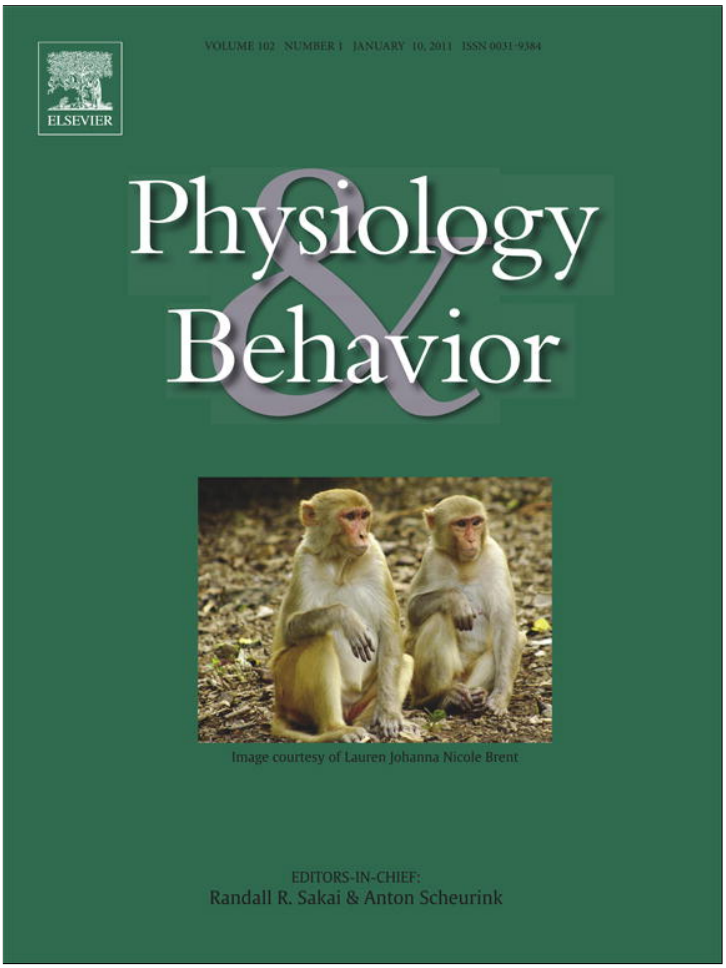

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Development of affinity to the stockperson in lambs from two breeds

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ABSTRACT

The interactions between genetic and environmental factors on the development of lamb affinity to humans was assessed on 48 animals from two breeds (Gentile di Puglia and Comisana) and two treatments (Gentled and Not Gentled) producing 4 groups (GPg, GPng, Cg, Cng, respectively). The Gentile di Puglia and Comisana gentled animals were subjected to a period of training consisting of gently handling each lamb for 5 min three times a day for the first week and then twice a week for three additional weeks. The gentling procedure included both tactile and visual/auditory interactions. At 30–32 days of age lambs were subjected to three arena tests conducted in a novel environment: i) isolation test (each animal was exposed to a novel environment, and isolated from tactile and visual contact with conspecifics for 5 min), ii) stationary human test (as previously but a human sitting in a corner of the pen), and iii) pen-mates' test (each animal was tested in the presence of 2 pen-mates whose behaviour was not recorded). During the 13 training sessions lambs exhibited an increasing number of contacts with the human and a decreasing number of bleats ($P<0.001$), although Gentile di Puglia lambs interacted more with the human ($P<0.001$) and tended to bleat less ($P<0.10$) than Comisana lambs. Lambs vocalised more and climbed more when tested in isolation as compared with lambs tested either with the human or with the pen-mates ($P<0.01$) and they vocalised more in the presence of the human than with the pen-mates ($P<0.001$). A higher number of contacts with the human stimulus was observed in gentled animals ($P<0.05$). However, differences between gentled and not gentled animals were only significant in Gentile di Puglia subjects ($P<0.01$). GPng lambs displayed the longest ambulatory activity during the isolation test ($P<0.05$) and exhibited more climbing attempts during the isolation test as compared with the human or the pen-mates' tests ($P<0.001$). A higher cortisol level was shown by GPng lambs in comparison with Cng subjects during the stationary human test ($P<0.01$), whereas no differences were detected between the two gentled groups. Gentling determined an improvement of the quality of human animal relationship in more reactive breeds such as Gentile di Puglia sheep.

1. Introduction

There are different forms of relationship between humans and animals. For wild animals a predator (human)/prey (animal) relation type is prevalent [1]. In fact, wild animals actively avoid human contacts as a consequence of their fear. Although in farmed animals the selection pressure over the animal fear of humans may have been reduced, some degree of fearfulness is still detectable. However, human animal relationship, as with any other behavioural trait, can be affected by previous experiences [2], with high learning abilities expressed by young animals [3]. Both associative and non-associative mechanisms have been proposed to explain their learning: habituation can reduce the fear response of farm animals to humans, but animals can also learn to associate humans with either rewards or punishments [4]. Numerous studies have been conducted where feeding has been used as a form of reward [5,6]. In particular, lambs are very sensitive to feeding, as it plays an important role in the development of mother–young bond [7]. Other studies focussed on the possibility to use humans as social support [5,8] and reported the possible formation of selective emotional bond (attachment) between the lamb and the caregiver. They highlighted the role of tactile stimulations for developing such emotional bond [9,10].

Although differences in behavioural reactivity can arise through domestication and husbandry, these differences may be also generated by selection for production traits with different farm breeds exhibiting genetic variability of biological stress responses [11]. In pigs, considerable differences between breeds have been described both in basal cortisol levels and after stress [11]. Breed differences in responses to people have been found in cattle [12,13] and sheep [14,15]. Due to its genetic basis, the level of fear of people may also be a function of the intensity of human selection, with higher levels of fear in less selected breeds. Romanov sheep extensively reared with little contact with

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humans exhibited greater flight distance from humans than intensively reared Laucane sheep [16]. Domestication is still occurring and domestic animals are still evolving to the presence of humans [17]. Therefore, it would be useful to assess the affinity of different breeds for humans or their sensitivity to management strategies aimed at improving affinity for humans.

In the present study two different breeds were chosen in relation to the intensity of human selection: Gentile di Puglia, a multipurpose sheep breed indigenous of the rural areas of Southern Italy where it is extensively reared, and Comisana, one of the most important Mediterranean dairy sheep originated in the Southeastern Sicily and either intensively or extensively reared. The experiment was conducted in conditions similar to those of a commercial farm, where lambs are trained to suck in the first days of life by the stockperson and then fed milk substitute from buckets. The aim was to assess the effect of the breed on the development of lamb affinity towards humans. We hypothesised that a breed such as Comisana would be more prepared to cope with an artificial environment, represented by social separation (e.g. from the dam) and human proximity, than Gentile di Puglia. Therefore, the former may be less sensitive to social disturbance, such as isolation, and less sensitive to additional positive humsan contact.

2. Material and methods

All procedures were conducted according to the guidelines of the Council Directive 86/609/EEC of 24 November 1986 on the protection of animals used for experimental and other scientific purposes [18].

2.1. Experimental design

The timeline of the experiment is depicted in Fig. 1. The experiment lasted 5 weeks and involved 48 lambs. Their mothers were all housed in the same experimental farm located in Puglia and subjected to the same management routine and rearing conditions. Lambs were divided into four groups of 12 subjects according to a 2×2 factorial design, with two breeds (Gentile di Puglia and Comisana) and two treatments (Gentled and Not Gentled). Each group was divided into two subgroups consisting of six animals. Subgroups were housed separately in 3 m×8 m straw bedded pens in the same building. Gentled and not gentled groups were housed in pens bounded by straw bales up to a height of 2 m to avoid visual contact between groups. Groups and subgroups were balanced for age, weight and sex of lambs. All the lambs were artificially reared: maintained with their dams 24–30 h after parturition to receive maternal colostrum and then separated from them, and offered a milk substitute. Milk replacer was available from two 10-l buckets fitted with six 50-mm long latex teats. Lambs were taught to suck from buckets by a stockperson. He caught each lamb between his legs, helped it to approach the artificial teat and trained it for 10 min a day to suck from the nipple until the animal was able to find the teat and to suck by itself. This occurred within the first 3 days of life. The lambs had free access to milk, which was administered twice daily (08:00 h and18:00 h). During the trial, the amount of milk given to the test animals was adjusted so that refusals would be less than 5% of the volume offered.

Immediately after separation, the Gentile di Puglia and Comisana gentled animals (Groups GPg and Cg, respectively) were subjected to a further period of training consisting of gently handling each lamb for 5 min three times a day for the first week of life and then twice a week for three additional weeks, as performed by Tallet et al. [10]. Two stockpersons (males, green dressed) with previous experience of lamb gentling were involved in the gentling treatment: for each session of treatment, one of the two stockpersons (each stockperson gentled one subgroup for each breed) carried out the treatment in each pen. The gentling procedure included both tactile and visual/auditory interactions. In particular, the stockperson entered the pen with slow deliberate movement, sat down and caught each lamb between his legs, and gently stroked the animal, with his hand resting on the animal while talking to it. In each subgroup of Gentile di Puglia and Comisana lambs the gentling procedure was performed always by the same stockperson to facilitate the development of the affinity with humans. Each of the two stockpersons carried out the gentling procedure in one subgroup within the same breed. During the gentling procedures (5 min) the number of bleats emitted by the treated animal and the number of contacts of the other members of the subgroup with the stockperson were recorded (27 recordings were performed for each animal in 13 days). The Gentile di Puglia and Comisana lambs in the groups not subjected to the gentling treatment (Groups GPng and Cng, respectively) were not in view of the humans during the gentling procedure and received minimal contact with stockpersons, except for routine management and testing procedures, which were consistent for all the experimental groups. Stockpersons involved in management and testing procedures were different from the stockpersons performing the gentling treatment and differently dressed (males, brown dressed), in order to avoid any confusion between response to gentling and response to humans in general. The training of artificially reared lambs to suckle from buckets was performed by the same stockpersons involved in the gentling treatment. In particular, animals from each

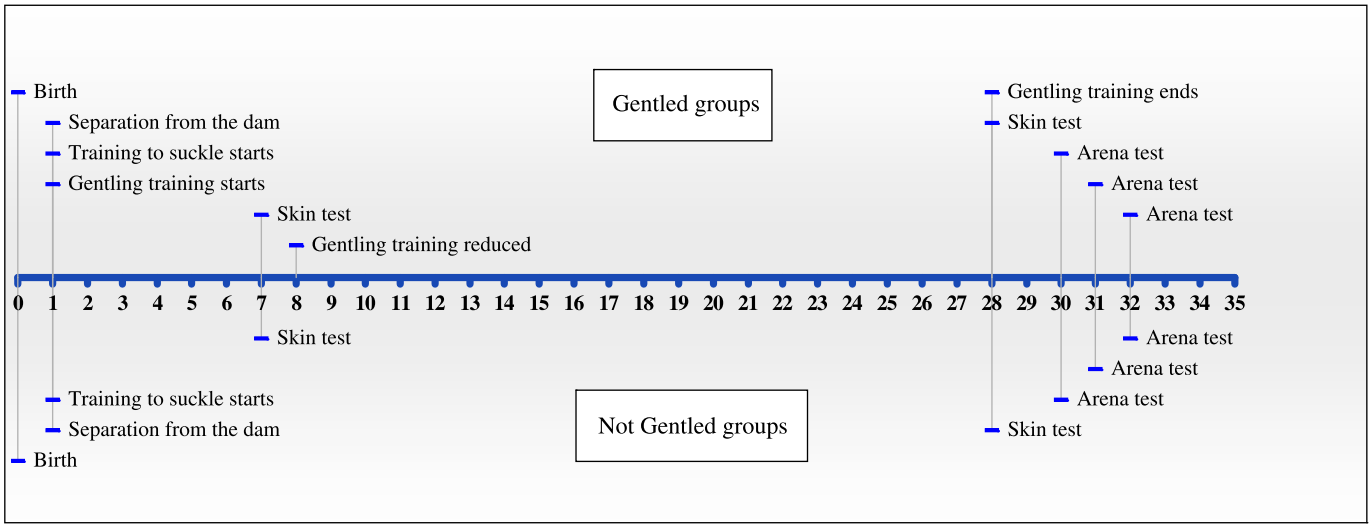

Fig. 1. Timeline of the experiment.

gentled subgroups were trained to suckle and gentled by the same person.

2.2. Arena testing and cortisol level determination

At 30–32 days of age, once the gentling treatment was terminated, lambs were subjected to three arena tests conducted in the same novel environment (a 4 m × 4 m pen). The three tests performed were: i) isolation test (each animal was exposed to a novel environment, and isolated from tactile and visual contact with conspecifics for 5 min during which the lamb could still receive auditory and olfactory stimuli from them), ii) stationary human test (as the previous one but a human sat in a corner of the pen), and iii) pen-mates test (each animal was tested in the presence of 2 familiar lambs from the same pen whose behaviour was not recorded; the three lambs were introduced into the box at the same time). The order of presentation of the animals to the three tests was randomised across groups, which means that all groups were exposed to each test in each position (first, second, third) with the same frequency. Lambs were subjected to the three tests at 1-d intervals. The tests were remote-video-recorded. In each test latency time till first movement, total duration of movement, investigative activity (walking slowly, often interrupted by stopping, with the head lowered while sniffing the ground) and the number of bleats and climbing attempts were recorded. During the test performed with a stationary human the time spent in proximity (within 1 m) of and the number of times the lambs came into contact with the human stimulus were also recorded (see Caroprese et al. for more details [19]).

Blood samples (10 ml) were collected in vacuum tubes from the jugular vein immediately before each test (i.e. isolation test, stationary test and pen-mates test) immediately after the tests and 60 min after to evaluate cortisol concentrations. Animals were caught and handled trying to keep disturbance to a minimum. As performed in previous studies [20], hormone concentration was determined by a competitive enzyme immunoassay kit for cortisol determination (Radim, Italy). Validation for ovine plasma was performed as described by Fisher et al. [21]. Samples were tested in duplicates. Aspecific binding, as monitored through blank samples, and sample to sample carry over were negligible. All samples from the same subject were run in the same assay. The sensitivity of the assay was 5 ng/mL. The inter- and intra-assay variation coefficients were 7.0 and 5.6%, respectively.

2.3. Immune response

At 7 and 28 days of age, 500 μg of PHA (phytohaemagglutinin, Sigma Aldrich-Italia, Milan, Italy) dissolved in 500 μl of sterile saline solution were injected intra-dermally into the centre of a 2 cm wide circle marked on shaved skin in the upper side of each shoulder. Skinfold thickness was determined before PHA injection and after 24 h with a calliper by the stockpersons involved in testing procedures. The average increase in skinfold thickness (24 h post-injection thickness - pre-injection thickness) of each animal was computed using the two measurements [22].

2.4. Weight gains

The mean weight of the lambs at the beginning of the trial was 4.26 ± 0.28 kg (mean ± sd). All the animals were weighed at the beginning, at d 7, 21 and at the end of the trial (d 28) using an electronic scale by the stockpersons involved in testing procedures.

2.5. Statistical analysis

All the variables were tested for normal distribution using the Shapiro–Wilk test. All the data were analysed using ANOVA for mixed models, having the lamb as a random factor nested in the treatment. Data collected during the gentling procedure were analysed having the breed, the time of sampling and their interactions as fixed effects. Behavioural responses to the Isolation test, to the Stationary Human test, and to the Pen-mates' test were analysed using the breed, the treatment, the type of test, and their interactions as fixed effects. Cortisol levels were analysed using the breed, the treatment, the type of test, the time of blood sampling (5 and 60 min from test onset) and their interactions as fixed effects, whereas pre-test blood samples (0 min from test onset) were used as covariate. Weight gains and cell-mediated immune response data were analyzed having treatment, breed, time of sampling, and their interactions as fixed effects. When significant effects were found (at $P<0.05$, unless otherwise noted), the LSD test was used to locate significant differences between means. Comparisons were performed either within factors, if their effects were significant, or between factors, if interactions were significant.

3. Results and discussion

3.1. Behavioural response to gentling treatment during training

Fig. 2 displays the number of bleats and the number of contacts performed by lambs during the 13 training sessions. For the first week the means of three daily treatments are reported. In gentled animals a significant effect of time was observed for the number of bleats and the number of contacts with the human ($P<0.001$). The latter was also affected by the interaction time x genotype, with values at 12 and at 13 d higher in Gentile di Puglia than in Comisana lambs (4.46 ± 0.97 vs 0.97 ± 0.93 at 12 d and 10.08 ± 0.97 vs 1.23 ± 0.97 at 13 d in Gentile di Puglia and in Comisana lambs, respectively; $P<0.001$), whereas the number of bleats only tended to be affected ($P<0.10$). The number of bleats decreased soon after the beginning of the gentling treatment: two sessions were able to markedly reduce the level of vocalization (differences between sessions 1 and 2 and all the other sessions were significant: $P<0.001$), thus indicating the effectiveness of the treatment in reducing the degree of fearfulness of lambs towards humans as a result of either habituation or age or both. The number of contacts increased after the first session of gentling ($P<0.001$) as a possible consequence of the positive reinforcement represented by the human stimulus and remained steady up to the twelfth session. A further increase was observed at session 13, when the lambs contacted the human more than in any other session ($P<0.001$). These results, although may well be a consequence of increased age, are in agreement with those reported by Markowitz et al. [3] indicating that training the lambs through gentle handling at an early age can positively influence the quality of human-animal relationship if handling is performed for 15 min/day at least during the first week of life.

The number of contacts increased with time more in Gentile di Puglia than in Comisana lambs, as significant differences between the two breeds were observed during the last two sessions (session 12: 0.97 ± 0.93 vs. 4.46 ± 0.97 for C and GP respectively, $P<0.01$; session 13: 1.23 ± 0.97 vs. 10.08 ± 0.97 for C and GP respectively, $P<0.001$). As a result on average Gentile di Puglia lambs had 5-fold more contacts with the human than Comisana lambs ($P<0.001$). For the number of bleats, significant differences between breeds were only observed during the first two sessions (session 1: 2.31 ± 0.45 vs. 3.83 ± 0.45 for C and GP respectively, $P<0.01$; session 2: 0.73 ± 0.47 vs. 2.5 ± 0.47 for C and GP respectively, $P<0.001$), as Gentile di Puglia lambs were initially more reactive to the human presence. Although confirming previous results on the lower degree of confidence in humans of sheep breeds characterized by shy behaviours [23], our data suggest that, in more reactive sheep (i.e. Gentile di Puglia), the gentle handling of lambs at an early age can be even more successful than in more confident breeds (i.e. Comisana). Gentile di Puglia lambs succeeded in overcoming the initial reactivity to the human presence and searched for contacts at the end of training.

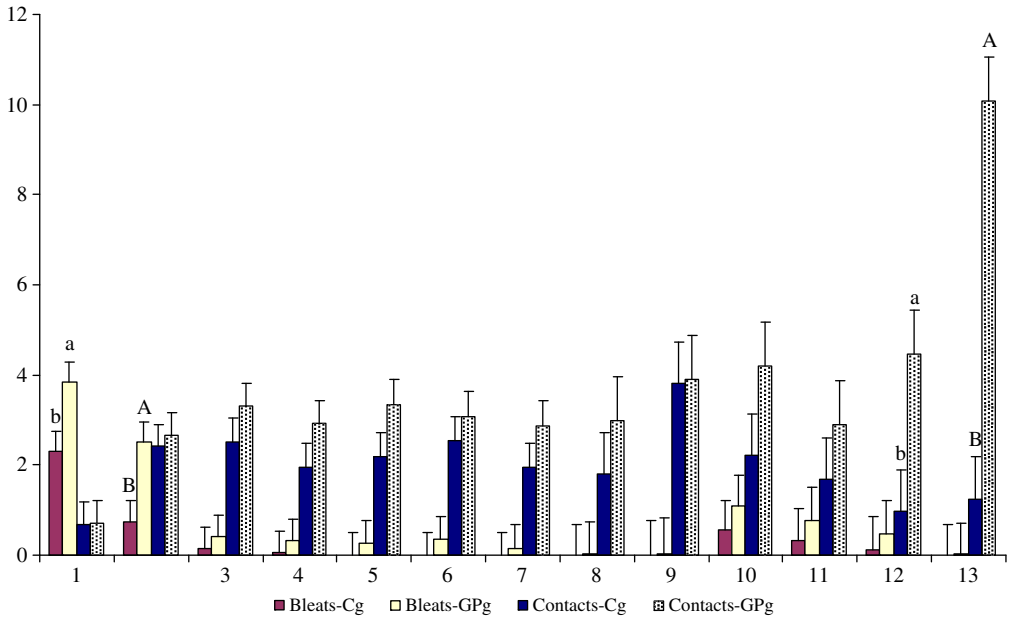

Fig. 2. Least square means ± SEM of the number of bleats and contacts with humans registered during the training sessions of gentling in Gentile di Puglia and Comisana lambs. For the first week the means of three daily treatments are reported. a,b and A,B indicate significant differences (P<0.01 and P<0.001, respectively).

3.2. Behavioural and cortisol responses to arena testing

Results on behavioural responses are reported in Table 1. Latency time to movement was affected by breed as Gentile di Puglia lambs on average exhibited shorter latency time than Comisana lambs (P<0.05). In addition, Gentile di Puglia lambs showed lower levels of investigative activities (P<0.001), a higher average number of climbing attempts (P<0.01) and emitted on average a higher number of bleats in comparison with Comisana subjects (P<0.001), thus exhibiting a higher motivation to re-join their group and experiencing more stress during the tests irrespective of the test conditions and the treatment. These results are confirmed by the cortisol response which tended to be higher (P<0.10) in Gentile di Puglia lambs overall and immediately after the tests (Fig. 3).

A significant genotype × treatment interaction was also detected because GPg lambs showed a lower number of climbing attempts than GPng lambs (P<0.05), whereas no differences were observed between Comisana lambs subjected to different treatments (P>0.05). Similarly, during the test with the human the number of contacts was affected by genotype x treatment (P<0.05). In fact, in Comisana lambs differences between gentled and not gentled animals were not significant (P>0.05), whereas gentled Gentile di Puglia lambs showed a significantly higher number of contacts with the human than Gentile di Puglia not gentled subjects (P<0.01). The time spent in proximity of the human had a similar trend, with a tendency to spend more time in proximity of the human of GPg as compared with GPng (P = 0.07), although no significant effects were detected (P = 0.30 for genotype × treatment interaction). These results confirmed those obtained during

Table 1
Least square means ± SEM of behavioural activities recorded in Comisana lambs subjected to gentling (Cg), or not (Cng), and in Gentile di Puglia lambs subjected to gentling (GPg), or not (GPng) during the isolation test, the stationary human test and the pen-mates test.

	Groups				Levels of significance		
	Cg	Cng	GPg	GPng	Breed	Test	Treatment
Latency time to movement, s							
Isolation test	4.78 ± 2.14	6.62 ± 2.28	6.77 ± 2.27	1.36 ± 1.95			
Stationary human test	6.18 ± 2.28	4.12 ± 2.28	4.73 ± 2.15	3.82 ± 1.95			
Pen-mates test	10.55 ± 2.28	5.62 ± 2.28	1.54 ± 2.28	3.73 ± 1.95	*	NS	NS
Duration of movement, s							
Isolation test	39.02 ± 8.11	49 ± 8.60	49.97 ± 8.60	78.64 ± 7.34			
Stationary human test	23.69 ± 8.60	27.25 ± 9.60	29.60 ± 8.16	19.64 ± 7.39			
Pen-mates test	18.57 ± 8.60	20.62 ± 8.60	23.46 ± 8.47	11.82 ± 7.34	NS	***	NS
Investigation, s							
Isolation test	51.28 ± 12.01	56.75 ± 12.75	37.43 ± 12.74	21.18 ± 10.88			
Stationary human test	48.88 ± 12.75	37.62 ± 12.76	34.55 ± 12.02	17.00 ± 10.88			
Pen-mates test	116.26 ± 12.75	142.75 ± 12.76	134.37 ± 12.75	119.91 ± 10.88	*	***	NS
Number of bleats							
Isolation test	59.08 ± 5.51	45.50 ± 5.86	67.78 ± 5.84	73.82 ± 4.99			
Stationary human test	38.08 ± 5.85	25.50 ± 5.86	49.38 ± 5.51	37.45 ± 4.99			
Pen-mates test	2.33 ± 5.85	0.01 ± 5.86	6.94 ± 5.81	1.82 ± 4.99	**	***	NS
Number of climbing attempts							
Isolation test	5.21 ± 2.47	1.37 ± 2.62	4.12 ± 2.62	18.00 ± 2.23			
Stationary human test	0.41 ± 2.62	0.37 ± 2.61	1.19 ± 2.47	4.55 ± 2.23			
Pen-mates test	0.03 ± 2.62	0.01 ± 2.62	0.71 ± 2.62	0.01 ± 2.23	**	***	NS
Number of contact with human	7.37 ± 24.3	6.50 ± 2.43	13.78 ± 2.29	4.00 ± 2.07	NS	NS	*
Time spent in proximity of person, s	105 ± 34.25	93.50 ± 34.25	167.22 ± 32.30	87.36 ± 29.21	NS	NS	NS

NS = not significant; * = P<0.05; ** = P<0.01; *** = P<0.001.

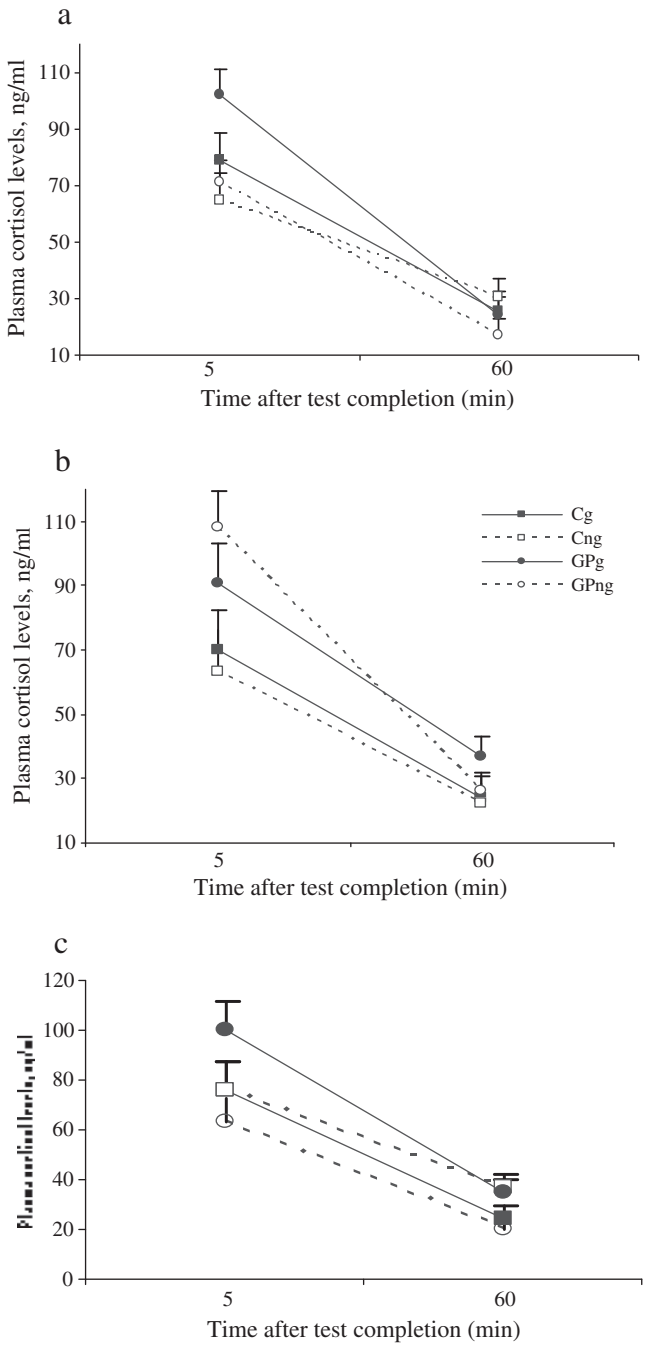

Fig. 3. a. Least square means ± SEM of Plasma cortisol levels, ng/ml in Comisana lambs subjected to gentling (Cg), or not (Cng), and in Gentile di Puglia lambs subjected to gentling (GPg), or not (GPng) when tested in the Isolation test. b. Least square means ± SEM of plasma cortisol levels (ng/ml) in Comisana lambs subjected to gentling (Cg), or not (Cng), and in Gentile di Puglia lambs subjected to gentling (GPg), or not (GPng) when tested in the Stationary Human test. c. Least square means ± SEM of plasma cortisol levels (ng/ml) in Comisana lambs subjected to gentling (Cg), or not (Cng), and in Gentile di Puglia lambs subjected to gentling (GPg), or not (GPng) when tested in the pen-mates test.

the training and indicate the effectiveness of the gentling treatment for the development of affinity to the stockperson in more reactive breeds such as Gentile di Puglia. As expected, a higher number of contacts with the human stimulus was observed in gentled animals as a consequence of the treatment ($P<0.05$).

A significant test × breed × treatment interaction was observed for duration of movement and climbing attempts ($P<0.05$), with GPng lambs displaying the longest ambulatory activity and exhibiting more climbing attempts during the isolation test as compared with the human or the pen-mates' tests ($P<0.001$). Gentile di Puglia lambs exhibited active avoidance strategies during stressful situations, such as isolation, but the presence of the stockperson helped the lambs to cope with the situation, particularly those subjected to the gentling treatment, which was able to render Gentile di Puglia lambs less fearful in aversive conditions. Accordingly, latency time to movement, duration of ambulatory activity and time spent in investigation taken together indicate that Gentile di Puglia lambs, in particular GPng lambs, dynamically reacted to the tests with increased walking and hyperactivity, particularly during the isolation test. The perception of a dangerous situation can induce panic reaction in sheep, as demonstrated by either increased locomotor activity and bleating or freezing [24].

As reported in Table 1, a significant effect of the type of test was detected for duration of movement, time spent in investigative activities, number of bleats and number of climb attempts ($P<0.001$). During the isolation test lambs showed a longer duration of movement as compared with lambs tested with either pen-mates or a human ($P<0.001$), whereas no differences were observed between lambs tested with pen-mates or a human. Previous studies showed that isolated lambs spent less time in ambulatory behaviours than ewe-reared lambs as a consequence of a stronger motivation in contacting the dam (e.g. [25]). The higher locomotive activity observed in isolated lambs may be interpreted in terms of stronger motivation in contacting social partners by animals with marked gregarious drive, whereas in the other two tests their social needs were, at least partly, satisfied by the presence of other social counterparts either belonging to the same species or not.

The investigative behaviour was lower during isolation than in presence of pen-mates ($P<0.001$), whereas no differences were observed between lambs tested in isolation and animals tested with a human $P>0.05$). Exploration is performed to locate resources such as cover and feed and to reach a sufficient level of control on the surrounding environment. Environmental control is an important component of good welfare because allows animals to predict changes in their physical and social environment [26]. In isolated subjects environmental control was likely to be lower and determined fear-induced behavioural inhibition [27], whereas animals in a group benefited from the social support provided by their companions and possibly felt more secure. Conversely, the low level of exploration observed during the test with a stationary human may be attributed to a change in lamb activity pattern with the animals finding the interaction with the human more attractive.

The highest level of vocalization was observed when lambs were tested in isolation as compared with lambs tested either with the human or with the pen-mates ($P<0.001$). However, the number of bleats was higher if lambs were tested in presence of the human than with the pen-mates ($P<0.001$). Vocalizations are used to locate and keep contact with the other components of the social group [28]. Obviously, isolation induced a higher expression of this behaviour, whereas the presence of the human, at least partly, mitigated the effect of separation.

Previous studies (e.g. [29]) reported reduced locomotor and vocal activity in response to a potential predator. However, in our study the animals were habituated to the human presence, at least through the suckling training. In addition, it was unlikely that the human was perceived, as a potential predator as during the human test exploration switched from the environment to the human, which means that the animals voluntarily approached him.

A higher number of climb attempts was performed by lambs tested in isolation than by those tested either with the human ($P<0.01$) or with the pen-mates ($P<0.001$), whereas no differences were observed between lambs tested with the human and those tested with the pen-mates ($P>0.05$).

Cortisol response (Fig. 3) showed a significant breed × test interaction ($P<0.05$), which can be attributed to the fact that during the stationary human test Gentile di Puglia lambs displayed a higher cortisol

level as compared with Comisana lambs ($P<0.01$). More importantly, the significant breed × test × treatment interaction ($P<0.001$) could be explained on the basis of a higher cortisol level shown by GPng lambs in comparison with Cng subjects during the stationary human test ($P<0.01$), whereas no differences were detected between the two gentled groups ($P>0.05$). Therefore, Comisana lambs tended to be less reactive, in terms of activation of hypothalamic–pituary–adrenal (HPA) axis, than Gentile di Puglia to the testing conditions, whereas Gentile di Puglia lambs were able to reduce their cortisol response to humans only if treated with gentling.

A significant effect of time ($P<0.001$) with the highest cortisol level registered immediately after the tests indicates that removal of lambs from their home pens, handling and exposure to a novel environment were able to induce a marked increase in cortisol concentration, whatever was the type of stimulus included in the testing (isolation, pen-mates, human). Therefore, these procedures imposed a certain increased demand to the lambs. However, cortisol production may be associated with both pleasant and unpleasant situations and secreted, either for defence or for reward, whenever some activation of the body is needed (e.g. [30]).

Results from the behavioural and cortisol responses to arena testing suggest an influence of genotype in coping with testing situations: Gentile di Puglia lambs displayed active avoidance reactions to field testing and were less involved in investigative activities. However, gentling by the stockperson in Gentile di Puglia lambs succeeded in making the animals more confident in aversive situations, positively influencing their behavioural response to stressful conditions. It can be hypothesized that more reactive animals either were more sensitive to human care or their exposition to isolation led to a stronger need for social support, such as the one represented by the human presence [31].

3.3. Immune responses and weight gains

All the lambs showed similar cell-mediated immune responses both at 7 and 28 days of age (data not shown). Neither the treatment nor the breed affected the growth rate of the lambs. At the end of the experiment average weight gains ranged from 0.24 ± 0.05 in group Cng to 0.36 ± 0.05 kg/day ($\pm$SEM) in group GPg.

4. Conclusions

Lamb gentling resulted in an improvement in the quality of human animal relationship, particularly in more reactive breeds, such as Gentile di Puglia sheep. In general the cortisol response matched the altered behavioural reaction in terms of number of contacts and time spent in proximity of the stationary human, thus validating the results concerning behavioural parameters and showing that the Gentile di Puglia breed was more sensitive to disturbance although these lambs more promptly built a positive relationship with humans, possibly because a higher disturbance generated a need to be reassured through social support. This study also indicated that genetic predisposition may play an important role in building positive human–animal relationship, thus providing a potential tool for animal selection based on possible genetic differences.

In both gentled and non gentled groups the negative effect of arena testing on lamb behaviour was at least partly mitigated by the presence of other social counterparts either belonging to the same species (i.e. pen-mates) or not (i.e. human).

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