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## Gentling and welfare of lambs

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**RIASSUNTO** – Gentling e benessere dell'agnello. Al fine di valutare l'effetto delle cure dell'operatore sul benessere dell'agnello allattato artificialmente è stata effettuata una prova della durata di 7 settimane condotta su 64 agnelli di razza Comisana. Gli agnelli in allattamento artificiale e sottoposti al trattamento di gentling (AR+G) hanno evidenziato valori medi di cortisolo ematico a 15 giorni più bassi degli agnelli allevati con le madri e sottoposti al gentling (ER+G), nonché una risposta immunitaria umorale migliore degli agnelli soggetti al solo allattamento artificiale (AR) e comparabile a quella degli agnelli in allattamento naturale (ER). I risultati ottenuti suggeriscono che le cure dell'operatore assumono un ruolo di rilievo nell'allevamento dell'agnello allattato artificialmente, influenzando positivamente l'assetto immunitario e riducendo lo stress connesso alla manipolazione degli animali imposto dalle normali procedure di management aziendale. Il gentling non sembra invece contribuire a migliorare lo stato di benessere negli agnelli allattati dalle madri.

Key words: gentling, lambs, artificial rearing, welfare.

**INTRODUCTION** – In traditional sheep production systems, lambs are suckled by their mothers and then gradually weaned at 35 days of age. However, the increased size of intensive dairy sheep flocks to obtain greater amounts of ovine milk for cheese making, is promoting the diffusion of artificial rearing programs involving early separation of lambs from their mothers. Maternal deprivation soon after birth can have detrimental effects on lamb immune functions, and lead to altered cortisol secretion and behavioural responses to isolation (Napolitano *et al.*, 1995). Human-animal interactions have been documented to play a role in sustaining the welfare and production of domestic animals (Hemsworth, 2003). In addition, gentled animals are less difficult to be handled and less susceptible to the stress induced by management practices involving human contacts (Lensink *et al.*, 2000). The aim of the present trial was to investigate the effects of gently handling newborn lambs on their behavioural, immune and endocrine responses, and on their growth rate when mothered or artificially reared.

**MATERIAL AND METHODS** – The experiment lasted 7 weeks and involved 64 Comisana twin-lambs, divided into four groups of 16. Ewe reared lambs (ER/ER+G) were kept with their dams throughout the experimental period. Artificially reared lambs (AR/AR+G) were maintained with their dams 24 to 30 h after parturition to receive maternal colostrum and then separated from them, and offered a milk substitute. Each group was housed separately in 3m x 8m straw bedded pens in the same building and balanced for age, weight and sex of lambs. ER+G and AR+G lambs were subjected to gentling treatment by trained stockmen. Gentling treatment consisted of gently handling the lambs for 5 minutes each, once a day throughout the trial. Two stockmen were involved in the gentling treatment to avoid the possibility of lambs' attachment to one particular person. The lambs in ER and AR groups received the minimal contact with stockmen except for the routine management and testing procedures. Stockmen involved in management and testing procedures were different from the stockmen performing the gentling treatment. AR lambs were taught to suck from buckets during the first three days of life.

All the animals were weighed at the beginning, at d 21 and at d 49 using an electronic scale. To determine lambs humoral response during the trial, at 3 days of age the animals received a subcutaneous injection of the antigen hemocyanin from keyhole limpets (KLH, Sigma-Aldrich Italia, Milan, Italy) to which the animals were not previously exposed. A second injection was repeated 12 days later. Antibody titres were determined in blood samples at d 3 and then at d 15 and d 45, and the concentrations of IgG anti-KLH in serum were determined by an ELISA test performed on microtiter plates according to Sevi et al. (2003). At d 13, 23, 33 and 43 behavioral activities of all lambs were video-recorded for 4 h and scan samples were taken every 5 min. Thus, behavioral variables were expressed as the proportion of subjects observed in each category of posture and activity. Grooming and playing activities were measured by continuous recording and expressed as the number of lambs involved in each activity. Lambs were tested for their responses to two stationary human tests performed at d 10 and 30 according to Markowitz et al. (1998). At d 15 and 45 lambs were subjected to two open field tests in a novel environment (a 4m x 4m pen), and isolated from conspecifics for 5 min. In each isolation test latency time to and duration of movement, and the number of bleats and of climbing attempts were recorded. Blood samples were collected immediately before the isolation test, immediately after the test and 60 min after to evaluate cortisol concentrations. Hormone concentration was determined by a radioimmunoassay. Cortisol levels were evaluated on  $\log_{10}$  transformed data to homogenize variance. Behavioural response to isolation and to stationary human test, antibody titres and cellular immune response were processed using ANOVA for repeated measures (SAS, 1999) having treatment, day of sampling and their interaction as source of variation. Differences among groups in the number of lambs which came into contact with the stockpersons were assessed using the  $\chi^2$  test in the FREQ procedure of SAS (1999). Weight gains were analyzed using ANOVA with one factor (treatment). When significant effects were found (P<0.05), the LSD test was used to locate significant differences between means.

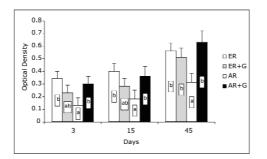
**RESULTS AND CONCLUSIONS** - Weight gains were found satisfactory in all groups and similar across treatments. Previous experiments have shown that early separation from the dam induces altered behavioural, immune and cortisol responses, which is not always associated with a reduced growth rate of lambs (Napolitano et al., 1995; Sevi et al., 2001). Accordingly, in the present study artificially reared lambs had weight gains comparable to dam-suckled lambs although displayed a significant reduction of humoral immune response, which was prevented by gentling. Antibody response to KLH was affected by day of sampling (P<0.001), and treatment x day of sampling (P<0.05), with AR lambs displaying antibody titres lower than the ER and AR+G lambs throughout the trial and than the ER+G animals at d 45 (Figure 1). Artificially reared lambs display altered behavioural reactions to isolation compared to dam-suckled animals during their early days of life (Napolitano et al., 1995). The magnitude of the pituitary-adrenal axis activation in response to open field tests can be ascribed to both pre-test procedures and isolation in a novel environment. When subjected to the open field test, handled and not handled lambs behaved similarly and so did mothered and artificially reared animals. At d 15 AR+G lambs displayed a moderate hormone release after pre-test manipulation (0.85 Logug/dl) and, together with AR lambs, a not significant plasma cortisol increase soon after isolation (1.34 Logug/dl) as well as the most marked hormone decline 60 min post-treatment (0.52 Logug/dl) (P<0.01). Moreover, averages of cortisol levels were found higher (P=0.07) in the group ER+G (1.25 Logug/dl) than in AR+G (0.90 Logug/dl) during the test performed at d 15. Thus, behavioural and cortisol responses from the present experiment suggest that gentle handling in AR+G group, and even careful training to suckle from the bucket performed by stockmen in AR group, helped the artificially reared lambs to cope with novel and potentially aversive situations. When monitoring animal behavioural activities, more AR+G and AR lambs were observed investigating (P<0.01) and playing with straw and objects (P<0.1) than ER lambs throughout the experiment (Table 1). Jensen et al. (1998) found that in dairy calves the reduction of space results in less activity and play behaviour, thus suggesting the use of play behaviour to reveal good welfare state in juveniles of farm animal species. In addition, group AR+G exhibited a higher percentage of lambs coming into contact with the shepherd than group ER+G (P<0.1) when tested at d 10 during the human stationary test thus supporting the hypothesis that gentling elicits the animal affinity to stockpersons in artificially reared but not in dam-suckled lambs. Group AR+G also showed higher mobility (62.56±6.79) compared to ER+G (27.06), AR (36.44) and ER (37.81) lambs, which can be regarded as the tendency of lambs to reach the stockperson at the opposite site of the experimental alley. Hence, based on previous findings and results from the present study, we can argue that the AR+G lambs were the most and the ER+G animals the least motivated in approaching the stockman. On the other hand, the good predisposition to interact with the shepherd displayed by AR lambs suggests that training to suckle from buckets was felt as a friendly interaction with stockman by the artificially reared not handled animals. Given that training to the artificial teat was performed during the first three days of life, our finding would lend support to the hypothesis that the most sensitive period for lamb socialization occurs within 3 days of life and has a durable effect on later lamb relationships (Markowitz *et al.*, 1998). In conclusion, gentling helped artificially reared lambs to cope successfully with the disruption of maternal environment, as suggested by immune and endocrine responses. Conversely, immune, cortisol and behavioural data suggest that cares of stockmen had no effects on dam-suckled lambs.

Table 1. Least square means  $\pm$  SEM of behavioural activities recorded in ewe-reared lambs when subjected to gentling (ER+G) or not (ER), and in artificially reared lambs when subjected to gentling (AR+G) or not (AR). Values are expressed as either proportion (p) or number (n) of animals.

	Groups					Levels of significance		
	ER	ER+G	AR	AR+G	SEM	Treatment	Time	Treat. x Time
Standing, p	0.38 a	0.45 ab	0.56 b	0.52 ab	0.05	*	NS	NS
Idling, p	0.61 b	0.55 ab	0.43 a	0.47 ab	0.05	*	NS	NS
Investigation, p	0.31 a	0.35 ab	0.46 b	0.45 b	0.03	**	*	NS
Object playing, n.	3.23 a	3.87 ab	5.17 b	4.69 b	0.38	+	+	NS

Means in the same raw followed by different letters are significantly different at P < 0.05. NS = not significant;  $^+$  = P<0.1;  $^*$  = P<0.05;  $^{**}$  = P<0.01.

Figure 1. Antibody response to hemocyanin from keyhole limpets (KLH) in ewe-reared lambs when subjected to gentling (ER+G) or not (ER), and in artificially reared lambs when subjected to gentling or not (AR+G) or not (AR).



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