EFFECT OF MILK SOURCE ON WELFARE AND WEIGHT GAIN OF LAMBS

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Abstract *Animal Welfare 2001, **10:** 163-172*

The possibility of lessening the adverse welfare impact of artificial rearing was assessed in thirty Comisana lambs under conditions of ad libitum *feeding. The lambs were assigned to a control dam-suckled group (DS) or to one of two test groups, each consisting of 10 animals. 18-24h after parturition, test lambs were separated from their dams: during the first post-separation week 10 subjects (EM) were offered ewe milk and the other 10 (MX) a 50:50 mix of ewe milk and a milk substitute. An artificial feeding system, to which the test lambs had free access, was filled with milk twice daily. After the first week, a transition from EM or MX to 100 per cent (reconstituted) milk substitute was carried out over 3 days in both test groups, gradually reducing the proportion of ewe's milk. In vivo cell-mediated immune response of lambs to phytohemagglutinin percutaneous injection were evaluated at 5, 15 and 25 days of age. At 10 and 20 days of age, all lambs were subjected to 15min isolation tests, during which latency to move, duration of movement and number of bleats were recorded. Blood samples were collected immediately before the test and 15 and 60min after, in order to assess the effect of differential rearing on the cortisol response of animals at different ages. In addition, the weight gain of lambs was recorded during the first 35 days of age at weekly intervals. Latency to move and duration of movement were not significantly affected by the experimental treatment, whereas the number of bleats was higher ($P < 0.001$) in dam-suckled lambs compared to test lambs at both 10 and 20 days of age. Neither immune response nor cortisol levels were affected by milk source. Although weight gain increased with increasing amounts of ewe's milk in the diet, the overall differences between the three groups were small and not significant. These findings suggest that the provision of ewe milk or a mix of ewe milk and a milk substitute during the first post-separation week can be a suitable strategy to sustain the welfare and production performance in the artificially reared lamb.*

Keywords: *animal welfare, artificial rearing, behaviour, production, performance, sheep*

Introduction

In conventional sheep production systems, lambs are suckled by their dams and gradually weaned at an average age of 35 days. However, the increased size of specialized dairy sheep flocks, especially in the northern countries of the Mediterranean basin, is leading to a gradual diffusion of lamb artificial rearing. As a result, separation from the dam is occurring 18-36h after birth, with the lambs experiencing an abrupt transition to substitute milk at this early stage of their development. This practice is aimed at releasing greater amounts of ewe milk to be processed into dairy products. However, it may result in increased morbidity and poor production performance of lambs, due to the reduced ability of young animals to cope with emotional and nutritional stresses arising from dietary change and separation from the ewe (Napolitano *et al* 1995). Thus, studies are required to assess the possibility of minimizing the adverse impact of artificial rearing on lamb welfare. Features of the artificial teat and milk dispenser, evoking maternal udders and teats, can induce spontaneous suckling in artificially reared lambs (Billing & Vince 1987a, b; Fraser 1987; Sevi & Casamassima 1996), but they are not effective in reducing nutritional stress due to the abrupt diet change and the lower nutritional value of milk substitutes compared to maternal milk. Nutritional stress may affect lamb endocrine functions because glucocorticoid production is stimulated to promote glucose synthesis in response to low nutritional levels (Wrutniak & Cabello 1987).

Oral-olfactory stimuli play a major role in the development of social and feeding behaviour of the young lamb (Nolte & Provenza 1991; Lynch *et al* 1992; Mirza & Provenza 1994); thus, giving lambs maternal milk during the days following separation from their dams may reduce the detrimental effect of both psychological and nutritional stresses associated with the change from maternal suckling to artificial rearing. Sevi *et al* (1998) observed that voluntary milk intake in new-born lambs increased by 40 per cent and 60 per cent respectively when passing from a milk substitute to either a mix of ewe milk and a milk substitute or to total ewe milk. Sevi *et al* (1999) also found that lambs receiving mixed milk showed a slightly decreased growth rate compared to dam-suckled lambs, whereas subjects only receiving milk substitute displayed altered endocrine, immune and behavioural responses, as well as a markedly inhibited growth.

In order to improve the well-being of lambs prematurely separated from their dams, we compared the behavioural, immune and endocrine responses and production performance of dam-suckled and artificially reared lambs when offered ewe milk or a mix of ewe milk and a milk substitute under conditions of *ad libitum* feeding.

Materials and methods

Experimental design

The experiment lasted 5 weeks and was performed on 30 Comisana lambs divided into two test groups (each n = 10) and a control group (n = 10). Control dam-suckled animals (DS) were maintained with their mothers in a 3x8 m straw-bedded pen for the whole experimental period. Test animals were separated from their dams 18-24h after parturition and housed in two separate 3x4 m straw-bedded pens. During the first week of the trial, ten subjects (EM) received ewe milk, while the other ten (MX) were offered a 50:50 mix of ewe milk and milk substitute (Lammeren Uniek milk powder [ALPURO BV, Uddel, The Netherlands] 200g per litre). In subsequent weeks, only the milk substitute was provided. The transition from ewe and mixed milk to milk substitute alone spanned over four days, during which the proportion of ewe milk was gradually reduced (the proportion of ewe milk to substitute milk was 0.75 to 0.25, 0.50 to 0.50, 0.25 to 0.75, 0 to 1 for EM lambs and 0.38 to 0.62, 0.25 to 0.75, 0.12 to

0.88, 0 to 1 for MX lambs). In each test group, milk was available from two 10 litre buckets each fitted with six, 50mm-long latex teats. Lambs had free access to milk, which was administered twice daily (at 0800 and 1800) at 37°C. During the trial, the amount of milk given to the test animals was adjusted so that refusals would be less than 10 per cent of the volume offered. At 21 days of age, all groups were supplemented *ad libitum* with alfalfa hay and a pelletted lamb weaning concentrate (Dell'Aventino Industria Mangimi, Fossacesia, Italy).

Chemical analysis of the milk substitute and the ewe milk

Milk powder samples (0.5kg) were analysed for fat, total protein, crude fibre and ash according to standard AOAC (1990) methods. Ewe milk samples (200ml), collected daily by mixing equal volumes of morning and evening milk, were analysed for total protein, fat and lactose by means of a Milko Scan 133 B infra-red spectrophotometer (Foss Electric, Hillerod, Denmark) and for ash according to standard AOAC (1990) methods. The chemical composition and nutritional value of ewe milk and of the mix of ewe milk and milk substitute are reported in Table 1.

Table 1 Chemical composition (gkg^{-1}) and nutritional value ($MJkg^{-1}$) of the ewe milk and of the 50:50 mix of ewe milk and milk substitute.

	Ewe milk	Mixed milk†
Total protein	56.0	50.0
Fat by ether extract	65.8	54.3
Crude fibre		0.1
Ash	9.0	13.2
Lactose	45.7	
Non structural carbohydrates		66.4
Gross energy	4.6	4.3

† Ingredients of the milk powder: dried whey, dried whey with no lactose, beef tallow, coconut oil, wheat starch, wheat meal, soybean lecithin, vitamin and mineral mix including vit. A 25,000 IU; vit. D_3 4,000 IU; vit. B_1 $2mgkg^{-1}$; vit. B_2 $7mgkg^{-1}$; vit. B_6 $1mgkg^{-1}$; vit. B_{12} $8\mu gkg^{-1}$; vit. K $1mgkg^{-1}$; vit. E $60mgkg^{-1}$; vit. C $100mgkg^{-1}$; Niacin $30mgkg^{-1}$; Colin $150mgkg^{-1}$; Beta-hydroxyl-toluene $10mgkg^{-1}$; Zinc $18mgkg^{-1}$; Manganese $22.5mgkg^{-1}$; Cobalt $2mgkg^{-1}$.

Isolation test and cortisol level determination

At 10 and 20 days of age, all lambs were subjected to an isolation test in order to assess the effect of rearing conditions on their behavioural and cortisol responses at different ages. Each animal was exposed to a novel environment (a 4x4 m pen), and isolated from tactile and visual contact with conspecifics for 15min. However, they could receive auditory and olfactory stimuli from other animals. Latency to move (s), duration of movement (s) and number of bleats per minute were recorded. Both a reduction in ambulatory behaviour and an increase in vocalizations are regarded as signs of distress in young lambs when they are subjected to social link disruption and isolation (Moberg & Wood 1982).

Blood samples (10ml) for evaluation of cortisol concentrations were collected in vacuum tubes, from the jugular vein, immediately before the isolation test and 15 and 60min after. Cortisol concentrations were determined by a radioimmunoassay (Cort-CT2™, Cis Diagnostici, Tronzano, Italy). The sensitivity of the assay was $0.2\ \mu gdl^{-1}$; the inter and intra-assay coefficients of variation were 7.0% and 5.3%, respectively. All procedures were conducted according to the guidelines of the Council Directive 86/609/ EEC of 24 November 1986.

In vivo cell-mediated immunity

The generation of inflammatory mediating T-lymphocytes in response to different mitogens, such as phytohemagglutinin (PHA), is a convenient indicator of an animal's ability to mount an active cell-mediated immune response (Burton *et al* 1989). At 5, 15 and 25 days of age, PHA (500μg, Sigma Aldrich-Italia, Milan, Italy) dissolved in 500μl of sterile saline solution was injected intra-dermally into the middle of two 2cm wide circles stamped on shaved skin in the upper side of each shoulder. The skinfold thickness (mm) was determined with calipers before PHA injection and 24h after. For each animal, an average increase in skinfold thickness (24h post-injection thickness – pre-injection thickness) was compared using the two measurements.

Weight gain

The mean (±SEM) weight of the animals at the beginning of the trial was 4.87 ± 0.59 kg. All animals were weighed every other day during the first trial week, and subsequently at weekly intervals, with an electronic scale.

Statistical analysis

Data were analysed using SAS®, version 6 (SAS 1990). Behavioural variables were transformed in $\log_{10}$ form to normalize frequency distributions and analysed using an ANOVA for repeated measures with group as the non-repeated factor and day of testing (day 10 & day 20) and the [group x day] interaction as repeated factors. For each test day (day 10 & day 20) the cortisol data were analysed by split-plot analysis with group as the whole-plot factor and sampling time (before and 15 and 60min after the isolation test) and [sampling time x group] as sub-plot factors. The increase in skinfold thickness data were analysed by split-plot analysis, with group as the whole-plot factor and day of injection (days 5, 15 and 25) and the interaction [day of injection x group] as subplot factors. Weight gains were analysed using an ANOVA with one factor (group) having lamb within group as the error term and initial body weights as co-variates. Where significant effects were found, a Student's *t*-test was used to locate significant differences between means.

Results

Behavioural and cortisol responses to isolation

Latency to move was not affected by the experimental treatment, although it increased slightly passing from DS to EM and MX lambs at both 10 and 20 days of age (Table 2). Duration of movement was also unaffected by the experimental treatment, whereas there was a significant effect of age. EM lambs showed 16 per cent ($P < 0.01$) longer and DS and MX lambs 11 per cent ($P < 0.05$) longer duration of movement at 20 days than at 10 days of age. During both isolation tests, the number of bleats was higher in DS than in EM and MX groups ($P < 0.001$), even though bleating had significantly decreased in the DS group ($P < 0.05$) at 20 days compared to 10 days of age.

At 10 (Figure 1a) and 20 days of age (Figure 1b), isolated lambs displayed the highest cortisol levels in samples taken 15min after the isolation test ($P < 0.001$). On both test days (day 10 and day 20), no effect of milk source on cortisol response to isolation was found (ns). In addition, there was no significant [group x time] interaction in either isolation test.

Table 2 **Mean (± SEM) latency to move (s), duration of movement (s) and number of bleats min^{-1} of dam-suckled lambs (DS) and test lambs, receiving ewe's milk (EM) or a mix of ewe's milk and milk substitute (MX), during the isolation tests conducted at 10 and 20 days of age. Test lambs were subjected to a gradual transition to substitute milk.**

	DS	EM	MX	Milk source *P* value	Days of age *P* value	Milk source x Days of age *P* value
Latency to move						
Day 10	4.2±0.9	4.8±0.7	5.6±0.6			
Day 20	4.0±0.7	4.4±0.5	5.2±0.4	0.1334	0.5215	0.8067
Duration of movement						
Day 10	412.6±12.8b	401.5±13.7B	393.5±16.7b			
Day 20	458.9±5.8a	467.5±8.7A	435.5±15.5a	0.7641	0.0196	0.3059
Number of bleats min^{-1}						
Day 10	26.4±1.2Aa	16.3±1.0B	16.1±1.3B			
Day 20	20.1±1.1Ab	13.6±0.6B	14.4±1.1B	0.0001	0.0072	0.2048

Means within rows or columns followed by different letters differ significantly; lower case $P < 0.05$ and upper case $P < 0.01$.

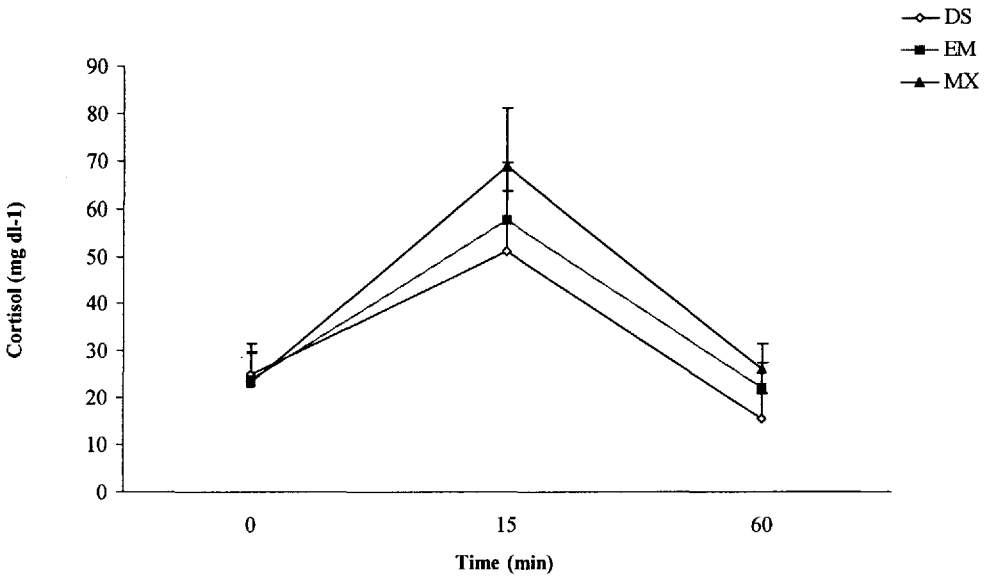

Figure 1a **Mean (±SEM) plasma cortisol levels at 10 days of age in dam-suckled (DS) and test lambs receiving ewes milk (EM) or a mix of ewes milk and milk substitute (MX) during the first post-separation week.**

In vivo cell-mediated immune response

Neither group, nor time, nor the interaction of [group x time] affected in vivo cell-mediated immune reactivity to intradermal injection of PHA (ns; Figure 2).

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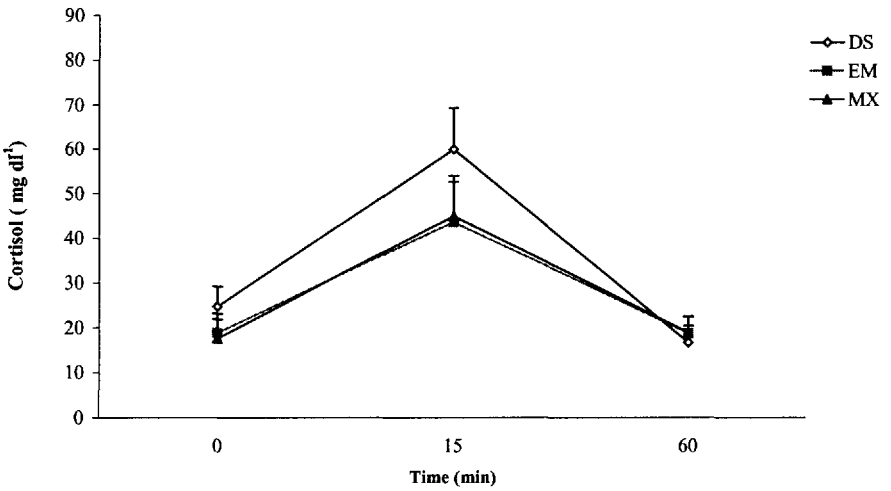

Figure 1b **Mean (±SEM) plasma cortisol levels at 20 days of age in dam-suckled (DS) and test lambs receiving ewes milk (EM) or a mix of ewes milk and milk substitute (MX) during the first post-separation week.**

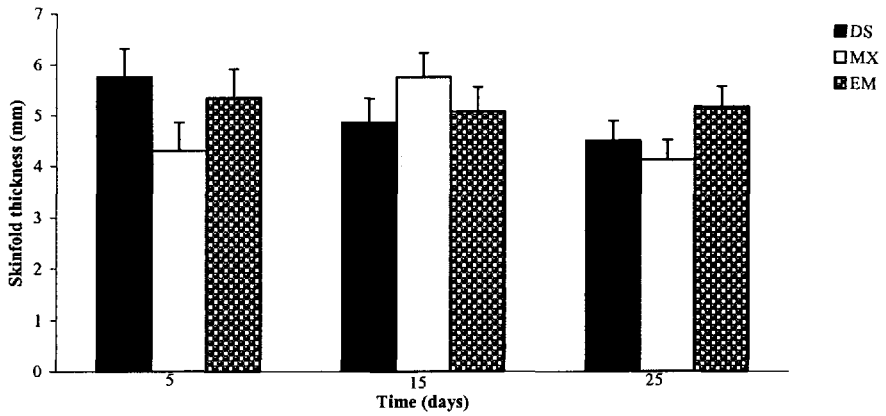

Figure 2 **Mean (±SEM) immune response to PHA at 5, 15 and 25 days of age in dam-suckled (DS) and test lambs receiving ewes milk (EM) or a mix of ewes milk and milk substitute (MX) during the first post-separation week.**

Weight gains

Growth rate was not significantly affected by the experimental treatment (Table 3), although DS lambs had slightly higher weight gains compared to EM lambs, which in turn gained slightly more weight than MX lambs during each 7 day period.

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Table 3 **Comparison of mean (±SEM) weight gain ($gday^{-1}$) of dam-suckled lambs (DS) and test lambs, receiving ewe's milk (EM) or a mix of ewe's milk and milk substitute (MX), during days 0-7, 8-14, 15-21, 22-28, 29-35 and 0-35.**

Day	Groups			*P* value
	DS	EM	MX	
0-7	221±54	207±42	196±51	0.9056
8-14	224±32	212±43	198±33	0.5112
15-21	242±33	224±29	209±26	0.3849
22-28	249±32	227±37	213±35	0.4007
29-35	255±33	232±29	216±28	0.3417
0-35	238±18	220±22	206±28	0.2914

Discussion

Lambs from both test groups behaved similarly to those from the dam-suckled group when exposed to the novel environment in the isolation tests. This finding is in agreement with previous reports (Sevi *et al* 1999), confirming that a gradual transition from maternal to substitute milk is effective in minimizing the detrimental impact of the disruption of the maternal/filial bond on the behavioural response of lambs to isolation.

An increase in vocalization is considered a sign of distress in adult animals (Lynch *et al* 1992) and has been used in the assessment of sheep responses to isolation (Grubb 1974). However, in young sheep, bleating is also a form of care-soliciting (Lynch *et al* 1992), which helps maintain the maternal/filial bond (Shillito-Walser *et al* 1982). This may explain why, when subject to isolation tests, DS lambs bleated significantly more than EM and MX lambs, which had been separated earlier from their dams. Sevi *et al* (1999) obtained similar results, assessing behavioural responses to isolation tests in lambs kept with their mothers or reared with their peers. Significantly less bleating occurred in the DS group at 20 days than at 10 days of age, probably as a consequence of either a progressive weakening of the mother/young bond (Hinch *et al* 1990) or a habituation effect to the procedure. However, the number of bleats remained higher in the control group with respect to test groups during the second isolation test. Decreased bleating with increasing age in lambs undergoing isolation tests was also found in other investigations (Zito *et al* 1977; Sevi *et al,* 1999).

Isolation tests have been widely used to assess the adrenal response to acute stress in lambs (Moberg & Wood 1982; Coppinger *et al* 1991; Minton *et al* 1992; Hashizume *et al* 1994; Minton 1994). Collecting a single blood sample by venipuncture, within 1min after first approaching an animal, is a suitable method for measuring baseline adrenal activity, at least in cattle (Hopster *et al* 1999). In the present study, cortisol levels 15min after separation from the group were greater than pre-separation levels in all groups of lambs. This increment was probably due to the reduced ability of young animals to cope with the emotional stress represented by the removal from their pen, isolation, exposure to a novel environment, jugular puncture and handling. Lambs are gregarious animals, therefore, when separated from the flock, they become very stressed. It has been stated that a graded cortisol response to different stressors may be attributed to the different stressfulness of stimuli. In a previous study (Sevi *et al* 1999), animals experiencing both separation from their dams and sudden diet change showed a higher cortisol response in comparison with the lambs which were either not separated or separated but progressively moved from maternal to substitute milk. In the present study, the three different treatments yielded similar adrenal responses.

Therefore, we hypothesised that for both test groups (EM and MX), gradual transition to substitute milk was suitable to sustain the welfare of artificially reared lambs. Sixty min after the first blood collection, cortisol levels returned to baseline concentrations, this may have occurred as a result of removing the stress of handling, bleeding and isolation. However, the decreased cortisol level observed at the third blood collection could partly be attributed to habituation. Novelty is one of many different stimuli that can induce fear (Gray 1987) and lambs can become accustomed to a novel stimulus after repeated exposure.

In lambs, many experiments (Minton & Blecha 1990; Napolitano *et al* 1995; Rhind *et al* 1998) have shown a link between circulating plasma glucocorticoids and impaired immune functions. In response to different stressful management practices, the hypothalamo-pituitary-adrenal axis becomes activated and the consequent reduction in the responsiveness of the immune system may determine an increased susceptibility to disease. In the present study, a progressive transition to milk substitute did not adversely affect cellular immune function in lambs, thus suggesting that the provision of ewe's or mixed milk during the first post-separation week can be a suitable strategy to sustain the welfare of artificially reared lambs. Orgeur *et al* (1998) found no significant differences in the hormonal and immune responses of lambs subjected to progressive or sudden weaning. However, in their study, the animals were older and close to the age of natural weaning; lambs display a higher frequency of independent activities at this age. Therefore, separation from mothers may not represent a very stressful management procedure for weaning lambs, as also stated by the authors, but this would not be the case for newborn lambs, whose attachment to their mothers is very strong.

Growth rate increased when increasing amounts of ewe's milk were offered to the lambs, but differences among groups were small and not significant throughout the study period. Sevi *et al* (1999) found that lambs that were fed a mix of ewe's milk and milk substitute during the first post-separation week had a markedly higher growth than lambs changing abruptly from maternal to substitute milk. Nonetheless, lambs benefiting from a gradual transition to artificial rearing had lower weight gains than dam-suckled lambs. Lambs used in that experiment only had access to milk for 3h a day, unlike the present study in which milk was available at all times. This probably resulted in a more efficient use of milk, which was consumed in smaller and more frequent meals.

Animal welfare implications

No differences were found in immune and endocrine responses and in growth between dam-suckled and test lambs, although artificial and dam-reared groups had different behavioural responses to isolation tests, significantly, the frequency of bleating. This latter result suggests that even a gradual transition to a milk substitute may have detrimental effects on the welfare of artificially reared lambs. In a previous study (Sevi *et al* 1999) it was also found that animals receiving mixed milk showed a slightly decreased growth rate. In the same study, however, subjects only receiving milk substitute displayed altered endocrine, immune and behavioural responses, as well as markedly inhibited growth. The results of the present study confirm that a gradual transition to milk substitute can, albeit partly, reduce the negative impact of artificial rearing, probably operating on both the nutritional and emotional component of stress.

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