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Brief communication

Positive or negative emotion induced by feeding success or failure can affect behaviors, heart rate and immunity of suckling calves

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ABSTRACT

This study investigated behavioral and physiological responses of suckling calves to either positive or negative situations. Twelve healthy female suckling Holstein calves at approximately 30 days of age were randomly selected and allocated to two groups: a) a positive situation (POS) group that received a feed reward and b) a negative situation (NEG) group that experienced food frustration. Behavior, heart rate (HR), and immune parameters were measured during the emotion-inducing periods and analyzed via ANOVA. The results indicated that the POS calves showed significantly increased locomotive play but decreased self-grooming, head shaking, and tongue-rolling behaviors as compared to the NEG counterparts ($p < .05$). The NEG calves had the highest HR ($p < .01$) after negative emotion induction. Salivary cortisol (S-CORT) level significantly increased following both positive and negative emotional induction ($p < .05$). In humoral immunity, serum concentrations of IgA were not significantly affected by either the positive or negative situation ($p > .05$), but the salivary SIgA level of the POS calves was higher than that of the NEG group ($p < .01$). Serum IL-2 and IL-3 levels in the POS calves were significantly higher than those of the NEG group ($p < .01$). However, the serum TNF-α level in the POS group was significantly lower than in the NEG group ($p < .05$). The results suggest that hunger satiation or food frustration may induce different behavioral and physiological responses in suckling calves, presenting an interesting set of reactions appropriate in response to the emotional situations elicited by this short-term stimulus

1. Introduction

Emotion, by definition, is a short-term psychological state triggered by an event or stimulus [1]. Emotion is an adaptive program designed through repeated encounters with situations, that are intended to either direct other physiological programs or to directly solve adaptive problems faced by a species over time [2]. Thus, evaluation of an animals' emotional state has been scientifically challenging due to its subjective nature [3]. For most mammals, physiological functions and brain chemical compositions are like those of human beings, and they can also express negative or positive emotion; therefore, esearch interest on animal emotion has increased in recent years [4]. Animals presumed to be experiencing positive emotion show a higher play and affinitive behaviors such as exploratory and licking behavior [2]. Animals presumed to be experiencing negative emotion or fear have been shown to display shivering, squealing, attempts to escape, frequently defecation and urination, or other behavioral phenomena, as the result of social isolation or unpredictable environmental disturbances [5]. When calves were fed concentrate feed and brushed, they became calm and rarely showed turning, self-grooming, and vocalizing behaviors; however, those behaviors occurred more often when calves were being frustrated [6].

Heart rate (HR) is one of the main physiological indicators of emotional response [7]. It has been reported that the food frustration treatment elicited a higher HR compared to both standard feed and concentrate feed treatments in cows [8]. However, when positive emotions were induced by stroking, cortisol (CORT) levels of dairy cows were reduced during stressful procedures [9]. Salivary CORT(S-CORT) is associated with emotional situations [10], which in turn is associated with immune functions. An increase in secretory immunoglobulin A (SIgA) and a decrease in S-CORT can be induced by pleasant emotion [11,12]. Evidence suggests that negative emotions

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trigger decrease of several cytokines such as interleukins (IL) 2, 3 and induce the secretion of tumor necrosis factor alpha (TNF-α) [13].

Emotion originates from the internal mental state of an individual in reaction to various stimuli, situation judgments, and expected responses [14]. Different stimuli, such as anticipation of food reward or gentle handling are presumed to be positive and have been used to induce positive situations in cattle [6]. Therefore, calves' feeding success or failure can be used as a stimulation to induce either positive or negative situations, respectively [15]. A deeper understanding is required of physiological changes, immune reaction, and behavioral expressions under specific conditions with respect to negative or positive situations induced by either food frustration or feeding success. This study induced positive or negative situations in calves by controlling success or failure to obtain food and quantified the associated behavioral, physiological, and immune responses of the animals.

2. Materials and methods

2.1. Animals and management

The experiments were conducted in a commercial dairy farm from June to November 2014. Management of the experiment complied with the guidelines of the Institutional Animal Care and Use Committee of Northeast Agriculture University. All calves were fed colostrum at postnatal 4 h and a one day later they were deprived from the dams, grouped in a herd of six calves received the same feeding regimes. The pens (36 m^2: 6.0 m (L) × 6.0 m (W) × 0.8 m (H)) consisted of an area with rice hull bedding (30 m^2) and a feeding area (6 m^2) with a solid concrete floor. Six stainless steel buckets were used to feed calves in each pen. The calves were fed at 07:30 am and 16:30 pm each day. The amount of milk fed to the calves was adjusted as follows: 6 L per day for 0–2 week of age; 8 L per day for weeks 3–4; 10 L per day for weeks 5–7. From the 4th day of age, the calves received starter feed (CP of 20%, energy level of 1.66 mcal/kg, crude fiber of 12%, crude ash of 10%, and lysine of 0.05%) and water ad libitum.

2.2. Emotion induction

Twelve female suckling Holstein calves of about one-month of age were randomly selected from 12 pens balanced for mean body weight of 63.34 ± 2.26 kg and divided into two groups(two pens): an induced positive situation (POS) group (N = 6 per pen) and an induced negative situation (NEG) group (N = 6 per pen). They were cared by two skilled technicians.

For emotional induction, individual calves were brought into a testing pen that was the same in structure as their own housing pens. During the testing, calves were exposed to one of two stimulation situations: a) feeding succeeded – POS, or b) feeding failed – NEG. To avoid potential stress induced by handling in the test, all the calves to be tested were individually brought into the six testing pens each day in two groups during the evening feeding time (16:00–16:30 pm) after they were regrouped until the day prior to the test (6 days of habituation), and their morning feeding was conducted in their own pens at 07:30 am. Emotion induction test was conducted for two consecutive days between 16:00–17:00 pm in a hunger state about 8 h after the first feeding at 07:30 am. On day one, six calves, three from the POS group and three from the NEG group were tested at the same time in six testing pens. All the behavioral and physiological parameters were measured from those six calves. On day two, same testing procedure was followed on the remaining six calves (three from the POS group and three from the NEG group) in exactly the same way as conducted on day one, recording all the behavioral and physiological parameters.

In the positive situation test, the calves in POS group were brought in the testing pens, fed and stayed there for about 60 min (16:00–17:00 pm) during which all measurements were made. Measurements of HR, saliva and blood parameters were conducted between 16:00–16:20 pm before the evening feeding (prior to POS test) –HR, saliva sampling, followed by blood sampling. At about 16:20 pm, the calves were fed with the buckets until they finished feeding (16:20–16:30 pm), HR measurements were taken during 16:25–16:30 pm; then saliva and blood samples were collected 5 min later – saliva sampling first, followed by blood sampling during 16:30–16:35 pm. 5 min after the blood sampling, the behavioral measurements were taken during 16:40–16:55 pm (15 min). To avoid disturbing the calves, which might lead to temporary stress during the saliva and blood sampling, all calves were fed and handled by the same two technicians during all physiological parameter measurement.

In the negative situation test, same as the POS group, the NEG calves were measured for all parameters (HR, saliva, and blood) before the evening feeding (16:00–16: 20 pm). At about 16:20 pm the feed buckets were brought in. The buckets had a cover containing 16 holes (diameter of 0.3 cm) to restrain the calves from feeding during 16:20–17:00 pm (same as for the POS test period). HR, saliva, blood samples, and behavior of the calves were collected, following the identical procedures as with the POS group testing. Once the NEG calves finished their test, they were allowed to feed (receiving the same amount of milk as the POS calves).

2.3. Behavioral sampling, HR, saliva, and blood measurement

For behavioral sampling, video camera (model: JX-8702, Jixingweishi, Taiwan,) were affixed on a post above each pen, allowing to record all behavioral activities of the calf during the observation days. During the testing period, the behavior sampling was conducted after the POS or NEG stimulation applied to the calves as described above for 15 min, and all occurrences of those behaviors such as self-grooming, exploring, and locomotive play [16], head shaking [17] and tongue-rolling [18] were recorded by an experienced experimenter, and they were regarded as event behaviors represented as frequency (n).

HR was measured with a heart rate sensor (Infineon Technology Co., Ltd. Model CS010, Tongxiang, Zhejiang Province, CHINA, 297 × 32 × 12 mm, 41.4 g), which was a blue-tooth belt tied around the forearm armpit. To ensure the accuracy of recording, all tested calves were trained to wear the belts for 20 min each time during evening feeding for two weeks before the test to achieve habituation. During the test period, HR was measured twice: one measurement was conducted prior to the emotion induction in calm surroundings (prior to the evening feeding); the other was conducted during either POS emotion induction or NEG emotion induction. A three consecutive readings from any records collected during the measurement was taken randomly, and the mean of the 3 readings was obtained and regarded as the HR value for that measurement period. By doing so, we could obtain all HR measurements of the calves before and during the POS or NEG situation.

Saliva and blood samples were obtained twice on each observation day, with one sample collected prior to the emotion induction and the other during the emotion induction as described above. The methods for collecting saliva and blood samples followed those described by Chacón et al. [19]. The contents of S-CORT, SIgA in saliva and the contents of serum IgA, IL-2, IL-3, and TNF-α samples were determined by using commercial ELISA test kits (Shanghai Jinma laboratory equipment Corporation., Ltd., Shanghai, China, LOT 201410), and the measuring procedures followed the manufacturer's instructions.

2.4. Statistical analysis

The data were processed with the Software Package of Statistical System Version 17.0 (SPSS, International Business Machines Corp, IBM). Behavioral, physiological, and immune parameters were analyzed with ANOVA. All behavioral parameters were examined for the normality of data before analysis, and none of all data was normally distributed, thus no data transformation was needed. The analysis model is as follows: $Yi = \mu + \alpha i + e$, where: Yi- individual observations,