TEMPERAMENT, BEHAVIOR, BODY TEMPERATURE, HEART AND RESPIRATORY RATE OF SHEEP TRAINED WITH TACTILE STIMULATION AND HALTER WALKING

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ABSTRACT: This study aimed to evaluate the influence of tactile stimulation and halter walking trainings in the temperament, physiology, behavior and body weight of sheep. A total of 80 sheep, males and females, of the Morada Nova (MN) and Santa Ines (SI) breeds, with initial ages of six to eight months, were trained with tactile stimulation (TS), halter walking (HW) or tactile stimulation and halter walking (TS+HW). The control (C) animals were not submitted to any kind of training. The behavioral aspects were measured by application of a open-field test, to quantify the traits movement, vocalization, escape attempt and digging, and the temperament test, with assignment of a temperament score for each animal. The animals were weighed and rectal temperature, heart rate and respiratory rate were measured. Males were more reactive than females to human. Animals trained with TS were the least reactive in the temperament test, while the most reactive animals were SI male from C and TS+HW treatments. Males of treatments C and HW were the heaviest during the study. The animals in the control group showed higher averages for heart rate and rectal temperature, while animals trained with TS and HW showed the lowest values. Females MN trained with TS and TS+HW moved more during the open-field test, while the SI females of the control group moved less. The SI animals vocalized more during the open-field test, both from treatment C and from TS+HW. The MN animals from C treatment vocalized less. Sheep trained with tactile stimulation were more docile. The training with tactile stimulation and halter walking in Santa Ines and Morada Nova sheep influenced the heart rate, rectal temperature, moviment, vocalization, escape attempt and body weight.

Key words: morada nova breed, open-field test, reactivity, santa ines breed

TEMPERAMENTO, COMPORTAMENTO, TEMPERATURA CORPORAL, FREQUÊNCIA CARDÍACA E RESPIRATÓRIA DE OVINOS TREINADOS COM ESTÍMULO TÁTIL E DESLOCAMENTO COM AUXÍLIO DE CABRESTO.

RESUMO: O objetivo desse estudo foi avaliar a influência dos treinamentos com estímulo tátil e deslocamento com auxílio de cabresto no temperamento, fisiologia, comportamento e peso corporal de ovinos. Um total de 80 ovinos, machos e fêmeas, das raças Morada Nova (MN) e Santa Inês (SI), com idade inicial de seis a oito meses, foram treinados com estímulo tátil (TS), deslocamento com auxílio de cabresto (HW) ou estímulo tátil e deslocamento com auxílio de cabresto (TS+HW). Os animais do grupo controle (C) não foram submetidos a nenhum tipo de treinamento. Os aspectos comportamentais foram mensurados no teste de campo aberto, para quantificar as variáveis movimentação, vocalização, tentativa de fuga e cavar chão, enquanto no teste de temperamento foram dados escores de temperamento para cada animal. Os animais foram pesados e foram aferidas as temperaturas retais, frequências cardíacas e respiratórias. Os machos foram mais reativos ao humano do que as fêmeas. Os animais treinados com TS foram menos reativos no teste de temperamento, enquanto os animais mais reativos eram machos SI dos tratamentos C e TS+HW. Machos dos tratamentos C e HW foram os mais pesados durante o estudo. Os animais do grupo controle mostraram maiores médias para frequência cardíaca e temperatura retal, enquanto animais treinados com TS e HW mostraram os menores valores. Fêmeas MN treinadas com TS e TS+HW se movimentaram mais durante o teste de campo aberto, enquanto fêmeas SI do grupo controle se movimentaram menos. Os animais da raça SI vocalizaram mais durante o teste de campo aberto, tanto do tratamento C quanto do TS+HW. Os animais da raça MN do grupo C vocalizaram menos. Ovinos treinados com estímulo tátil se mostraram mais dóceis. O treinamento com estímulo tátil e deslocamento com auxílio de cabresto em ovinos das raças Morada Nova e Santa Inês influenciou a frequência cardíaca, a temperatura retal, a movimentação, a vocalização, a tentativa de fuga e o peso corporal.

Palavras-chave: raça morada nova, teste de campo aberto, reatividade, raça santa inês

INTRODUCTION

The study of cognitive processes related to emotional states in livestock has great potential to advance and improve our understanding of animal welfare (Baciadonna and McElligott, 2015). Individual variation in animal behavior is often interpreted in terms of temperament (Beausoleil et al., 2012) and the reactivity to humans could be considered as a temperament trait (Lansade and Bouissou, 2008).

Temperament has often been restricted to express the way in which individuals perceive and react to fear-eliciting events. However, fear not only includes the subjective feeling of terror, it is also associated with changes in heart rate, raised blood pressure and increased tendency for fleeing or freezing behavior (Baciadonna and McElligott, 2015). Stressful interactions where cognition is impaired may compromise the welfare of sheep in the short term, and also increase the risk of injury to the animals when they are panic. In the longer term, experiences of repeated negative handling and repeated exposure to fear-inducing stimuli can be remembered by sheep and may influence future behaviour making them more reactive and harder to move and handle (Ferguson et al. 2017).

In an "open-field" test, an animal is released into an open area for a period of time during which its behavior is observed (Mintline et al., 2012). The expression of behaviors reflects the integration of various motivational states experienced by the individual during the test (fear, social motivation, curiosity) and the relative influence of each motivational state of the behavior can vary among individuals (Beausoleil et al., 2008).

Most veterinary and handle procedures are seen as aversive by the animals, so identifying effective strategies to induce positive emotions and taking into account animals' individual characteristics could promote animal welfare (Baciadonna et al., 2016). Earlier studies have focused on tactile stimulation as a simple handle change that can improve human-animal interaction. During the tactile stimulation the animal is petted over the whole body surface, including the back legs, with standardized pressure (medium) and speed of stroking (between 40 and 60 strokes per minute) (Coulon et al., 2015).

Halter-training is another simple possibility to minimize the stress associated with animal handling (Neary et al., 2017), in addition to being a useful management in a sheep farming. However, there was no available information about relationship between the halter training and reactivity of sheep to humans. This study aimed to evaluate the influence of tactile stimulation and halter walking trainings in the temperament, physiology, behavior and body weight of sheep, trying to understand which manipulation would be better to improve farm handle.

MATERIAL AND METHODS

Animals and housing

This study was approved by the ethics committee on animal use of the Animal Science Institute (CEUA-IZ), finding number 179/2013. The experiment was conducted from May to September 2013 at the Animal Science Institute, located in Nova Odessa, state of São Paulo, Brazil (latitude 22 ° 46 '39' 'S and longitude 47 ° 17' 45 '' W). We used 80 purebred sheep (*Ovis aries*) of Morada Nova (MN) and Santa Ines (SI) breeds, which are rustic Brazilian naturalized breeds. The animals were born in this facility, were only used for the experiment, had initial ages of six to ten months at the start of experiment (born between July and November 2012) and were entire males and females. They had previous experience with humans, but only to general handles, such as feeding, weighing and veterinary treatments. We selected animals with similar body weights and ages for the experiment, but there was a random separation of the animals into the treatments. They ate corn silage and commercial concentrate and had *ad libitum* access to water.

The animals were housed at a shelter for animals with straw bedding and a feeding area with a solid concrete floor. The control animals remained separated from treatment ones and females were separated from males. Daily handles were not performed by the same stockperson who trained the animals. Before the training the animals were not used to be restrained or moved with the halter. Each day of the training period, only treatment animals were displaced to a handle corral located near the shelter, where they had no access to food or water. At the end of the daily training the animals returned to the shelter. The animals were divided into four groups of 20 animals, balanced by sex (10 males and 10 females) and breed (10 Santa Inês and 10 Morada Nova), subject only to tactile stimulation (TS); only to halter walking (HW); the two types of training combined (TS + HW) and no training (control group - C). The training of the animals was performed for 15 consecutive days.

Training

The tactile stimulation was applied to each animal daily at the handling system pens. Before the start of the experiment, the caregiver has trained to harmonize speed of stroking (40 strokes per minute) and to use a standardized medium pressure (Coulon et al., 2015). The tactile stimulation was carried out with the aid of a small brush for a period 5 minutes/animal/day. During this training, the animals remained in physical contact with the other animals of the pen, still (standing on all four legs) and were contained by the human as follows: the caregiver passed one legs over the animal's neck, so that each leg was leaning on the sides of the neck, cranial to the shoulder blade. The tactile stimulation was performed on the head, neck, back and sides, randomly.

The halter walking was conducted in a corridor beside the pens. It was carried out with an adjustable halter for 10 minutes/animal/day. During this time the caregiver walked with just one animal, back and forth in the corridor. There was no other animal in the corridor during this training, but the training animal had visual, olfactory and auditory contact with the rest of the group that was in the pen.

In the tactile stimulation plus halter walking training, the animals were subjected to the same techniques, i.e., 5 minutes/animal/day of tactile stimulation and soon after halter walking for 10 minutes/animal/day.

Tests

During the experiment the animals were weighed and submitted to open-field test, temperament testing and measurement of physiological characteristics (heart rate, respiratory rate and rectal temperature). The evaluations and measurements were performed before the training (D0), in the middle of training (D8), at the end of training (D17), 45 days after training (D62) and 90 days after training (D107).

Open-field test and temperament test were carried out in the handling system pens (tamped earth, measuring 3.25 m long by 2.50 m wide). The sides of the test area were covered with black plastic sheets to prevent viewing of other animals from the same group. The floor of the cage was subdivided with the white paint, forming rectangles 80 cm long by 1.10 m wide, with the objective of measuring the animal’s movement. The tests were performed in the same place, without water or food (the animals were not familiar with this place before the first test).

In open-field test, the animal was taken to the test site and left there for two minutes with no extra stimulus beyond physical and visual isolation. The animals were sent to the test site randomly. Each test was recorded with a video camera. Filming was always done in the same place, outside the pen, so that the animal that was being evaluated could not see the person doing the filming (the same person who performed the training and entered the pen during the temperament test). The following behavioral traits were taken into consideration:

- Movement: number of rectangles on the floor entered by the animal. Change of rectangle was defined as at least one foreleg touched the area of the rectangle (not just the rope dividing the areas). A change was also recorded when the animal touched the ground after trying to escape (after placing the forelegs on the fence surrounding the area or trying to jump over it, or when returning to the standing position after kneeling and put the head under the fence).
- Vocalization: number of times the animal bleated, regardless of its characteristic.
- Attempt escape: number of times the animal tried to escape. The animal placed the two forelegs on the fence or knelt and put the head under the fence, or when it jumped over the fence.
- Digging: number of times that the animal dug the floor with any foreleg.

The temperament test was performed immediately after the open-field test, at the same place, for 4 minutes. The woman (always the same person) who entered the pen during the temperament test was the same person who carried out the training and dressed the same clothes in all tests (green pants and shirt and black rubber boots) and was trained as an evaluator to validate the scale. She always entered the pen by the single gate, stood standing and without moving in front of the gate for three minutes. Then, for about one minute, she moved towards the animal and moved her arms slowly trying to touch and stroke the animal, ending the test. If the animal tried to invested against the evaluator (head butting), the test was ended immediately. A scale of subjective scores was used to describe the reaction of the animal to the presence and closeness of the evaluator. The temperament scores were defined as follows:

1 - Very reactive: the evaluator tried to touch the animal, but it moved back and invested against the evaluator, succeeding in head butting.

2 - Reactive: the evaluator tried to touch the animal, but it moved back and invested against the evaluator. In this case the animal did not succeed in head butting, only threatened.

3 - Slightly reactive: the evaluator tried to touch the animal, but it moved back and run away. The animal did not invest against the evaluator, just ran away.

4 – Docile: during the period the evaluator stood still and without moving, the animal did not stayed close to the evaluator (in the same or neighboring rectangle), but allowed be touched at least one time.

5 - Very docile: during the period the evaluator stood still and without moving, the animal stayed close to the evaluator (in the same or neighboring rectangle) and allowed itself to be touched continuously for at least 45 seconds.

After the temperament test, the physiological parameters of the animals were measured outside the temperament test pen. The rectal temperature was measured using a digital clinical thermometer with range up to 42 ° C, introduced directly into the animal's rectum until a beep was emitted. Heart rate was measured with phonendoscope and respiratory rate was determined by observing the breathing movements of the thorax and abdominal region. The heart and respiratory rates were measured for 15 seconds and then multiplied by four (to transform into frequency/minute). After the measurement of the physiological characteristics the animals were weighed (kg) in an electronic scale.

Statistical analysis

The statistical analysis was carried out using the SAS version 9.3 program, 2012 (SAS, 2012). Normality test was realized in all quantitative traits. The traits movement, vocalization, attempt escape and digging were normalized by a square-root transformation before statistical analyses.

We performed variance analysis (PROC GLM) to evaluate the treatment (T), sex (S) and breed (B) fixed effects with simples interactions between the effects and with the triple interaction between the effects of T, S and B at the physiological parameters (respiratory rate, heart rate and body temperature), behavior traits (movement, vocalization, attempt escape and digging) and body weight. The body weight was considered as covariate in the model. Differences among averages were compared by the Student-Newman-Keuls (SNK) test, with significance of 5% ($P<0.05$).

The categorical trait digging was showed as frequencies (PROC FREQ) and the temperament score variable was analyzed by the chi-square test ($\chi 2$) as frequency per treatment, sex and breed.

The statistical model used for continuous traits was:

$$Y_{ijkl} = \mu + T_i + S_j + B_k + TSB_{ijk} + e_{ijkl},$$

where:

Y_{ijkl} = average value observed for each evaluated trait;

μ = general average;

T_i = fixed effect of i^{th} treatment;

S_j = fixed effect of k^{th} sex;

B_k = fixed effect of l^{th} breed;

TSB_{ijk} = interaction between the fixed effects T, S, B;

e_{ijkl} = random error inherent in each observation ~ NID (0, σ^2_e).

RESULTS

During the open-field tests, 87% of animals did not manifest the digging behavior and 7% expressed only once. The same animal (M/SI/

TS) manifested this behavior 0, 1, 6, 8 and 18 times on the measuring days D0, D8, D17, D62 and D107, respectively.

The animals M/MN/C attempted to escape more times during the open-field test when compared to F/MN/C (2.6±3.4 and 3.7±4.7, respectively). F/MN/TS+HW attempted to escape more times than M/MN/TS+HW (4.4±4.7 and 2.6±3.4, respectively).

Differences between means and standard deviations (SD) of sex, breed and treatment for respiratory rate, heart rate and rectal temperature in sheep are shown in Table 1.

The weight differed due to treatments (F=8.84, P<0.0001), sexes (F=45.43, P<0.0001) and breeds (F=64.04, P<0.0001). Animals from the C and HW treatments showed similar means, larger than TS and TS + HW. The mean weight of males was higher than that of females and the SI breed had greater mean weight than the MN. The heaviest animals were males SI from C treatment and the MN females showed the lighter body weight in all treatments.

There were differences in respiratory rate

Table 1. Differences between means and standard deviations (SD) of sex, breed and treatment for body weight, respiratory rate, heart rate and rectal temperature in sheep. Different letters indicate significant differences between mean values (P<0.05).

Sex	Breed	Treatment	Body weight Mean±SD	Respiratory Rate Mean±SD	Heart Rate Mean±SD	Rectal temperature Mean±SD
Male	Morada Nova	Control	34.2±7.2^{b}	53,2±24,2^{abc}	149,8±15,1^{a}	40,2±0,4^{ab}
Male	Morada Nova	Tactile stimulation	27.1±5.4^{cde}	33,7±9,5^{bcd}	126,2±23,6^{cde}	38,9±0,4^{f}
Male	Morada Nova	Halter walking	34.7±5.8^{b}	34,6±8,4^{bcd}	117,4±18,6^{de}	39,1±0,2^{def}
Male	Morada Nova	Tactile stimulation + Halter walking	25.7±3.7^{ed}	38,2±9,1^{abcd}	123,4±21,1^{cde}	39,1±0,7^{def}
Male	Santa Ines	Control	41.2±13.2^{a}	42,0±18,8^{abcd}	144,2±20,9^{ab}	40,1±0,5^{b}
Male	Santa Ines	Tactile stimulation	29.8±9.3^{bcd}	29,6±6,1^{d}	106,6±21,3^{e}	39,1±0,5^{def}
Male	Santa Ines	Halter walking	36.9±4.3^{ab}	32,6±9,7^{cd}	115,6±20,0^{de}	39,2±0,6^{def}
Male	Santa Ines	Tactile stimulation + Halter walking	32.1±12.6^{bcd}	32±9,3^{cd}	115,4±18,0^{de}	39,0±0,5^{ef}
Female	Morada Nova	Control	22.7±4.3^{e}	58,0±31,6^{a}	144,8±12,1^{ab}	40,5±0,5^{a}
Female	Morada Nova	Tactile stimulation	23.3±3.8^{e}	43,2±17,9^{abcd}	137,0±22,8^{abc}	39,3±0,6^{def}
Female	Morada Nova	Halter walking	25.1±4.2^{de}	44,3±18,6^{abcd}	123,1±15,7^{cde}	39,1±0,6^{def}
Female	Morada Nova	Tactile stimulation + Halter walking	20.7±5.1^{e}	39,8±18,0^{abcd}	129,8±22,4^{bcd}	39,2±0,7^{def}
Female	Santa Ines	Control	30.3±4.9^{bcd}	54,6±46,2^{ab}	121,4±9,9^{cde}	39,6±0,5^{c}
Female	Santa Ines	Tactile stimulation	32.9±10.6^{bc}	41,6±22,7^{abcd}	117,0±30,4^{de}	39,5±0,4^{cde}
Female	Santa Ines	Halter walking	30.8±7.9^{bcd}	42,7±14,6^{abcd}	132,6±21^{abcd}	39,2±0,4^{def}
Female	Santa Ines	Tactile stimulation + Halter walking	31.5±4.0^{bcd}	47,2±26,6^{abcd}	115,8±16,6^{de}	39,5±0,7^{cd}

Means with the same letter are not significantly different between the different variables, at 5% in SNK test.

due to treatment (F=14.15, P<0.0001), and sexes (F=31.89, P<0.0001). The mean rate of C group was higher than for TS, TS + HW and HW. Females MN and SI from the control group showed higher respiratory rate and male SI from TS treatment showed the smaller rate.

Heart rate differed according to treatment (F=16.53, P<0.0001), and breeds (F=29.09, P<0.0001). The mean for treatment C was higher than for treatments TS, HW and TS + HW. The MN breed showed higher means than the SI breed. The heart rate of males MN from the C group were the highest and the heart rate of males SI from TS treatment were the lowest.

There were differences in rectal temperature between treatments (F=67.45, P<0.0001), and sexes (F=16.07, P<0.0001). The mean was highest for treatment C and females had higher mean than males. Females of the MN breed from the C group showed the highest rectal temperature, but males of the MN breed from the TS treatment showed the lowest temperature.

Differences between means and standard deviations (SD) of sex, breed and treatment for movement, vocalization and temperament in sheep are shown in Table 2.

There was difference for movement (number of entered rectangles during the open-field test) between sexes (F=10.52, P=0.001) and breeds (F=13.38, P=0.0003). Females presented higher movement means than males and the MN breed showed higher mean than the SI breed. Females MN, from all treatments moved more and females SI from the C group moved less.

There were differences for vocalization (number of vocalizations during the open-field test) between treatments (F=5.37, P=0.001), and breeds (F=95.23, P<0.0001). The TS + HW treatment showed highest mean, while the TS and C treatments had the lowest. The SI breed showed higher mean vocalization than MN.

The temperament test determines that higher scores (4 and 5) are given to docile animals, while animals with low scores (1 and 2) showed reactive behaviors. The results for temperament showed that it was different between treatments ($\chi 2$=30.5, DF=12, P=0.0023) and sex ($\chi 2$=50.49, DF=4, P<0.0001), but the was no difference for breed ($\chi 2$=7.22, DF=4, P=0.12). Females and males of Santa Ines breed trained with TS were more docile, while the most reactive animals were male Santa Ines from the control group.

For females, the percentage in the categories 1 (very reactive), 2 (reactive), 3 (slightly reactive), 4 (docile) and 5 (very docile) were respectively 0, 0, 9.6, 26.4 and 14.13%. The temperament score docile was more frequent in females (26.4%). On the other hand, for males, the percentage in the categories 1, 2, 3, 4 and 5 were respectively 8, 2.13, 4.53, 18.67 and 16.53. The temperament score very reactive was more frequent in males (8%).

The temperament scores docile and very docile were more frequent in HW (13.87%) and TS + HW (10.93), respectively. These scores were less frequent in C treatment (9.33% and 4.8%, respectively). The temperament scores reactive and slightly reactive were more frequent in HW (1.6% and 4%, respectively). These scores were less frequent in TS and TS + HW (0%, 3.20% for both, respectively).

DISCUSSION

In sheep production systems, the human-animal relationship should be optimized since negative interactions can bring unpleasant consequences for both humans and sheep (e.g. damage the health, decrease welfare, animal productivity and human satisfaction about the environment work). The temperament evaluation of sheep through specific tests enables knowledge of animal behavior towards humans and subsequent selection of individuals easier to handle, that do not cause injury to humans or other animals and that do not cause damage to the livestock facilities. It is desirable that sheep are not reactive to humans. However, handling of extremely docile animals can be slow and difficult, as they may not respond to human commands.

The method of handling sheep can affect sheep behaviour at short and long term (Dodd et al., 2014). This statement was confirmed in our study, where sheep trained with tactile stimulation showed higher temperament scores (more docile), which is a positive result. The presumably positive situation of being gentle human tactile stimulated has a calming effect (Coulon et al., 2015) and induces an optimistic-like judgment (Destrez et al., 2014). This may reflect positive emotions during stroking because if the sheep were simple habituated

Table 2. Differences between means and standard deviations (SD) of sex, breed and treatment for movement, vocalization and temperament in sheep. Different letters indicate significant differences between mean values (P<0.05).

Sex	Breed	Treatment	Moviment Mean±SD	Vocalization Mean±SD	Temperament Mean±SD
Male	Morada Nova	Control	40,1±17,6[ab]	6,8±5,4[e]	3,3±1,5[abc]
Male	Morada Nova	Tactile stimulation	38,7±10,0[ab]	14,0±7,6[bcd]	3,4±1,7[abc]
Male	Morada Nova	Halter walking	33,2±15,8[ab]	12,2±7,9[cde]	3,6±1,5[abc]
Male	Morada Nova	Tactile stimulation + Halter walking	38,7±24,8[ab]	15,2±5,9[bcd]	4,4±0,7[ab]
Male	Santa Ines	Control	39,8±18,2[ab]	24,5±6,7[a]	3,2±1,6[c]
Male	Santa Ines	Tactile stimulation	32,1±13,2[ab]	17,5±9,6[abc]	4,5±1,0[a]
Male	Santa Ines	Halter walking	43,2±8,8[ab]	17,8±7,4[abc]	3,5±1,3[abc]
Male	Santa Ines	Tactile stimulation + Halter walking	36,6±14,4[ab]	22,3±5,8[a]	3,3±1,8[bc]
Female	Morada Nova	Control	45,7±21,8[ab]	9,5±4,8[de]	3,9±0,8[abc]
Female	Morada Nova	Tactile stimulation	49,1±17,9[a]	13,0±6,2[cde]	4,0±0,6[abc]
Female	Morada Nova	Halter walking	45,9±21,4[ab]	17,8±4,8[abc]	4,0±0,5[abc]
Female	Morada Nova	Tactile stimulation + Halter walking	51,0±21,9[a]	15,0±6,6[bcd]	4,3±0,7[abc]
Female	Santa Ines	Control	29,7±20,8[b]	17,9±8,1[abc]	4,1±0,7[abc]
Female	Santa Ines	Tactile stimulation	34,2±18,2[ab]	20,4±13,8[ab]	4,5±0,7[a]
Female	Santa Ines	Halter walking	42,3±12,8[ab]	21,1±8,9[ab]	3,9±0,6[abc]
Female	Santa Ines	Tactile stimulation + Halter walking	39,3±18,8[ab]	23,9±8,6[a]	4,3±0,8[abc]

Means with the same letter are not significantly different between the different variables, at 5% in SNK test.

to repeated human presence and contact, they should stop react or react only slightly to tactile stimulation (Coulon et al., 2015).

In this study females never showed temperament score 1 (very reactive) or 2 (reactive), which indicates that females are less reactive to humans. Less fearful animals, which are easier to handle, produced better meat quality, so an economic benefit can be obtained, mainly through quality seal programs (Probst et al., 2012).

On the first temperament test the males invested against the human (1 and 2 scores) at the beginning of the test. In the fourth and fifth tests (last tests), some males began rubbing the head on the human's leg and then began to move back and try to head-butt the human. (Reefmann et al., 2009a) presumed that some attention-seeking behaviour at the evaluater (e.g. establishing body contact and placing their heads under her hand) was an attempt to solicit stroking.

After being subjected to stressful stimuli, such as open-field and temperament tests, sheep can show variations in measurements of physiological parameters, such as heart rate and body temperature. These variations are proportional to the intensity of the stimulus, but are often of short duration (Reefmann et al., 2009b).

The reference values of adult sheep for heart rate are 90-115 beats per minute and for rectal temperatures are 38.5-40°C (Feitosa, 2008). The average heart rate and rectal temperature can rise due to fever, exercise or environmental temperature (Feitosa, 2008). In this study, sheep at C treatment showed higher mean values for heart rate and rectal temperature, while animals at TS and HW treatments showed the lowest averages. This result agrees with those obtained by (Coulon et al., 2015), where lambs stroked showed slower heart rate and it can be seen that they experienced positive emotions during stroking.

In our study the females MN in the TS and TS + HW treatments showed the highest moviment average during the open-field test, whereas females SI of the C treatment showed the smallest ones. Our findings were opposite to those described by (Coulon et al., 2015), where stroked sheep moved less during open-field test, which is a sign of positive calming effect. Increased movement in a test area can be due to negative emotional states (Reefmann et al., 2009b). The breed can also influence the movement, since some breeds displays an active coping mechanism (high levels of locomotion, high-pitched bleats and escape attempts) and others a passive mechanism (immobilization, quiet bleating and retreat from stimuli) (Dodd et al., 2014).

The SI animals vocalized more, both from treatment C and from TS + HW, while MN breed from C treatment vocalized less. In gregarious animals, such as sheep, vocalization can express distress or can be a way of maintain social contact with other flock members at a distance (Ligout et al., 2011).

The digging behaviour was showed by the same animal (M/SI/TS) 0, 1, 6, 8 and 18 times on the measuring days D0, D8, D17, D62 and D107, respectively. We found no reference in the literature about digging behaviour related to open-field test, only describing foraging or agonistic behaviours (Blackshaw and Wash, 1986). However, it is interesting to note that this behaviour always occurred in front of the gate during the open-field test, which may reflect social motivation (attempt to rejoin the flock). Another interesting observation is related to the average temperament score of that animal, which was 1.4 and it means he was a very reactive animal towards human.

As expected, males were heavier than females and SI was heavier than MN breed. The serum testosterone level, the male sex hormone, is positively correlated with body weight gains of sheep (Karakuş et al., 2017). The SI breed had the highest average body weight, because it has larger body size and percentage of bone than the MN breed (Araújo Filho et al., 2010).

During the displacement of the animals we observed that sheep from control group were more agitated than the animals from the others treatments and they were more difficult to handle. This agitation may have been due to the lack of habituation to the handling, which can be avoided with more frequent displacements to the handling system by the shepherd, without invasive procedures, only to inspect the sheep health, for example. Manipulated animals are easier to handle, since they are less afraid of humans (Becker and Lobato, 1997).

The use of black plastic to cover the sides of the test area was not good, as some animals jumped on the plastic, tearing it. Moreover, strong wind in contact with the plastic produced a sound that often frightened the animals. Thus, it could be interesting to use a stronger material to cover the sides of the test area.

As cited above, during the temperament test some males became more reactive during the measurement days, showing more head-butting behaviour. This is potentially dangerous for both the evaluator and the animal. Therefore, it would be interesting to develop another type of sheep temperament test, in which the human does not need to have direct contact with the sheep.

CONCLUSION

The training with tactile stimulation and halter walking in Santa Ines and Morada Nova sheep influenced the heart rate, rectal temperature, moviment, vocalization, escape attempt and body weight. The results also indicated that sheep trained with tactile stimulation showed higher temperament scores, so they were more docile. This suggests the importance of positive interactions with sheep in order to improve welfare of both humans and animals.

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