other treatment. The space allowance in the weaning pens was 10 m² to which 0.9 m² per kid was added. The weaning pens’ walls were made of adjustable iron fences and allowed visual and physical interactions with the weaned kids housed in the adjacent pens. After weaning, the kids were only handled to be re-marked once per week using a mixture of turmeric and water.

2.2. Treatment allocation

Three days after birth (ranging from 59 to 83 h old), 40 healthy female kids were allocated to one of two treatments following a randomized matched-pair design. Treatments were balanced for kids’ age and weight at two days of age (average weight ± standard deviation: 4.27 ± 0.70 kg for the artificially-reared kids, 4.30 ± 0.62 kg for the dam-reared kids). If female twins were born, they were separated and allocated one to each treatment, which occurred for 64.5% of the kids.

2.2.1. Artificial-rearing

Twenty focal kids were separated from their dams three days after birth and raised together in a pen, physically and visually isolated from the lactating goat herd. The group size grew from two to 20 individuals through the trial as kids were progressively born and allocated to treatments. The pen, built with adjustable iron fences, originally measured 11.2 m², and 0.6 m² of floor-space was added for each kid introduced in the group by moving the pen’s walls.

These artificially-reared kids (‘AR’ treatment) were fed from teated buckets filled with whole goat milk three times per day at 6:30 h, 11:30 h, and 17:00 h. After allocation, the kids were helped to suckle until they were able to feed properly on their own and each meal was supervised to ensure that all the kids found a teat to suckle. In order to eliminate any nutritional disparity between treatments, the distributed quantities were calculated for the kids to aim for complete satiation after each meal. The milk was warmed up to 40 °C before being distributed.

2.2.2. Dam-rearing

The other twenty focal kids were kept together in the lactating herd along with their dams and other non-focal dam-reared kids until weaning, in a group size ranging from five to 56 dams and from 12 to 50 kids. These dam-reared kids (‘DR’ treatment) were able to suckle their dam ad libitum. Each dam and its kid were introduced in the pen, with the fences moved to provide an additional 1.8 m³ of floor space. The kids had also access to a 10 m² creche-area in the pen inaccessible to the adults to be able to rest without being disturbed by adults. Before milking, the adults were moved to a collecting area and the kids were left alone in the home-pen until the adults came back from the milking parlour.

2.3. Disbudding and pain-related behavioural observations

As per the routine practice on that farm, the kids were thermally disbudded with a hot iron when they were between seven and 28 days of age. Nine kids (four dam-reared and five artificially-reared kids) were disbudded one week after the beginning of the trial whereas 28 kids (13 dam-reared and 15 artificially-reared) were disbudded four weeks later
(Fig. 1), according to the veterinarian’s availabilities, and three dam-reared kids were genetically polled. The kids were provided with sedation, local anesthesia, and analgesia, before disbudding, by an injection of 6 mg of xylazine (SEDAXYL® 20 mg/mL, Eurovet Animal Health, Netherlands), 40 mg of procainhydrochlorid (PROCAMIDOR® 20 mg/mL, Richter Pharma, Austria, 2 mL per side), and 0.2 mg of meloxicam (METACAM® 20 mg/mL, Boehringer Ingelheim Vetmedica GmbH, Germany). The lateral and longitudinal diameters of their horn-wounds measured using a Vernier-caliper before the kids recovered from anesthesia. Each kid’s horn-wounds’ diameters were measured a second time after the kid went through the behavioural tests detailed in the next section, 11 to 25 days after disbudding.

Four pain-related behaviours were recorded using continuous focal sampling [39]. The kids disbudded on the first session (n = 9) were aged from seven to nine days and were observed five minutes twice a day, once in the morning and once in the afternoon, for the six days following disbudding. The kids disbudded on the second session (n = 28) were older (from 17 to 28 days old) and could only be observed for five minutes twice a day every third day, from three days before to seven days after disbudding (Fig. 1). The pain-related behaviours (Table 1) were recorded by one trained observer using Animal behaviour pro 1.4.4 (Friard and Gamba 2016).

2.4. Behavioural tests

2.4.1. Test setting

Prior to weaning, the focal kids were subjected to a series of behavioural tests composed of a novel arena test, a novel goat test and a novel object test on a total of four testing days with eight, 11, 12 and seven kids per testing day, respectively. These 38 animals were aged from 30 to 39 days during their tests and were allocated to one testing day based on their age, from oldest to youngest. Two dam-reared kids died from diseases unrelated with the study before being tested. Treatments were balanced on each testing day.

The testing arena consisted of a 2.8 m × 2.8 m set up on the side of a pen housing nulliparous goats unrelated to the study. The walls of the testing arena were made of see-through wire-mesh. A fence was placed around the testing area to keep the nulliparous goats 1.5 m away from the arena. On the day of the test, eight of these nulliparous goats were randomly chosen and segregated and enclosed between this fence and the rest of the herd (Fig. 2), from which five of them were used as the stimulus goat for the novel goat test, alternating in a random order.

2.4.2. Testing procedure

The tests were conducted between 13:00 h and 16:30 h. Each focal kid was subjected individually to the three tests in a consecutive order, with each test lasting 180 s and starting when the researchers were out of the animal’s sight. During the novel arena test, the kid was left alone in the arena. Then, one of the stimulus goats was brought in the area surrounding the testing arena for the duration of the novel goat test (Fig. 2). The stimulus goat was then brought back to the intermediate pen and the novel object, a yellow chicken feeder with colorful horizontal lines made of electric tape and silver tape (Fig. 2), was placed in the middle of the arena for the novel object test using a pick-fork. In case the focal kid started screaming or tried to escape more than once, the tests were terminated for ethical reasons and the kid was excluded from the dataset as detailed in the behavioural data section. This occurred for five artificially-reared and two dam-reared kids.

2.4.3. Behavioural data

An experimenter recorded live vocalizations (Table 2) while the kid was in the testing arena using the Animal behaviour pro 1.4.4 software [40]. The software Boris v. 7.7.4 [41] was used by one trained observer to record the other behaviours from the video recordings of the behavioural tests post-hoc, with six categories of mutually-exclusive behaviours (Table 2). This last observer was blind to treatment.

The videos of the kids tested on the third day were accidentally lost, reducing the sample size to 22 kids (10 dam-reared and 12 artificially-reared) for this dataset.

2.4.4. Salivary cortisol concentration

For each focal kid, two saliva samples were collected using...
Salimetrics Children Swabs (SalivaBio Children’s Swab, Salimetrics). Saliva samples were collected right after retrieving the kid from its home pen to assess baseline salivary (free) cortisol concentration, and right after retrieving the kid from the testing arena to quantify the effect of the tests on salivary cortisol concentration. For each sample, half of a swab (6.25 cm long \( \times \) 0.8 cm diameter) was introduced in the kids’ lower cheek for 45 s, letting it free to open or close the mouth. The sample were then withdrawn, put back in a plastic tube case and stored immediately in a cooling Styrofoam box filled with dry-ice before being transferred to a -20 \( ^\circ \)C freezer. The sampling procedures were performed within 2 min after the experimenter entered the pen to catch the kid, before the salivary cortisol concentration may start changing due to the human handling. After all the behavioural tests were performed, the samples were brought back to the laboratory and spinned with the supernatant separated in Eppendorf tubes, carefully avoiding food particles if present in the sample. Samples with quantities \( \geq 30 \) µL were analyzed at half-dilution for their saliva cortisol concentration using the Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit from Salimetrics\(^{\circledR}\) (USA, Item No. 1–3002, 96-Well Kit). Sixteen artificially-reared and four dam-reared kids gave enough saliva, before and after the tests, to be analysed. All the samples were processed on the same plate, with an intra-assay CV \( \leq 12.6\% \).

2.5. Human–animal relationship test

Two sessions of human avoidance distance tests separated by 48 h were performed in the kids’ home pen during the 5th week following weaning (Fig. 1), when the kids were aged from 72 to 103 days old. The trained assessor was familiar to the kids, as she supervised the meals of the artificially-reared kids one to three weeks before weaning and handled the kids of both treatment once per week after weaning during marking, but she wore a white overall and rubber boots that were novel to them. Before starting the avoidance distance tests, the assessor stood stationary for two minutes in each of the four corners of the pen for the kids to lose interest and stop interacting with the assessor.

The kids were tested for their avoidance distance following the method used by Mattiello et al. (2010). The testing order was predetermined following a pseudo-random design alternating between treatments. To test a kid, the assessor placed herself at a distance of 2 m in front of the tested kid and waited until the kid was standing and gazing towards her direction. Then, the assessor started moving towards the kid at a speed of one step per second, one step being about 60 cm long. The assessor kept her arm inclined at 45\(^\circ\), her fingertips oriented towards the floor and the back of her hand oriented towards the kid while walking, and looking at the animal’s muzzle while avoiding looking into the kid’s eyes. When the kid withdrew, the assessor estimated the distance between her hand and the muzzle of the animal with a resolution of 10 cm, and recorded it as the avoidance distance of the kid. If the assessor was able to establish physical contact with the tested kid but the kid withdrew right after being touched, the avoidance-distance was recorded as 0.01 m. If the kid allowed the assessor to stroke it on its head, the avoidance-distance was recorded as 0 m. If the assessor could not distance herself 2 m away from the kid without the animal withdrawing, the avoidance distance was recorded as 2 m. If any disturbance happened during a test, such as the tested kid stopped gazing at the observer, another kid crossed the path of the assessor or interacted with the tested kid and thus potentially would have affected the outcome of the test, the test was restarted.

2.6. Statistical analysis

Statistical analyses were performed using RStudio (version 1.2.5033; RStudio Team, 2019). Linear mixed models and generalized linear mixed models were used to estimate the effects of treatment on each of the response variables measured [41]. These statistical methods made it possible to analyze related response variables using models that
Table 2

Behaviours recorded and parameters analysed as behavioural tests outcomes.

| Cat. | Behaviour Description Parameter | Parameter analyzed |
|------|----------------------------------|--------------------|
| 1    | Vocalisation | Emission of a short and segmented contact call with open mouth. | Number of events |
| 2    | Urination | Crouching to urinate | Presence/absence |
| 3    | Self-grooming | Licking, grooming, nipping or rubbing its body | Number of bouts |
| 4    | Exploration | Nose at less than a muzzle distance from the arena’s walls or floor, with all four feet on the floor | Total duration |
| 5    | Rearing | Two front feet in contact with the arena’s walls | Proportion of time |
| 6    | Standing | All four feet in contact with the floor while neither grooming nor exploring | Proportion of time |
| 7    | Running | Galloping for more than 2 steps while not exploring | Number of bouts |
| 8    | Walking | Walking or trotting for more than 2 steps while not exploring | Excluded (Excluded) |
| 9    | Jumping | Either two or four feet raising from the floor before landing on it again | Number of events |
| 10   | Step | One forepaw initiating contact with the floor after being lifted | Number of events |
| 11   | Square 1 to 9 | Right forepaw touching the floor of one of the nine 0.9 m x 0.9 m squares dividing virtually the arena for the first time. | Total duration |
| 12   | Social proximity | Kid’s nose in the square closest to the adult goat’s front feet | Number of events |
| 13   | Gaze | Rostral-caudal axis crossing the adult goat or the novel object while standing or walking | Latency to first event |
| 14   | Contact | Kid’s nose at less than a muzzle distance from the novel object or the adult’s body through or over the fence | Proportion of time |
| 15   | Tail up | Tail oriented above the longitudinal axis of the kid’s body | Proportion of time |
| 16   | Ears backward | Tip of both ears oriented towards the back of the kid | Proportion of time |
| 17   | Ears asymmetrical | Left and right ears oriented differently | Proportion of time |
| 18   | Ears horizontal | Ears parallel to the frontal plane and perpendicular to the rostral-caudal axis | Proportion of time |
| 19   | Ears forward | Tip of both ears oriented towards the front of the kid | Proportion of time |
| 20   | Head movement | Rapid movements of the head | Number of events |

- Variable only recorded in the novel goat test.
- Only recorded in the novel goat and novel object tests.

included the same factors and that were suited to the different response variable distributions.

Pairwise scatter plots were checked to ensure that the dependent variables were not correlated prior to fitting the models.

2.6.1. Disbudding pain-related measures

The models used for the pain-related behaviours included the fixed factors treatment, day since disbudding, age and the interaction between treatment and day since disbudding as the amount of pain-related behaviour was expected to decrease faster in dam-reared kids compared to artificially-reared kids. Kid and their dams were included as random effects. The frequency of ear-flicking was analyzed using a negative binomial model while the beta-transformed proportions of time spent head-scratching and head-shaking were analyzed using a beta regression. To avoid the models being overconfident regarding the precision of fixed effects estimates, and to keep type I error rate at the nominal level of 5%, age and day since disbudding within kid and dam were included as random intercepts [42,43]. Head-rolling was only observed 14 times and therefore was not analyzed.

The linear mixed model used to analyze horn-wound size included treatment, day since disbudding, side (left or right horn-bud), orientation (lateral or longitudinal diameter), age at disbudding and the interaction between treatment and day since disbudding as fixed factors. The interaction between treatment and day since disbudding was included as we expected the size of the horn wounds to be similar between treatments on the day of disbudding but to be smaller in dam-reared kids compared to artificially-reared kids on the day of the second measurement. Kid, their dams and date were included as random effects, and day since disbudding, side and orientation within kid and dam were included as random slopes with the correlations between them and the random intercept.

2.6.2. Behavioural tests

The models included treatment, test, age and the interactions between treatment and test as fixed effects for all the response variables except ‘social proximity’. The interaction between treatment and test was included as we expected the difference between treatments to vary between social (novel goat test) and non-social challenges (novel arena test and novel object test). To control for their potential effects, kid, the kid’s dam and date of test were included as further random effects. The models used to analyze ‘social proximity’ included the same factors except for the fixed effect of test and the interaction between treatment and test given that this behaviour was only possible in the novel goat test. The random factor ‘dam’ had to be removed from the models analyzing the proportion of time spent rearing and the proportion of time spent with the tail up to simplify the model and allow it to converge.

2.6.3. Statistical method

The linear mixed model used to analyze salivary cortisol concentrations included the fixed effects of treatment, sampling time point, and the interaction between treatment and sampling time point, as the increase in salivary cortisol concentration during the tests was expected to be higher in artificially-reared kids compared to dam-reared kids. Kid was also included as a random effect to control for its potential effects. Considering the small sample size, dam was not included as a random effect to reduce the model complexity.

2.6.4. Human–animal relationship tests

The models used to analyze the avoidance distance included treatment, weaning batch, day and age as fixed effects. Kid and dam were included as random effects. The avoidance distance was divided by two and beta-transformed to be analyzed using beta regressions.

2.6.5. Statistical method

When included in a model as a fixed effect, age was included after being z-transformed to ease the interpretation of the model [45]. For each response variable, a full-null model comparison was conducted to
test for the effect of treatment and its interactions with test, with the null model lacking these two effects but otherwise identical to the full model. The full and the null models were compared using a likelihood ratio test [46]. If the full model included a non-significant interaction, this last step was repeated with a reduced model lacking the non-significant interaction but otherwise being identical to the full model. After fitting a linear mixed-model, the assumptions of normality and homogeneity of the residuals were checked by visual inspection of a QQ-plot [47] of the residuals and the residuals plotted against the fitted values [48]. The collinearity between the fixed effects of each model was checked after being determined for a model lacking the random effects and the interaction but otherwise being identical to the full model [48]. The stability and standard deviation of each model on the level of the estimated coefficients were assessed by excluding the levels of the random effects one at a time [49]. The overdispersion coefficient was also calculated and estimates of overdispersed models were corrected for it. This last step was done for the models analyzing contact, latency to contact, latency to gaze, ears horizontal and head movements.

3. Results

The behavioural tests’ dataset included the vocalisations of 15 artificially-reared kids and 16 dam-reared kids, and the activity, locomotion, proximity, attention and approach, tail and ear positions of 12 artificially-reared kids and 10 dam-reared kids. The cortisol dataset included 16 artificially-reared kids and 4 dam-reared kids. The
3.1. Behavioural tests

3.1.1. Vocalisation

Beats occurred more frequently in artificially-reared kids than in dam-reared kids (full-null model comparison: \( \chi^2 = 23.8, df = 3, P < 0.001 \)), and kids bleated the most during the novel goat test and the least during the novel goat test (reduced model: \( \chi^2 = 36.7, df = 2, P < 0.001 \); Fig. 3.a), but the interaction between treatment and test, and age, had no significant effects.

3.1.2. Activity

Urination occurred less frequently in artificially-reared kids than in dam-reared kids (full-null model comparison: \( \chi^2 = 6.2, df = 2, P = 0.046 \); Fig. 4.a), but test, the interaction between treatment and test, and age, had no significant effects.

Running (Fig. 4.b) and jumping (Fig. 4.c) occurred more frequently in artificially-reared kids than in dam-reared kids (full-null model comparison for running: \( \chi^2 = 10.5, df = 3, P = 0.015 \); full-null comparison for jumping: \( \chi^2 = 24.7, df = 4, P < 0.001 \)), but test, the interaction between treatment and test, and age, had no significant effects.

Rearing was longer in artificially-reared kids than dam-reared kids (full-null model comparison: \( \chi^2 = 14.8, df = 3, P = 0.002 \)), and kids reared for the longest time during the novel goat test and the shortest time during the novel object test (reduced model: \( \chi^2 = 18.8, df = 2, P < 0.001 \); Fig. 3.b), but the interaction between treatment and test, and age, had no significant effects.

Self-grooming occurred less often in artificially-reared kids than dam-reared kids (full-null model comparison: \( \chi^2 = 8.8, df = 3, P = 0.032 \)), and kids self-groomed the most during the novel goat test and the least during the novel object test (reduced model: \( \chi^2 = 9.8, df = 2, P = 0.007 \); Fig. 3.c), but the interaction between treatment and test, and age, had no significant effects.

Standing tended to be shorter in artificially-reared kids than in dam-reared kids (full-null model comparison: \( \chi^2 = 6.8, df = 3, P = 0.077 \)), and kids stood for the longest time during the novel goat test and the shortest time during the novel goat test (reduced model: \( \chi^2 = 11.5, df = 2, P = 0.003 \); Fig. 3.d), but the interaction between treatment and test, and age, had no significant effects.

Exploration did not significantly differ according to treatment, test, the interaction between treatment and test or age (estimated mean \( \pm \) standard error: 69 \( \pm \) 8.1 s).

3.1.3. Locomotion

Stepping tended to occur most frequently during the novel arena test and the least frequently during the novel goat test (reduced model: \( \chi^2 = 5.3, df = 2, P = 0.071 \); novel arena test: 81.4 \( \pm \) 0.2 steps, novel goat test: 93.4 \( \pm \) 0.2 steps, novel object test: 72.3 \( \pm \) 0.1 steps), but none of the other fixed effects had a significant effect on steps and squares entered (7.1 \( \pm \) 0.1 squares entered).

3.1.4. Proximity

Social proximity only differed according to age (reduced model: \( \chi^2 = 4.6, df = 1, P = 0.033 \)), as the time spent in social proximity with the unfamiliar goat during the novel goat test was shorter for older kids (intercept: 87.9 \( \pm \) 10.5 s, estimated effect of being 1.6 days older: \( -16.8 \pm 8.0 \) s).

3.1.5. Attention and approach

Latency to contact the novel goat and the novel object was higher in dam-reared kids than in artificially-reared kids (full-null model comparison: \( \chi^2 = 6.2, df = 2, P = 0.045 \); Fig. 4.d), but test, the interaction between treatment and test, and age had no significant effects.

Gazing at the novel goat and at the novel object occurred less frequently in artificially-reared kids than in dam-reared kids (full-null model comparison: \( \chi^2 = 6.9, df = 1, P = 0.009 \); Fig. 4.e), and was lower in older kids (\( \chi^2 = 7.5, df = 1, P = 0.006 \)), but the kids did not gaze at the novel goat more than they gazed at the novel object, and the interaction between treatment and test was not significant.

Contact with the novel object occurred longer in artificially-reared kids than in dam-reared kids (\( \chi^2 = 6.6, df = 2, P = 0.037 \); Fig. 3.e), and this difference tended to be reduced during the novel goat test (full model: \( \chi^2 = 3.2, df = 1, P = 0.071 \)), and age had no significant effect.

The latency to gaze did not differ according to any of the fixed effects (18.5 \( \pm \) 0.2 s).

3.1.6. Tail position

Tail up occurred for longer in artificially-reared than in dam-reared kids (full-null model comparison: \( \chi^2 = 13.6, df = 3, P = 0.004 \)), and kids kept their tail up the longest during the novel goat test and the shortest during the novel arena test (reduced model: \( \chi^2 = 7.1, df = 2, P = 0.028 \); Fig. 3.d), but the interaction between treatment and test, and age, had no significant effects.

3.1.7. Ear positions

Ears horizontal occurred for longer in artificially-reared than in dam-
reared kids (full-null model comparison: $\chi^2 = 8.8, df = 3, P = 0.032$), and kids spent the highest amount of time with their ears forward during the novel arena test and the lowest amount of time during the novel object test (reduced mode: $\chi^2 = 13.3, df = 2, P = 0.001$; Fig. 3g), but neither the interaction between treatment and test nor age had significant effects.

Ears asymmetrical differed according to treatment (full-null model comparison: $\chi^2 = 8.9, df = 3, P = 0.030$), and the interaction between treatment and test (full model: $\chi^2 = 8.1, df = 2, P = 0.017$), as artificially-reared kids spent less time with the ears forward than the artificially-reared kids during the novel arena test, with the opposite result during the novel goat and novel object tests (Fig. 3h).

Ears backward tended to differ according to treatment (full-null model comparison: $\chi^2 = 6.7, df = 3, P = 0.082$), and the interaction between treatment and test (full-model: $\chi^2 = 5.8, df = 1, P = 0.055$), as artificially-reared kids tended to spend less time with the ears backward than the dam-reared kids during the novel arena test, with the opposite result during the novel goat and novel object tests (Fig. 3i).

Head movement and ears forward did not differ according to treatment but differed according to the test. Kids of both treatment made head movement the most during the novel arena test and the least during the novel object test (reduced model: $\chi^2 = 8.1, df = 2, P = 0.017$; novel arena test: $0.1 \pm 0.5$ movements, novel goat test: $0.9 \pm 0.5$ movements, novel object test: $0.7 \pm 0.5$ movements), but kids spent the highest amount of time with this ear position during the novel object test and the lowest amount of time during the novel arena test ($\chi^2 = 7.1, df = 2, P = 0.028$; novel arena test: $58.1 \pm 26.9$% of the time, novel goat test: $25.7 \pm 26.8$% of the time, novel object test: $66.6 \pm 28.7$% of the time).

3.2. Physiology

The salivary cortisol concentration significantly increased from $0.1 \pm 0.02 \mu g/dL$ to $0.2 \pm 0.02 \mu g/dL$ after the tests ($\chi^2 = 12.1, df = 1, P < 0.0001$; Fig. 5), but treatment and the interaction between treatment and time of sampling had no significant effects.

3.3. Disbudding pain-related behaviours

The proportion of time spent head-scratching tended to increase across the days in artificially-reared kids but it tended to decrease in dam-reared kids (interaction between treatment and day since disbudding: $\chi^2 = 3.0, df = 1, P = 0.083$; intercept: $1.3 \pm 0.98$% of the time, estimated effect of 2.4 days in artificially-reared kids: $+16.9 \pm 8.3$%, estimated effect of 2.4 days in dam-reared kids: $-2.4 \pm 11.1$%) but none of the other fixed effects had a significant effect. Horn-wound size significantly increased with the number of day since disbudding ($\chi^2 = 163.7, df = 1, P < 0.001$; disbudding day: $12.3 \pm 3.1$ mm, estimated effect of 8.4 days: $+0.6 \pm 0.04$ mm), but none of the other fixed effects had a significant effect. The frequency of ear-flicking and the proportion of time spent head-shaking were not significantly affected by treatment, day since disbudding, the interaction between treatment and day since disbudding or age (ear-flicking: $1.6 \pm 0.3$ flicks, head-shaking: $2.0 \pm 9.9$% of the time). Head-rubbing was observed only six times in dam-reared kids and eight times in artificially-reared kids.

3.4. Human–animal relationship

The avoidance distance was shorter for artificially-reared kids than for dam-reared kids (full-null model comparison: $\chi^2 = 58.9, df = 1, P < 0.001$; artificially-reared kids: $0.9 \pm 0.4$ m, dam-reared: $2.9 \pm 0.5$ m). Older kids during the avoidance-distance test had greater avoidance distances ($\chi^2 = 5.9, df = 1, P = 0.015$; estimated effect of being 5.2 days older: $1.2 \pm 0.2$ m), and kids from the first batch had significantly higher avoidance distance than kids from the second batch ($\chi^2 = 4.2, df = 1, P = 0.042$; first batch: $1.6 \pm 0.4$ m, second batch: $2.2 \pm 0.4$ m). Kids also tended to let the human approach closer on the first day than on the second day of testing ($\chi^2 = 3.8, df = 1, P = 0.052$; first day: $1.5 \pm 0.4$ m, second day: $2.3 \pm 0.3$ m).

4. Discussion

4.1. Behavioural tests

During the behavioural tests, artificially-reared goat kids vocalized, reared, ran and jumped more than dam-reared kids, while dam-reared kids self-groomed and urinated more than artificially-reared kids. Therefore, artificially-reared kids appear to show a higher behavioural arousal, usually interpreted as a more active coping strategy, whereas dam-reared kids showed a lower behavioural arousal, usually interpreted as a more passive coping strategy, during these challenging situations [50,51]. Coping style has been described as an aspect of personality, defining the physiological and behavioural strategy individuals employ to reduce the effect of aversive stimuli on them [52-55]. A more proactive or active coping strategy will be expressed by bolder individuals actively exploring their environment, whereas more reactive or passive coping strategy will be shown by shyer individuals less prone to active exploration [55]. The coping strategies of individuals of a population will be normally distributed over a spectrum from proactive to reactive, as shown in pigs and cows [52,56,57]. Active pigs show a greater motivation to escape an arena than passive pigs during an open-field test [52], and active cows escape a restraint device faster than passive ones [56]. This difference in coping strategy between treatments is in line with previous studies on goats, which found that kids reared without their dam have greater excitability and are bolder when confronted to novelty [34,58]. This seems to contradict findings in calves, as calves reared without their mothers were less active during social isolation than calves reared with their mothers [32]. However, in this experiment calves were visually isolated from any conspecific and this complete social isolation may have played a larger role than in the current experiment on kids.

There was however no significant difference in exploration time, number of steps, number of squares entered, or salivary cortisol
Ears and tail positions also varied across treatments and tests. Artificially-reared kids kept their tail up and their ears asymmetrical and backward more than dam-reared kids. The kids performed head movements and had the ears horizontal the most, and kept their tail up, their ears asymmetrical, forward and backward the least during the novel arena test. They kept their tail up and the ears asymmetrical the most during the novel goat test. The kids kept their ears forward the most, and kept their ears horizontal and performed head movement the least during the novel object test. We interpret these differences in terms of arousal of the valence of the kids’ internal state [69], which was the closest to positive during the novel goat test and the most negative during the novel object test. In terms of arousal, we interpret it as the highest during the novel object test and the lowest during the novel arena test and artificially-reared kids as less aroused than dam-reared kids during the tests. Indeed, the time goats spent with ears asymmetrical and tail up increases with a more positive valence of their emotions, whereas the time spent with ears forward is positively correlated with arousal and time spent with ears horizontal is negatively correlated with arousal [69, 70]. Reactive individuals have already been found to be more aroused by a changing environment than proactive ones in pigs [71]. However, each group of kids had not increased with a more positive valence of the situation [69] but with the degree of isolation or the degree of uncontrollability of the situation, as proposed in sheep [72,73]. The frequency of head movements decreased over time and therefore might indicate habituation to the testing arena. We cannot rule out a potential carry-over effect between the consecutive tests, but the change of response over the course of the test procedures was similar between groups.

Besides differences in coping strategies, other factors may have contributed in different degrees to the behavioural differences of the two treatments. Firstly, dam-reared kids showed a higher level of fear of humans, as reflected in their higher avoidance distance, than artificially-reared kids and thus the pre-test handling could have induced a higher level of stress in dam-reared than in artificially-reared kids [74]. This may have affected their perception of the test situation and thus their behaviour, as well as the quantity of saliva collected through the cotton swab. Secondly, dam-reared kids had experienced a more complex environment not only socially but also regarding other environmental stimuli. Due to the higher number of animals in their home pen, the size of the space that dam-reared kids could explore both physically and visually was also larger. They also had to deal with intermittent separation from their mothers and the other adult goats twice daily during milking. In contrast, artificially-reared animals were living in a relatively small pen, visually and physically separated from all other goats and the rest of the barn. Thirdly, dam-reared goats likely had experienced agonistic interactions from other adult goats and thus may have developed social skills to avoid aggression by keeping more distance from other adult goats, as described for dam-reared calves [75–77]. This is in agreement with higher levels of gazing and lower duration of contact with the adult goat in our experiment. Lastly, this study performed within a farm setting only allowed to have one group for each treatment, all purebred Saanen goats. The generalization of these findings would require replicability with more groups, in different farms, and with different breeds.

To be able to make definite conclusions on an individual’s coping style, one must show the consistency over time of the same individual’s response. We only assessed the kids’ behavioural response at one time-point in infancy, and therefore further research is necessary to assess the stability of their behavioural phenotype over time [78], as a similar study found that differences reduced with age [34].

4.2. Human–animal relationship tests

Rearing kids artificially with bucket feeding improved the human–animal relationship later on as assessed five weeks after weaning between 11 and 15 weeks of age. The artificially-reared kids showed less avoidance of a familiar human than dam-reared kids, in accordance with the literature in goat kids [34,36,37], lambs [35], and calves [38,79]. In dairy farming, the human-animal relationship is an important component of welfare as the animals have to interact with humans daily, and fear of humans can lead to chronic stress and decrease in production [80, 81]. To develop a good human–animal relationship, regular positive interactions between humans and animals, during or outside the feeding context, are important [74,82,83]. In this study, the artificially-reared kids experienced daily encounter with a human until weaning, including positive physical interactions during feeding such as gentle stroking, and thus received frequent and close positive contacts. In contrast, the dam-reared kids only rarely interacted with humans and usually for the imposition of aversive husbandry procedures, such as marking, veterinary intervention and moving. Increasing positive interactions with dam-reared kids could decrease their fear of humans [84], and therefore the difference in human-animal relationship quality between treatments, as shown in calves [38]. Performing this test with an unknown human would allow assessing whether the kids generalize their response toward other humans.

4.3. Disbudding and pain-related behaviour

We found only minor effects of treatment on the kids’ response to disbudding, with head-scratching tending to increase across the days in artificially-reared kids but tending to decrease more quickly across the days in dam-reared kids than in artificially-reared kids. However, none of the other pain-related behaviours differed or varied over time. The amount of head-scratching and head-shaking were similar to what has been described few hours after hot-iron disbudding with anesthesia and analgesia [85,86], therefore suggesting that the anesthesia and
analgesia procedures were effective, which might have overcome the potential beneficial effect of the dams’ presence on their kids’ pain, and therefore likely prevented us from detecting an effect of our rearing treatments. Disbudding the kids without anesthesia and analgesia might have led to different results, but would not be ethically justified as it induces highest grade pain and is prohibited in Austria.

5. Conclusion

Rearing kids with or without their mothers affected their behavioural responses to challenging situations when being individually confronted to a novel arena, novel goat and novel object tests. The kids reared with peers but without their mothers responded in a more behaviourally active way than the kids reared with their mothers, and appeared bold. Kids reared artificially also had a better human–animal relationship than kids reared with their mothers, likely due to the regular and close positive interactions with humans in early life. However, the presence of an unknown adult had the same stress-mitigating effects on the kids of both treatments, suggesting a general change in response rather than a change specific to social situations.

Declaration of Competing Interest

None.

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