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Play and other behaviour indicative of affective state

Välfärdsproblem hos lamm runt avvänjning

Lek och andra beteenden som indikerar känslotillstånd

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Skara 2014

Msc Animal Science

Photo: Joyce Broekman

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Student report ***No. 591***
Swedish University of Agricultural Sciences
Department of Animal Environment and Health

ISSN 1652-280X

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Course title: Degree project in Animal Science, EX0567, Advanced level E, 30 credits
Program: Master in Animal Science

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Keywords: lambs, weaning, separation, play, vocalisations, affective state

Serie: Studentarbete/Sveriges lantbruksuniversitet, Institutionen för husdjurens miljö och hälsa, nr. 591, ISSN 1652-280X.

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Contents

Summary

The abrupt and early weaning of intensively kept lambs may have serious animal welfare implications, but relatively little is known about this. Weaning includes the breaking of the mother-young bond and abrupt replacement of milk by solid food, often coinciding with changes in the (social) environment. Altogether, these changes are likely to affect lamb welfare significantly. This study aimed to establish how strong and persistent lamb welfare is impaired following abrupt weaning by means of measuring behaviour parameters indicative of affective state. Also, we investigated the existence of a diurnal rhythm in play behaviour, which is considered an important indicator of positive affect and good welfare. Play behaviour was studied in combination with other (potentially) useful welfare parameters, mainly indicative of negative affective state.

In order to investigate the effect of weaning on welfare, behaviour was recorded of 15 twin lambs that were weaned in two steps at 50-65 days of age. Firstly, the mother was removed from the home pen and placed within hearing distance of the lambs, and secondly the mother was removed from the barn. Durations of behavioural occurrences were scored for body movement, feeding, ear posture and tail posture and frequencies were recorded for play behaviour, social contact and vocalisations. Moreover, the lambs were weighed every week. Diurnal rhythms in locomotor and social play behaviour of lambs were investigated in 5 pre-weaned twins that were camera recorded for 24h. Statistics focussed on testing for effects of weaning period, sex and an interaction of these two on the different behaviours.

There was a significant effect of weaning period (i.e. *before* weaning, *during* the time the ewe was moved from the lambs' pen but still in the same barn, and *after* removal of the ewe from the barn) on total play (P=0.006), social play (P=0.018) and locomotor play (P=0.019), with a reduction in play during weaning. Social contact with the twin lamb and vocalisations increased in the period during weaning to regain its previous level after weaning (both P<0.001). Increases during weaning lasted respectively 2 and 3-4 days. The duration that the lambs were lying reduced during weaning (P=0.032). The plane ear posture was shown less in the period during weaning than before weaning (P<0.001), whereas raised and asymmetrical ear postures both increased rapidly during weaning.

Play behaviour was affected by sex differences, playground size, sex ratio of the playmates, and hour of the day. For example, pre-weaning total play (P=0.004), social play (P=0.013) and locomotor play (P=0.003) occurred especially during the 7th (7:00-8:00), 9th (9:00-10:00) and 19th (19:00-20:00) observation hour. The removal of the mother from the home pen to another pen in the same barn, and concurrent reduction of space and separation of playmates by bars (i.e. during weaning), evoked behavioural changes indicative of negative affect that lasted 2 to 4 days. The removal of the mother from the barn after another 5 days (i.e. after weaning) had minimal effects on behavioural parameters and weight gain. Play behaviour was reduced by weaning, but in a rather subtle way which does not make it a powerful indicator for on farm welfare assessment. Alternative indicators of welfare seem to be vocalisations, social contact with the twin, raised and asymmetrical ear posture and, possibly, the percentage of time lying. Pre-weaning, the lambs appeared to have a diurnal cycle of play, though this probably reflected in part the activities of the caretakers. The present study shows that

impaired lamb welfare is likely to follow from the conventional weaning method as indicated by a number of behavioural parameters.

Sammanfattning

Den abrupta och tidiga avvänjningen av intensivt hållna lamm kan ge allvarliga konsekvenser på djurvälfärden, men det finns ganska litet kunskap om detta. Avvänjning innebär att moder-ungebandet bryts och att mjölken plötsligt ersätts med fast föda, vilket ofta sker samtidigt med förändringar av (den sociala) miljön. Sammantaget är det troligt att dessa förändringar avsevärt påverkar lammens välfärd. Denna studie syftade till att bedöma hur mycket och hur länge lammens välfärd påverkas efter abrupt avvänjning genom att mäta beteendeparametrar som indikerar känslotillståndet. Dessutom undersöktes om det finns en dygnsrytm i lekbeteende, vilket anses vara en viktig indikator på positiva känslor och god välfärd. Lekbeteende studerades i kombination med andra (potentiellt) användbara välfärdsparametrar, som huvudsakligen indikerar negativa känslotillstånd.

För att kunna undersöka effekten av avvänjning på välfärden registrerades beteendet hos 15 tvillingslamm som avvandes i två steg vid 50-65 dagars ålder. Först flyttades modern från hemboxen och placerades inom hörselavstånd för lammen, och sedan flyttades modern ut ur ladugården. Durationen på beteenden registrerades för kroppsrörelser, foderintag, öronhållning och svanshållning, medan frekvenser registrerades för lekbeteende, social kontakt och vokalisering. Utöver det vägdes lammen varje vecka. Dygnsrytmer för rörelselek och social lek hos lamm undersöktes hos fem tvillinglamm innan avvänjningen från videofilmader under 24 timmar. Statistiken fokuserade på att testa effekter av avvänjningsperiod, kön och interaktionen mellan dessa två på de olika beteendena.

Det var en signifikant effekt av avvänjningsperiod (dvs. *före* avvänjning, *under* tiden tackan flyttades från lammens box men fortfarande vistades i samma byggnad, och *efter* flyttning av tackan från byggnaden) på total lek (P=0,006), social lek (P=0,018) och rörelselek (P=0,019), med en minskning av lek under avvänjningen. Social kontakt med tvillinglammet och vokalisering ökade i perioden under avvänjning för att återta sin tidigare nivå efter avväjning (båda P<0,001). Ökningar under avvänjningen varade 2 respektive 3-4 dagar. Durationen för liggande hos lammen minskade under avvänjningen (P=0,032). Plan öronhållning visades mindre under avvänjningsperioden än innan avvänjningen (P<0,001), medan rest och asymmetrisk hållning av öronen båda ökade snabbt under avvänjningen.

Lekbeteende påverkades av könsskillnader, lekområdets storlek, könsfördelning hos lekkamraterna och tid på dagen. Till exempel förekom total lek innan avvänjning (P=0,004), social lek (P=0,013) och rörelselek (P=0,003) speciellt under den 7:de (7:00-8:00), 9:de (9:00-10:00) och 19:de (19:00-20:00) observationstimmen. Flyttningen av modern från hemmaboxen till en annan box i samma byggnad, och samtidig minskning av ytan och separation från lekkamraterna med rör (dvs. under avvänjningen), utlöste beteendeförändringar som indikerar negativa känslor som varade 2-4 dagar. Flyttningen av modern från byggnaden efter ytterligare 5 dagar (dvs. efter avvänjning) hade minimal effekt på beteendeparametrar och viktökning. Lekbeteenden minskade vid avväjning, men på ett ganska subtilt sätt vilket inte gör dem till en kraftfull indikator vid välfärdsbedömningar på gårdar. Alternativa indikatorer på välfärd verkar vara vokaliseringar, social kontakt med tvillingen, rest och asymmetrisk öronhållning och, möjligen, procent tid lammen ligger. Innan avvänjning verkade lammen ha en dygnsrytm i lekbeteendet, men detta reflekterade troligen

delvis aktiviteter hos skötarna. Denna studie visar att minskad välfärd hos lamm troligtvis sker med de vanliga avvänjningsmetoderna vilket indikeras av ett antal beteendeparametrar.

Introduction

Farm animal welfare is of high relevance to society; people care about the way animals are kept and treated. Also, good animal welfare can positively affect food quality and safety, being another incentive for people to prioritise good animal welfare. Insight in the causes of impaired welfare in the different farm animal species, as used for different purposes and in different stages during their life, can contribute in finding strategies to safeguard farm animal welfare. On the basis of species-specific indicators like play and vocalisations, positive and negative affective states can be assessed. Domestic lambs are exposed to many stressors under conventional conditions of production in intensive systems. One of them is early artificial weaning which includes separation from the mother (e.g. Orgeur *et al.* 1998). With a total global sheep stock of 1,169,004,916 individuals in 2012 (FAOSTAT, UN Food and Agriculture Organisation), of which a considerate proportion is kept in intensive systems, it is important to find out how strong and persistent the welfare of the domestic lambs is impaired in response to multiple abrupt changes around artificial weaning in intensive systems. This information can be used in determining whether strategies should be developed to improve welfare of weaned lambs. In this report the term weaning is used to describe the contemporary artificial weaning method that entails separation from the ewe, making suckling no longer possible and additional changes in the (social) environment, specified in the description of methods.

Welfare impairment by weaning and separation

Soon after the lamb is born, a psychological attachment is formed between the mother and her young. Initially, the interest of the mother towards the young is very strong. The mother can soon recognise her lamb, while the lamb cannot recognise its mother before its sixth day of age (Arnold *et al*. 1975). After this age, gradually the attachment is more dependent on the lamb's interest in its mother (Arnold *et al*. 1979; Hinch *et al*. 1987). The Merino mother actively searches for her lamb during the first month of the lamb's life. During the first month, the mean distance between the mother and her lamb increases during some behaviours., like when lambs interact as groups, and stays the same for behaviours like lying or walking (Morgan and Arnold 1974). After one month the Merino mother prefers the flock over her lamb, while the lamb still actively searches for its mother (Hinch *et al*. 1987). Although there are differences between breeds (Walser *et al*. 1983), this change in the mother-lamb role in the maintenance of the mother-lamb attachment probably applies to many other breeds than the Merino (Hinch *et al.* 1987). Therefore, a broken mother-lamb bond at an age of around eight weeks, maintained by the contemporary weaning method, is probably affecting the lamb in particular. Under natural circumstances, lambs are usually weaned from milk at five months of age, depending on the breed and the maternal milk supply (Arnold *et al*. 1979). The mother starts to refuse suckling by the lamb more and more and may show agonistic behaviour towards it (Dwyer 2009). The feeding habits of the lamb change gradually until it is completely weaned from milk. Natural weaning from milk does not coincide with a completely broken bond between the mother and lamb (Arnold and Pahl 1974; Arnold *et al.* 1979; Shackleton and Shank 1984). Hinch *et al*. (1990) report that associations between Merino mothers and their offspring, although weakening over time, may last for more than two years.

The time of artificial weaning depends on the production system. In intensive as well as extensive systems, the time of weaning of domestic lambs is characterised by several changes that occur relatively abrupt, early and in concert. Weaning occurs generally at around eight until twelve weeks of age (Dwyer 2008), though dairy sheep may already be weaned within one or two days of birth (described by Napolitano *et al*. 2008). Weaning implies that the psychological mother-young bond, which was established soon after parturition (Dwyer 2009), is abruptly broken. Moreover, milk is replaced abruptly by solid food. Generally, the lambs are not fed milk replacer when they are weaned at eight weeks, because ruminant digestion and gut development is almost complete at this age (Dwyer 2009). Additional changes during the time of artificial weaning might occur in the (social) environment of the lamb (e.g. number of playmates, size of the enclosure). The abrupt and early changes may cause undesirable effects for the lamb. The presence of social companionship may alleviate the stress. Porter (1995) has found a reduction in distress calls due to separation from the mother in 3-week-old lambs that had access to an unfamiliar lamb and even more so if they had access to their twin. Also Rault *et al*. (2011) has found that artificially-reared lambs show strong distress behaviours when isolated, but less distress was shown by lambs that were placed with a penmate. Post-weaning group sizes of three lambs and above even nearly prevented stress-related vocalisations during separation. Number of penmates after weaning might therefore have a significant effect on welfare of the lamb. Orgeur *et al.* (1998) has studied the behavioural, hormonal and immune-pathological response in lambs that were progressively or suddenly weaned at three months of age using among other parameters, behaviours indicative of impaired welfare. They concluded that welfare is impaired even after weaning at 3 months of age, although not strongly. Behavioural disturbances were shown on the day of weaning and the two days after weaning. Another study found that lambs weaned at 1 or 2 days of age showed a lower weight gain (although rapidly recovering afterwards), a lower immune response, higher cortisol levels after isolation and changed behavioural response to an open field test (Napolitano *et al.* 1995; Napolitano *et al*. 2002). These lambs however seem to cope much better than lambs that are only prevented from suckling (Napolitano *et al*. 2003).

Altogether, impaired welfare during weaning is multicausal. Firstly, it is caused by the broken mother-lamb bond. Secondly, impaired welfare during weaning is caused by the unfulfilled need of lambs to suckle for non-nutritive comfort reasons. Thirdly, it is caused by the swift change in feeding habits, which depends on the level of milk yield of the ewe and the amount of solid feed the lamb was already used to (Arnold *et al.* 1979). Fourthly, the coinciding changes in the environment of the lamb, like changed pen (size) or (number of) playmates, can cause additional welfare impairment during weaning. Fifthly, infectious disease as a result of immunosuppression (a reduction in efficiency of the immune system) due to stress caused by the above four factors, can impair the welfare of the weaned lamb (Watson 1991; Dwyer 2008).

Animal welfare and affective states

In order to assess animal welfare, one should know what an animal's affective experience is in a certain situation. It is however difficult to judge if behaviour in certain situations represents emotional behaviour or just a functional response (e.g. Cockram 2004; Boissy *et al*. 2007). Some studies have moved beyond the simple description of an animal's behaviour towards an understanding of their emotional states (Désiré *et al.* 2002, 2004; Mendl and Paul 2004) by using an individual's appraisal of a situation and by focussing on manipulating and measuring cognitive processes. The more we know about an animals' affective experience, the better we can assess their welfare. Good welfare predominantly means the presence of positive affective states, i.e. a mere lack of poor welfare does not mean that the animal has good welfare (Seligman and Csikszentmihalyi 2000; Boissy *et al*. 2007). Therefore, the investigation of animal welfare should not only involve discovering indicators to assess negative affective states, but also indicators that assess positive affective states.

Negative and positive emotions both have the function to increase survival. However, negative affective states have evolved in 'need situations', as proposed by Fraser and Duncan (1998). In these situations an action is needed to cope with a threat to its fitness, e.g. dehydration or the approach of a predator. Positive affective states have evolved in 'opportunity situations'. In these situations they guide the animal's behaviour towards acquiring resources and experiences that may later enhance fitness, e.g. playing and exploring. They argue that the costs of performing this behaviour must be sufficiently low. Studies on finding indicators of positive affective states in farm animals to assess and improve their welfare are being increasingly performed (Harding *et al*. 2004; Paul *et al.* 2005; Boissy *et al*. 2007; Yeates & Main 2008). Earlier studies on sheep identified candidate indicators of negative and positive affective states (e.g. Reefmann *et al*. 2009a; Sanger *et al*. 2011).

Indicators of impaired sheep welfare

Several studies have looked at behavioural disturbances in sheep to different stressors, as for example cold or hot weather, shearing of the mothers, and tail docking (which is not allowed anymore in Sweden and The Netherlands). Behavioural and physical parameters shown to be related to events that impair welfare of sheep or that have the potential to be a useful parameter in assessing sheep welfare are: increased number of vocalisations (Porter *et al*. 1995; Dwyer *et al.* 1998; Orgeur *et al*. 1998), lower weight gain (Orgeur *et al*. 1999; Caňeque *et al*. 2001), less time spent feeding (Greiveldinger *et al.* 2007), less time spent ruminating (Fraser and Broom 1990), less time spent resting (Cockram *et al.* 1993; Orgeur *et al*. 1998; Réale *et al*. 1999 in mouflon), less time spent on comfort behaviour, the ears positioned more often in a backward posture, raised position and asymmetrical position and less often in a plane position (Reefmann *et al*., 2009a,b; Boissy *et al*. 2011), tail positioned more often in a posture more than 10 degrees from perpendicular to the backbone (Reefmann *et al*. 2009a), change in frequency of social contact (which can be hypothesized from Porter *et al*. 1995, Napolitano *et al*. 2003) and lower frequency of different play behaviours (Réale *et al.* 1999 in mouflon; Thornton & Waterman-Pearson 2002).

Play as indicator of good welfare

Play behaviour has been identified in a variety of species as a possible indicator of a positive affective state (Boissy *et al.* 2007). This is because play behaviour is sensitive to physical and environmental conditions (e.g. Newberry *et al*. 1988). Play decreases in conditions associated with poor welfare. Boissy *et al.* (2007) argue that play has a long-term payoff and therefore does not have an immediate high priority. Hence, if an animal shows play, it would mean that its other needs are met. It is a typical sign of satisfaction according to Boissy *et al*. (2007). Although play is present in almost all mammalian orders, suggesting a common ancestry, play behaviour is very diverse between as well as within species and scientists have a hard time explaining how and why it has evolved (Spinka *et al*. 2001). There are numerous definitions of play. The one that is most widely cited is from Bekoff and Byers (1981). *Play is all motor activity performed postnatally that appears to be purposeless, in which motor patterns from other contexts may often be used in modified forms and altered temporal sequencing.* According to Spinka *et al*. (2001), the main function is to allow animals to develop the physical and psychological skills to handle unexpected events in which they experience a loss of control. In addition, play behaviour is thought to train social skills (Van den Berg *et al*. 1999) and to develop the ability to express and understand intraspecific communicative signals, which inhibits aggression and increases group stability (Van der Schuren *et al*. 1997).

Since play behaviours and their accompanying triggers are very diverse, some play behaviours might be better indicators of good welfare in some species than in others. To understand what play in a species tells exactly about welfare, more fundamental and proximate questions should be answered and species-specific play-patterns should be described (Held and Spinka 2011).

Play in lambs

As in other animals, play behaviour in lambs entails locomotor play, social play and object play (Dugatkin 2010). Gambolling, i.e. jumping with stiff legs which leave the ground at the same time, is a very typical type of locomotor play in sheep. Locomotor play moreover involves jumping, jumping and kicking in the air, running, rearing and bucking. Social play includes mounting, butting, touching each other with a paw and standing on (most often) their mother with two or four paws. They form play bands of peers. Object play involves the use of inanimate object, such as stones, sticks, leaves etc. (Sachs and Harris 1978; Orgeur *et al*. 1995; Dugatkin 2010).

Play is probably a very promising indicator of a positive affective state in lambs, as play fighting rarely changes to fighting in this species, in contrast to piglets (Newberry *et al*. 1988). Play behaviours in lambs are relatively easy to define, which is not always the case in other species. Moreover, lambs have the reputation to play vigorously, which makes it easier to observe and analyse (Sachs and Harris 1978). Therefore, this behaviour appears to be advantageous to observe when investigating lamb welfare. Even more so because sheep are often described as behaviourally cryptic; assumingly the developed subtle behaviours as prey animals (Dwyer 2008), which makes it more difficult to assess their welfare. Little is known yet about the frequency and the time during the diurnal cycle that pre-weaned lambs perform play.

The relationship between play and good welfare in lambs has been shown by Chapagain (2012). She observed that, when given access to a play arena, both the anticipatory and consummatory phase of the reward system and possibly also the post consummatory phase (not obvious due to experimental methodology) are present in lamb pairs which were moved to a play pen that was larger than their home pen and enriched with some toys. Behavioural parameters indicative of the three phases were used in combination with cortisol measurement from saliva samples. Keeling *et al.* (2008) have hypothesized that the presence of the complete reward cycle results in the strongest positive affective state. Therefore, it can be hypothesized that play is very rewarding for lambs and thus a good indicator of good lamb welfare.

An example of how play is affected by poor welfare in lambs has been shown by Thornton and Waterman-Pearson (2002). The time spent performing play behaviour (gambolling) was reduced when one-week-old lambs were castrated. Moreover, play is reduced following the shearing of the mothers, amputation of the lamb's tails, and during cold weather, hot weather or rain (Sachs and Harris 1978). Gradual weaning at 14-15 weeks of age was found to have a negative effect on mounting behaviour, which is a form of play behaviour (Orgeur and Signoret 1984). No other literature was found about the effect of weaning on play behaviour in lambs. However, weaning has been found to reduce play behaviour in piglets (Donaldson *et al*. 2002) and calves (Krachun *et al*. 2010). In these animals weaning also consisted of both weaning from milk and separation from the mother, although in the calf study both procedures happened gradually over nine days.

Ontogeny of play in lambs

Lambs perform locomotor play behaviour within a few hours of birth, the onset is dependent on the breed (Dwyer 2009), as is play intensity, with dairy lambs mounting more often than mutton lambs (Górecki and Kieltyka 2012). It is reported that the play repertoire of lambs is well developed at one month post-partum (Orgeur and Signoret 1984; Lynch *et al*. 1992). Although Lynch *et al.* (1992) found that play behaviour can be seen in lambs of up to ten weeks of age, Orgeur and Signoret (1984) and Broom and Fraser (2007) reported, and this was also the experience of researchers at SLU (Chapagain, 2012), that play behaviour does not stop after ten weeks. Play is reduced as lambs continue to grow, and becomes rare by four months of age (Broom and Fraser 2007) or even later (Orgeur and Signoret 1984). It is also seen in young ewe groups and mature non-pregnant ewes, mostly with a positive energy balance (Lynch *et al.* 1992), i.e. when their energy intake is larger than their energy requirement. Only the study by Sachs and Harris (1978) has looked systematically at the ontogeny of play behaviour in domestic lambs. They found a bimodal pattern describing the ontogeny of play in lambs kept with their mother in groups in the period until ten weeks post-partum, with peaks of play (gambolling, mounting and butting) during week two to three and eight to nine. Although this study was done on lambs living in bigger groups and in bigger, partially roofed, pens outdoors, this pattern potentially occurs under circumstances of intensive systems as well. Yet extrapolation should be made with care. Górecki & Kieltyka (2012) did not find an effect of age on mounting behaviour in lambs of both a dairy and mutton breed of around 2.5 until 6.5 weeks of age kept in a building together with their mothers. Moreover, the study by Orgeur and Signoret (1984) did not show any such pattern in their observations of mounting behaviour.

Sex differences in play

In wild mountain sheep, males play more than females (Hass and Jenni 1993), and this is also known for domestic sheep. Lambs of domestic sheep clearly show sex differences in the types of play they exhibit. More males engage in more frequent mounting and butting and more females engage more frequently in locomotor play (Sachs and Harris 1978; Orgeur and Signoret 1984; Dwyer 2009; Górecki and Kieltyka 2012). This difference between the sexes may represent a difference in function; preparation for social and sexual interaction and animal maintenance (Lynch *et al.* 1992). In wild mountain sheep the butting difference between the sexes is reverse though, with females butting more (Geist 1971). It is suggested that the higher incidence of locomotor play in females of domesticated lambs ensures good physical fitness as the predation pressure on this sex would be greater due to their smaller size and the fact that they give birth out of the herd (Sachs and Harris 1978). But it might also result just from the lower butting and mounting activity. The sex of the twin lamb does not influence the play behaviour of females (Sachs and Harris 1978). Males however, mount females more often than males (Orgeur and Signoret 1984).

Weaning and welfare

Despite sex differences in play frequency and play type frequency, and insufficient knowledge of the ontogeny of play in lambs, play promises to be a good parameter for the assessment of lamb welfare. The contemporary method of artificial weaning in intensive systems, namely weaning from milk, separation from the ewe and additional coinciding (social) environmental changes, may compromise lamb welfare. A detailed description of the compromised welfare of lambs at weaning is not present in scientific literature. This study examines how strong and persistent changes in play behaviour are in relation to other behavioural disturbances, in order to give a founded answer on how welfare is compromised at weaning at around 8 weeks of age in intensive systems.

Aim

The aim of this study was to investigate in what way and for how long the welfare of lambs was affected by artificial weaning (including separation from the mother and concurrent changes in social environment and pen size) at around 8 weeks of age, by means of behavioural observations and measurement of weight gain. The focus was on play as an indicator of positive affect. Also, the existence of a diurnal cycle in play was investigated in order to be able to improve research methods. Regarding the main points of investigation, the following predictions are proposed. It is hypothesized that during and after weaning the number of recordings of play will decrease, the proportion of recordings of lying and ruminating will decrease, the recorded number of vocalisations and social contact with the twin lamb will increase and the proportions of recordings of backward, raised and asymmetrical ears and a high tail posture will increase. On the basis of the study by Orgeur et al. (1998), it is hypothesized that all changed behavioural parameters during and after weaning will attain their pre-weaning frequency again after two or three days. On the basis of observations on the farm and of the study by Sachs and Harris (1978), which describes an increase in play frequency around feeding, it is hypothesized that pre-weaned lambs will start to play when the staff arrives at around 07:00 a.m., as the arrival of staff is assumed to be associated with feeding. Also, they will play more frequently before and after actual feeding between 9:00 a.m. and 11:00 a.m. each day.

The study was conducted on sheep being part of a silage additive experiment on two types of additives to silage. Although there were no reasons to believe that the silage additives would have an effect on the behaviour of lambs, the study sample (including sexes) was balanced for the silage additive treatments to minimize a potential effect. The used models tested for possible effects of silage treatment and controlled for potential effects when testing other effects.

Material and methods

This study was carried out at Götala Beef and Lamb Research Centre outside Skara in Sweden. The Research Centre is managed by the Section for Production Systems at the Department of Animal Environment and Health, SLU. Both ewes and lambs were involved in a silage additive experiment and the present study was purely observational, therefore the approval from the Ethical Committee for Research on Animals in Gothenburg (Dnr. 212-20011) for using these experimental animals for the silage additive experiment sufficed.

Animals

Fifteen twins, 16 males and 14 females, with their dams (Finewool x Dorset) were used in the study (Table 1). The sire breed was Texel. The compositions of twins was five twins of two males, four twins of two females and six twins of one female and one male. The twins were born at the research farm, between 9 and 23 January 2012, after the ewes were brought there. The lambs were sprayed with green and purple pig paint (Kruuse, Porcimark) depicting different signs to be able to identify them quickly during observations.

Table 1. Individual lamb characteristics

Pen (Original pen)	Study	Silage additive	Weaning group	Sex	Weaning age (days)[a]
1 (1)	1,2	Control	3	M	60
	1,2	Control	3	M	60
2 (4)	1,2	Control	3	M	61
	1,2	Control	3	M	61
3 (5)	1,2	Control	2	F	62
	1,2	Control	2	F	62
4 (7)	1,2	Control	2	F	63
	1,2	Control	2	F	63
5 (8)	1,2	Control	2	M	54
	1,2	Control	2	F	54
6 (10)	1	Acid	3	F	59
	1	Acid	3	F	59
7 (11)	1	Acid	1	M	51
	1	Acid	1	M	51
8 (12)	1	Acid	1	F	55
	1	Acid	1	M	55
9 (13)	1	Acid	2	F	55
	1	Acid	2	M	55
10 (14)	1	Acid	1	F	50
	1	Acid	1	M	50
11 (17)	1	Salt	2	M	54
	1	Salt	2	M	54
12 (18)	1	Salt	2	M	57
	1	Salt	2	M	57
13 (19)	1	Salt	2	M	65
	1	Salt	2	F	65
14 (21)	1	Salt	3	F	62
	1	Salt	3	M	62

15 (23)	1	Salt	2	F	52
	1	Salt	2	F	52

a. The age of the lambs when the mother was removed from the home pen.

Housing and management

The twins were housed with their mother in pens of 2 x 3 m separately (Fig. 1). Only the lambs could walk out of the home pen trough a small gate to enter a larger area, called the playground (Fig. 2). On the playground they could meet other lambs from home pens that were also connected to the playground. The size of the playground and the number of lambs they could meet, ranging from 8 to 16 m^2 and from 1 to 6 lambs respectively, depended on the number of home pens that were connected. The substantial differences were due to limitations of the barn (Table 2). The gate to the playground was closed after the mother was removed from the home pen (see "Weaning procedure") to prevent the lambs from going to other ewes to suckle. The concrete floor was bedded with a thick layer of straw. More straw was added every week. The amount of straw added depended on the condition of the bedding. The dirtier, the more straw was provided, always resulting in a dry upper layer of straw. Natural light could penetrate through the windows.

Figure 1. Separate home pens with playground areas on the left.

Figure 2. Gate connecting home pen with playground for only the lambs.

Tabel 2. Distribution of experimental, subject and situational variables per individual lamb

Pen (Original pen)	Initial playgound size (m^2) (no.)	Initial playgound size/animal (m^2)[a]	Weaning area (m^2)	Initial no. of playmates (present study)[b]	Initial max. no. of playmates (present study)[c]	No. of playmates at weaning	Sex ratio m : f of initial playmates $\left(\frac{M-F}{M+F}\right)$.	Playmate age (days) on day before weaning (mean age)
1 (1)	12 (1)	6	6	1 (1)	4 (1)	1	1 : 0 (1)	59 (59)
	12 (1)	6	6	1 (1)	4 (1)	1	1 : 0 (1)	59 (59)
2 (4)	12 (2)	6	6	1 (1)	5 (3)	1	1 : 0 (1)	60 (60)
	12 (2)	6	6	1 (1)	5 (3)	1	1 : 0 (1)	60 (60)
3 (5)	12 (2)	3	6	3 (3)	5 (3)	1	2 : 1 (1/3)	53-61 (55.7)

	12 (2)	3	6	3 (3)	5 (3)	1	2 : 1 (1/3)	53-61 (55.7)
4 (7)	8 (3)	2	6	3 (3)	3 (3)	1	1 : 2 (-1/3)	53-62 (56)
	8 (3)	2	6	3 (3)	3 (3)	1	1 : 2 (-1/3)	53-62 (56)
5 (8)	8 (3)	2	6	3 (3)	3 (3)	1	0 : 3 (-1)	53-62 (59)
	8 (3)	2	6	3 (3)	3 (3)	1	1 : 2 (-1/3)	53-62 (59)
6 (10)	8 (4)	4	6	1 (1)	1 (1)	1	0 : 1 (-1)	58 (58)
	8 (4)	4	6	1 (1)	1 (1)	1	0 : 1 (-1)	58 (58)
7 (11)	12 (5)	2	6	5 (5)	5 (5)	1	3 : 2 (1/5)	47-54 (50.4)
	12 (5)	2	6	5 (5)	5 (5)	1	3 : 2 (1/5)	47-54 (50.4)
8 (12)	12 (5)	2	6	5 (5)	5 (5)	1	4 : 1 (0.6)	47-54 (49.6)
	12 (5)	2	6	5 (5)	5 (5)	1	3 : 2 (1/5)	47-54 (49.6)
9 (13)	12 (5)	6	6	1 (1)	5 (5)	1	1 : 0 (1)	54 (54)
	12 (5)	6	6	1 (1)	5 (5)	1	0 : 1 (-1)	54 (54)
10 (14)	12 (6)	3	6	3 (1)	5 (1)	1	2 : 1 (1/3)	47-49 (47.7)
	12 (6)	3	6	3 (1)	5 (1)	1	1 : 2 (-1/3)	47-49 (47.7)
11 (17)	12 (7)	2	6	5 (5)	5 (5)	1	4 : 1 (0.6)	53-64 (58.6)
	12 (7)	2	6	5 (5)	5 (5)	1	4 : 1 (0.6)	53-64 (58.6)
12 (18)	12 (7)	2	6	5 (5)	5 (5)	1	4 : 1 (0.6)	53-64 (58)
	12 (7)	2	6	5 (5)	5 (5)	1	4 : 1 (0.6)	53-64 (58)
13 (19)	12 (7)	2	6	5 (5)	5 (5)	1	4 : 1 (0.6)	53-64 (56.4)
	12 (7)	2	6	5 (5)	5 (5)	1	5 : 0 (1)	53-64 (56.4)
14 (21)	16 (8)	4	6	4 (0)	10 (3)	1	3 : 1 (0.5)	59-61 (62.5)
	16 (8)	4	6	4 (0)	10 (3)	1	2 : 2 (0)	59-61 (62.5)
15 (23)	16 (8)	2.67	6	6 (3)	10 (3)	1	3 : 3 (0)	51-56 (54.5)
	16 (8)	2.67	6	6 (3)	10 (3)	1	3 : 3 (0)	51-56 (54.5)

a. Playground size (m^2) divided by the number of playmates + 1 (the lamb itself) before weaning step one.

b. Number of playmates during the week before the mother was removed from the home pen; when the observations were done.

c. Maximum number of playmates that could reach the playground in the entire period until weaning step one.

Feeding

The sheep were fed between 9:30-11:00. They received grass silage, consisting of *Festulolium* and *Phleum pratense*, with 26% of red clover which was harvested on 2 June 2011 at Brogården. Depending on the treatment of the silage additive experiment, the sheep received silage treated with a salt-based additive (Kofasil Ultra K, 2 litres/ton forage at harvest); Addcon Europe GmbH), silage treated with an acid (GrasAAT Plus, 3 litres/ton forage at harvest; Addcon Nordic) or silage treated with no additive at harvest. The following feeding scheme was used. Before weaning the ewe was fed 0.5 kg of ewe concentrate (Tacka Lantmännen) per day, from which the lambs nibbled a bit as well. Subsequently they got silage, which both the lambs and ewe ate. Additionally, the lambs got 0.8 kg of lamb concentrate (Lamm 500, Lantmännen) per twin per day, which the ewe could not reach. The day before weaning no more ewe concentrate was provided. After weaning, the lambs were fed silage and lamb concentrate. They always had access to water in a trough, a mineral lick and a bucket with mineral supplements. They ate the straw from the floor of their pen as well.

Silage additive experiment

For the silage additive experiment the twins were divided into three groups; a group receiving silage treated with the acid additive, a group receiving silage treated with the salt-based additive and a control group receiving silage without additive (Table 1). The twins and sexes of the sample in the present study were evenly distributed among the control group and silage treatment groups with 5 males and 5 females in the control group, 5 males and 5 females in the acid silage group and 6 males and 4 females in the salt silage group.

Weaning procedure

Weaning was performed in two steps dividing three periods. The first period involved the period before weaning, therefore called 'before weaning'. The second period involved the period after weaning step one when the mother was removed from the home pen and placed in a pen in the same barn, within hearing distance but out of sight of her lambs. This period lasted 5 days and was called 'during weaning'. The third period involved the period after weaning step two when the mother was removed from the barn. This period was called 'after weaning'. The terms 'before weaning', 'during weaning' and 'after weaning' are extensively used in this report, to differentiate between the three periods.

The moment of weaning step one depended on the weight of the lambs. The yardstick for this moment was around 25 kg. Due to practical reasons actual weaning weights deviated somewhat from this value. The mean weight at weaning step one was 28.83 kg (± 0.55 SE, *N*=30). This resulted in substantial differences in weaning age, which was on average 57.33 days (± 0.85 SE, *N*=30), and entailed a range of 50 days until 65 days post-partum. At weaning step two the lambs were between 55 and 70 days old.

In order to keep to the yardstick of 25 kg, the 15 twins were weaned over a period of three weeks (Fig. 3). This resulted in 3 weaning groups, one group per week. Weaning step one took place on Thursday at 13:30 p.m. Weaning step two took place every subsequent Tuesday at 13:30 p.m. The first weaning group consisted of three twins, the second weaning group of eight twins and the third weaning group of four twins (Table 1).

Experimental design

In order to investigate both (1) the effect of weaning on welfare and (2) the diurnal cycle of play in lambs, two different recording methods were used. (1) To investigate the effect of weaning, direct behavioural observations and weight measurements were done over a period of around 4 weeks respectively on all 15 twins. The behavioural observations took place before weaning, during weaning and after weaning, to be able to analyse potential differences between these periods. (2) The diurnal cycle of play was investigated by means of indirect observations pre-weaning through video recordings of the 5 twins (Table 1) receiving silage without additive. In both studies, one person was responsible for the observations.

(1) Lamb welfare: Direct observations

In order to be able to compare the behaviour (potentially) indicative of affective state of the lambs between the weaning periods, observations were done before (3 sessions, spread over 3 days), during (5 sessions, spread over 6 days) and after weaning (5 sessions, spread over 6 days). These periods were chosen as Orgeur *et al.* (1998) found that lambs show behavioural disturbances for only two days after the day of weaning.

Before the observations took place, the observer had habituated the lambs to her presence. This habituation phase entailed standing near the home pens and walking alternately between pens. During the observations the observer was only standing in the alley in front of the home pens; hence this is what was practiced. No contact with the sheep was made. The habituation phase took around seven days during which a decline in fear responses was noticed until no fear response at all was visible, with complete ignorance in some lambs.

As there were three weaning groups (see Weaning procedure), one group weaned each week, and a maximum of one session per weaning group per day was done, the schedules of the separate groups ran parallel to cover all periods for all twins (Fig. 3). The lambs were observed over a period of 26 days, from 6 March until 1 April 2012. The youngest lamb was observed at an age of 43 to 69 days and the oldest was observed at an age of 56 to 82 days.

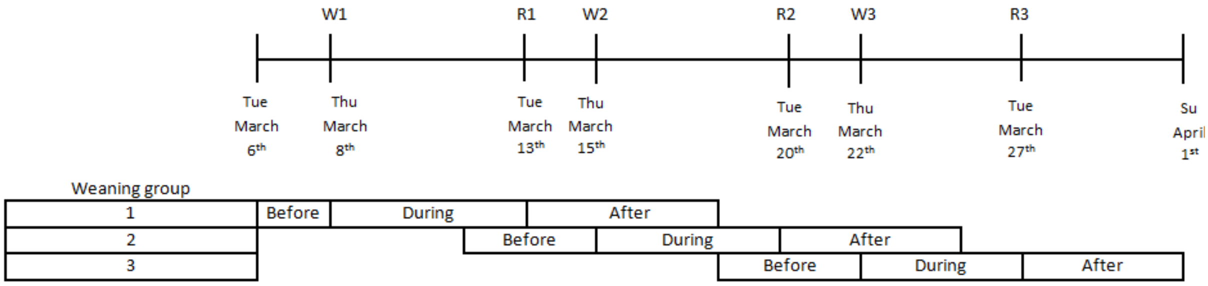

Figure 3. Time schedule of weaning of the three weaning groups defined in Table 1 and the corresponding periods before (3 sessions), during (5 sessions) and after weaning (5 sessions). W stands for withdrawal of the mother from the home pen, R for removal of the mother from the barn. The number of the weaning group concerned is added.

Sessions were scheduled in the morning (from 9:30) and/or afternoon (from 16:00). During one session the number of twins in the focal weaning group (see Weaning procedure) were observed in order of distance to the barn entrance, i.e. the shortest distance for the observer to walk through the barn, in order to disturb the lambs the least. One minute was taken to walk from one pen to the next and habituate the twin to the presence of the observer. This was repeated in six consecutive rounds (Fig. 4).

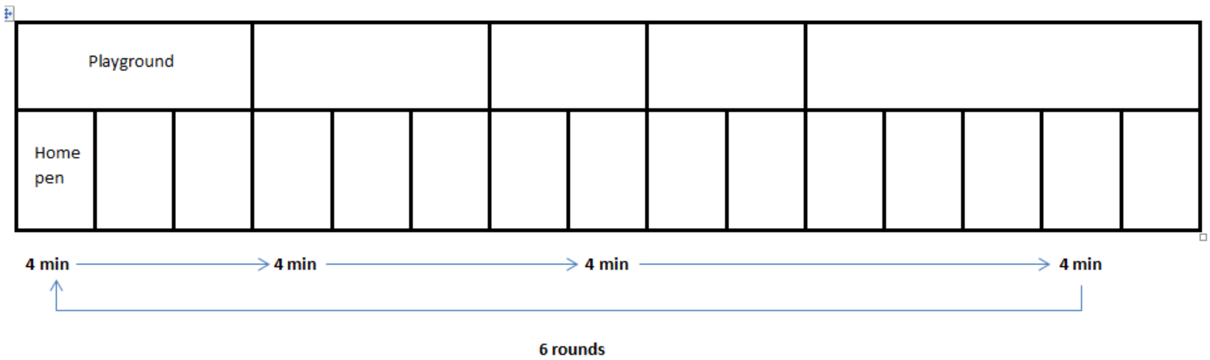

Figure 4. Example of recording schedule. In reality other home pens or empty space could be between the 15 home pens of the present study.

Four minutes of observation on one pen (twin) gave 24 (6x4) minutes of observation per session on one twin.

Behavioural measurements

The four minutes of observation were divided into 16 15s intervals during which two individuals of a twin were alternately observed. Therefore, the measurement unit was the individual lamb. During 15 s of observation per lamb, two techniques were used to record behaviour of the focal lamb.

First, time sampling was performed at the beginning of each 15 s by instantaneous observation of the focal lamb. With one sample point per 30 s, every individual was scored (8x6) 48 times per session. This resulted in a total score per lamb of 144 sample points before weaning, 240 sample points during weaning and 240 sample points after weaning. The behaviours that were scored were part of four categories, i.e. body movement, behaviours, ear posture and tail posture. Within these groups behaviours are mutually exclusive and cover 100% of the recordings (see the ethogram in Table 3).

Second, the frequency of play behaviours, social contact and vocalisations were noted during 15 s (see the ethogram in Table 4), as these were events of short duration that were easily missed with an instantaneous scan sampling technique. This meant that each lamb was observed for a total of two min (8 times 15 s) per round, 12 (6x2) min per session. In that way each lamb was observed 36 min before weaning, 60 min during weaning and 60 min after weaning. The diverse play behaviours were categorized for locomotor play and social play. For the behaviours of social play and for social contact, additionally the recipient sheep was noted. Before weaning this could be the twin lamb, another lamb, the mother or another ewe. During and after weaning this could be the twin lamb (in the same home pen) or another lamb or ewe (the neighbours through the fence).

Table 3. Ethogram of lamb behaviours recorded instantaneously

Behaviour	**Definition**
Body postures	
Lying	The weight of the lamb is supported by the belly and/or side of the lamb which contact(s) the ground.
Standing	All feet touching the ground, body not moving.
Moving	At least three legs are moving and the body is displaced in a forward or backward movement. Also the transitions between standing and lying are included, i.e. standing up or lying down.
Behaviours	
Ruminating	Bringing up swallowed food, chewing it and swallowing it again. The period between swallowing and bringing it up is included, as long as it does not exceed 3 s.
Eating concentrate	Head of the lamb is or has been in the bucket which is filled with lamb or ewe concentrate and the lamb is chewing.
Eating Silage	Head of the lamb is or has been in the bucket filled with silage and the lamb is chewing.
Eating straw	Lamb is chewing on straw or something undetermined which it has picked up from the ground.
Drinking	The muzzle of the lamb touches the water in the water bowl and makes drinking movements. Drinking is often audible.

Sniffing pen	Olfactory exploration of the pen. The lambs nose is at maximum 5 cm. from the pen bars, drinking bowl or feeding bowls while the nostrils move. The lamb often stretches its neck a bit.
Comfort behaviour	Rubbing the body against the pen, objects or pen mates. Scratching the body with the hind leg is also included. Stretching the body is only included after resting (Wemelsfelder and Farish 2004), as this looks like displacement behaviour.
Other behaviour	All other behaviours not defined above.
Postures	
Ear posture	1) Raised 2) plane 3) backward or 4) asymmetrical ears (after Boissy *et al*. 2011).
Tail posture	1) Tail not raised 2) Tail raised more than 10 degrees until 90 degrees from the perpendicular to the backbone 3) Tail raised more than 90 degrees from the perpendicular to the backbone (partly after Reefmann *et al*. 2009a).

Table 4. Ethogram of lamb play and social behaviours recorded in frequency per 15 s

Behaviour	**Definition**
Locomotor play behaviours	
Running	A gait in which all the feet can leave the ground and in which the feet touch the ground only shortly. Faster than walking. Movement in forward direction. Not scored when chased away by (another) mother or running to the mother to drink milk, nor when running away from the staff.
Jumping	The forelegs leave the ground before the hind legs. The body does not touch the floor. In contrast to gambolling, not all legs leave the ground at the same time.
Jumping and kicking	Kicking one or both hind legs in the air while jumping.
Gambolling	Jumping, but with the four legs leaving the ground at the same time. The legs are rigid.
Rearing or bucking	Only the front legs or hind legs leaving the ground and kicking.
Pawing object	Using one of the front legs to touch an object, like the ground, pen bars or feed bucket.
Social play behaviours	
Mounting	Animal stands on hind legs and raises front to come on top of another sheep and touch it with its chest. The place where it touches the sheep could be the head, front, side or back. Attempts are included as long as the two front legs have left the floor.
Butting	Any contact in which the head is involved with some strength. The head is lowered. It can be directed to a head or to another part of the body of the recipient. When head to head, it can be unidirectional but is mostly bidirectional.
Standing	Standing with two or four legs on another sheep.
Pawing Sheep	Using the front leg to touch another sheep.
Other behaviours	

Social Contact	Sniffing another sheep, with the nose within 5 cm of any part of the body of the other sheep and moving the nostrils. Often stretching the neck. To score a second time, the nose should have left the 5 cm. area.
Vocalisations	Each audible bleat.

Weighing and feed intake

The lambs were weighed once a week on Thursdays in a scale standing in the alley outside the pens.

Feed intake was measured per pen per day by deducting the weight of the feed left at the beginning of the next day from the weight of the feed given the previous day. As this meant that the number of sheep eating from the food differed between weaning periods (before weaning feed intake was measured for the mother, twin and an occasional unrelated lamb together, and after weaning only for the twin), feed intake was not included in the analysis.

Subject and situational variables

There are a number of variables that might have had an effect on the behaviours measured in the present study and that potentially have affected the results of the present study (Table 2). These will be analysed and discussed.

(2) Diurnal play cycle: Indirect observations

In order to investigate the existence of a diurnal play cycle in lambs, the frequency and moment during the day of locomotor and social play of pre-weaned lambs were recorded over 24 h. For this purpose video recordings over 24 h (from 0:00 to 23:59:59) were used that were made at a maximum of five days before weaning of the 10 lambs that did not receive a silage treatment (control group) (Table 1). The video recordings were made between 12 and 21 March 2012, when the age of the lambs ranged between 53 and 61 days at the day of recording.

Cameras were placed above the home pens as well as above the playgrounds. They were connected to a computer with monitor. For recording and analysis the programs MHS-video server and MHS-video client (M. Shafro & Co., Latvia) were used respectively.

Behavioural measurements

The behaviours that were observed and categorized as social play were the same as in the direct observations (Table 4). However, the behaviours that were observed and categorized as locomotor play were only running and jumping, as only these behaviours could be recognized on the videos. The frequency of these behaviours was recorded over 24 hours, divided in 24 blocks of one hour, for each lamb.

Data analysis

To analyse the results Genstat for Windows (2004) 7th Edition VSN International Ltd., Hemel Hempstead, UK, was used. All behaviours were analysed statistically.

(1) Lamb welfare: Direct observations

During 192 minutes of observation per twin, (24 min. x 13 days), 368 observations of play, 1187 observations of social contact and 1885 observations of vocalisations were recorded and analysed in the study on the effect of weaning on lamb welfare in 15 twins (N=15). Moreover, 1248 scans per twin (96 scans x 13 days) were made of body, ear and tail posture and feeding behaviour. This resulted in a total of 18720 scans for 15 twins. All the behaviours were analysed, as were the different categories that they included.

All behaviours, recorded as frequencies or instances, were analysed using a Restricted Maximum Likelihood analysis (REML), which accounts for repeated observations on the same individual or twin in the same treatment (e.g. period of weaning). All behaviours were analysed independently (no multivariate analysis). The mixed model consists of a fixed and a random part as to correct for repeated measures on an experimental unit.

First, a basic model was used to find out whether weaning period and/or sex had an effect on the recorded behaviours. The fixed part of the basic model consisted of the independent variables 'silage additive treatment + (sex * weaning period)'. Silage treatment was entered in the model first to correct for its potential effect, and was shortly described subsequently as it is the least important in the present study. Sex is put in the model, because it is expected to have an effect on behaviour. Weaning period is the most important variable tested. The random part consisted of the repeated measurements 'twin, individual', to correct for observations on the same units lamb and twin. The same tests were done for the frequency observations as the instantaneous observations. Although the instantaneous observations were first converted into percentages per lamb and session, see below.

Predicted means of the constant were generated for all behaviours using the basic model. Predicted means and standard errors for the behaviours that were recorded in frequencies were calculated from 36 min. observations before weaning (12 min. x 3 days) and 60 min. (12 min. x 5 days) during and after weaning. This resulted in a total of 2340 records. The predicted means were given in number of recordings per 2 minutes per lamb. Predicted means and standard errors for the behaviours that were recorded instantaneously were calculated in percentage of time that the behaviour was performed. Instantaneous recordings were originally recorded as 48 sample points per lamb and session. In order to analyse the instantaneous recordings, they were first converted to percentages per lamb and session (number of observations of behaviour/total number of observations per lamb and session*100) resulting in 3, 5 and 5 values per lamb for the period before, during and after weaning respectively. This means that the percentages are based on 288 scans (3 days, 96 scans per day) before weaning and 480 scans (5 days, 96 scans per day) during and after weaning. For the analysis of the tail postures, the observations were deleted when the lamb was lying, defecating or urinating. Lying observations were deleted because the tail posture depended on how the lamb was lying and if it was lying against the fence, or a little pile of straw. Therefore, 9357 scans were deleted as the lamb was lying, 332 scans were deleted as the body posture was missing and an additional 156 scans were deleted as the tail posture was most probably a result of defecating or urinating. This left 8876 scans for the analysis. With these data the REML analyses was done and predicted means were calculated. In case there was an effect of a variable (as result of a Wald test for fixed effects), the standard errors of differences (sed) per contrast made clear which predicted means differed. There was a significant difference if the difference between the predicted means was larger than twice sed.

Since the chance on low values of behavioural recordings was higher than on high values in the dataset, plots of residuals against fitted values were made to check whether they tended to look like a loudspeaker shape and deviated from normality, i.e. whether the model could estimate the lower values better than the higher values. For all analyses, natural logarithm transformations of the data were conducted in order to find out whether these data were better described by the model. For the frequency data (log (behaviour +1)) was used, and for the instantaneous data (log((behaviour+0.01)/(100-(behaviour-0.01)))). When the log data were better described by the model than the raw data, this p value was used, however, predicted means were used from the analysis of the raw data. The method without log transformation most often appeared robust enough.

Second, to evaluate whether the effects measured were indeed caused by the variables themselves and not by any potentially confounding subject and situational variables (Table 2), Fisher exact tests were employed on cross tabulations of the variables sex and these variables. No variables could be confounding weaning period, as all the lambs went through all the weaning periods. Chi-square test for independence was not possible, since >20% of the expected values in the cross tabulation were <5. Therefore, Sisa's Five by Two exact Fisher table program was used (http://www.quantitativeskills.com/sisa/statistics/fiveby2.htm). It resembles the Chi-square test for independence, and accounts for small cell values (<5) and very uneven marginal values. Although the test can handle small cell values, the results should not be over-interpreted.

Third, in order to investigate which subject and situational variables (Table 2) had an effect on the performance of the behaviours recorded, the basic model was modified. The variable ‘sex’ in the basic model was changed into the given subject or situational variable, all variables were entered as (co)variates as they were continuous and a linear effect was expected. Analyses for every variable were run on all behaviours, again with the raw data and with log transformation. For the variable sex ratio values of -1, -0.5, 0, 0.5 and 1.0 were used.

Fourth, in order to evaluate whether the effects measured in the third step were indeed caused by the variables tested, Chi square tests were carried out on cross tabulations of rxc (rows x columns). Cells deviated significantly from expected values when the residuals where larger than 2 or smaller than -2. Cell values were at times small, and results should not be over-interpreted.

Fifth, REML analyses were also employed to investigate whether some measured behaviours that were affected by weaning period, were also affected by days around weaning. In this way it could be investigated how long a behavioural change lasted after the mother was removed from the home pen. In order to do this, data of some sessions was removed and of some other sessions was added to the dataset, as the original dataset included 2 sessions that were conducted on the same day as another session. In order to get sessions per lamb on different days covered for all twins, 7 sessions were removed and 1 other sessions was added, i.e. the datasheet included behavioural measurements on 2 days before weaning and 10 days after weaning with only one session a day. This dataset included 17280 records. The model used was silage additive + (days around weaning * sex) and a spline function was used. The latter optimizes the use of degrees of freedom and allows curves other than the linear function.

(2) Diurnal play cycle: Indirect observations

In the study on the diurnal cycle of play in pre-weaned lambs, a total of 1027 recordings of play were noted and analysed from a 24h video (from 0:00 till 23:59:59) of 10 lambs (5 twins). The units that were analysed were the individual lambs (*N*=10). The lambs of a twin were observed on the same day pre-weaning. Because the two lambs of a twin also played with each other, the data were not independent. This was not considered a problem, as the lambs also played with other playmates. And as the video observations were time-consuming, observing 5 twins (10 lambs) was beneficial over 5 lambs, i.e. one per twin. Data were analysed as if they were not interrelated.

Again a Residual Maximum Likelihood analysis (REML) was used to see whether differences in play were found between hours of the day. The model used was Age + Number of playmates + Sex + Hour of the day. The random part consisted of twin and lamb.

First, a spline function was used for the raw datasheet, and for datasheets describing values per 2 and 3 hours. This did not seem to work, as the predicted mean of hour 0:00 was nothing like hour 23:00. Therefore, Hour was converted into a Factor and the normal analysis was done on the datasheet with data per hour.

Results

(1) Lamb welfare: Direct observations

Behavioural observations

Most behaviours were affected by weaning period and some by sex or silage additive (Table 5 and 6). There were however a few behaviours that were neither affected by weaning period nor sex.

Weaning effect

There was a significant effect of weaning period on total play, social play and locomotor play (Fig. 5). The number of recordings per 2 minutes of total play and social play decreased during weaning and subsequently reached the level of before weaning again in the period after weaning. The number of recordings per 2 minutes of locomotor play also decreased during weaning, and after weaning it was in between the levels before and during weaning and did not differ significantly from either one. Per hour, the numbers of recordings of the behaviours before, during and after weaning were respectively: total play: 6.870, 2.160 and 5.742; social play: 4.188, 1.725 and 4.098; and locomotor play: 2.681, 0.436 and 1.646 per hour.

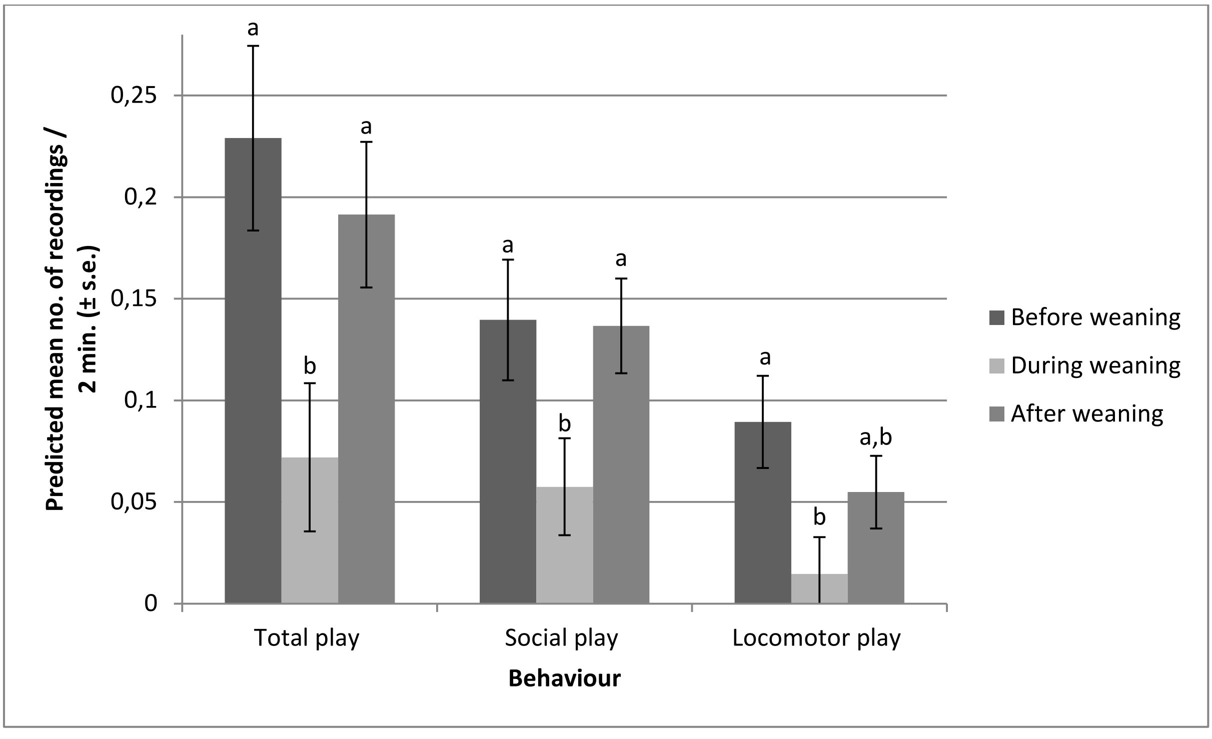

Figure 5. Predicted mean number of recordings (± s.e.) of play behaviour per 2 minutes over the weaning periods, based on 2340 records on 15 twins. Total play = Social play + Locomotor play. Letters denote differences in number of recordings of a behaviour between weaning periods. (Wald tests, Total play: X^2_2= 10.17, *P*=0.006; Social play: X^2_2=8.06, *P*=0.018; Locomotor play: X^2_2=7.97, *P*=0.019).

Social play with the twin lamb was steady over the periods before (0.049±0.028/2 min.) and during weaning (0.053±0.024/2 min.) and significantly increased in the period after weaning (0.119±0.023/2 min.) (*P*=0.019).

The number of recordings per 2 minutes of social contact was constant over the period before and during weaning and decreased in the period after weaning (Fig. 6). Social contact with the twin lamb increased in the period during weaning to decrease again to the level of before weaning in the period after weaning (Fig. 6). Social contact with other lambs decreased in the period after weaning (0.091±0.017/2 min.) compared with the periods before (0.138±0.022/2 min.) and during (0.148±0.018/2 min.) weaning (P=0.021). The number of recordings per 2 minutes of vocalisations increased in the period during weaning, while it attained the same level as before weaning in the period after weaning (Fig. 6).

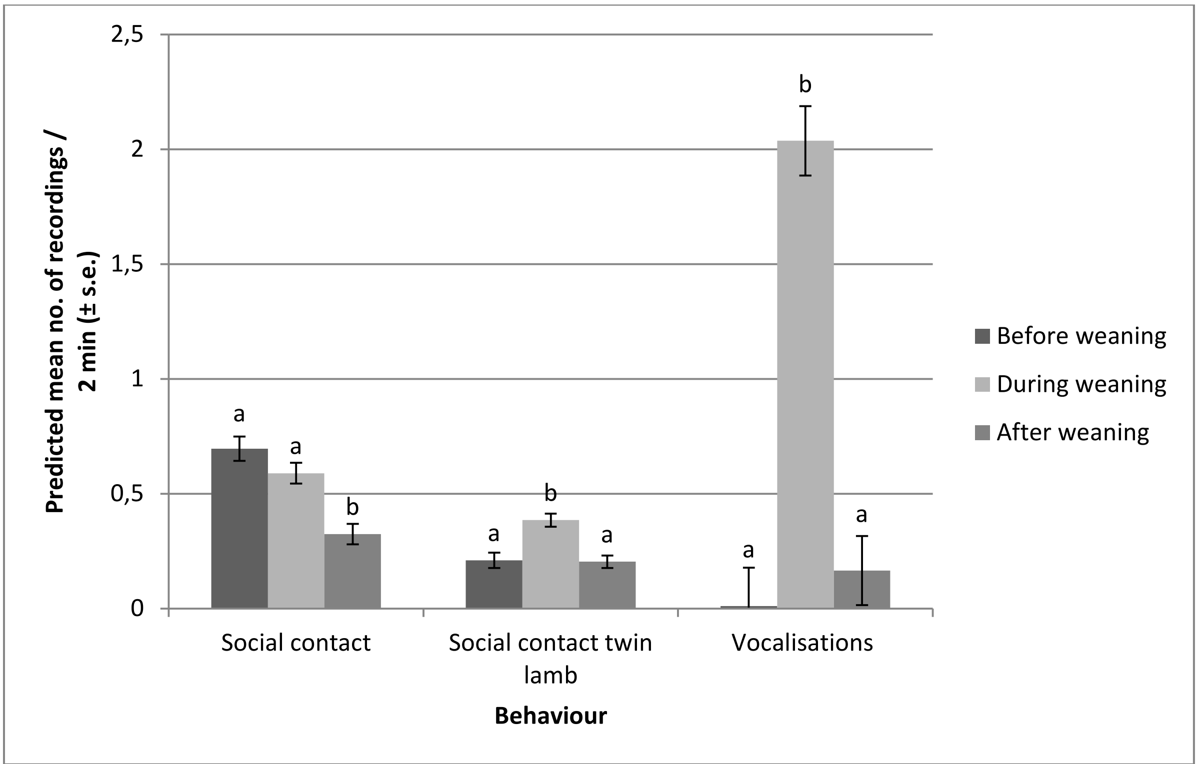

Figure 6. Predicted mean number of recordings (± s.e.) of social behaviour per 2 minutes over the weaning periods, based on 2340 records on 15 twins. Letters denote differences in number of recordings of a behaviour between weaning periods. (Wald tests, Social contact: X^2_2=47.56, P<0.001; Social contact with the twin lamb: X^2_2=38.49, P<0.001; Vocalisations: X^2_2=283.74, P<0.001).

The percentage of observations that the lambs were lying decreased in the period during weaning (Fig. 7). After weaning it was in between the percentage before weaning and during weaning and did not differ significantly from either one. The percentage of observations standing tended to increase in the period during weaning (Fig. 7). After weaning it was again between the percentages of before and during weaning and did not differ significantly from either one. The percentage of observations moving was steady before (4.3±3.6%) and during (4.8±0.4%) weaning and decreased in the period after weaning (3.6±0.4%) (P=0.037).

The plane ear posture was shown less in the period during weaning than before weaning (Fig. 7). After weaning the lambs showed the posture more than during weaning, but not as much as before weaning. The raised ear posture was shown more in the period during and after weaning than before weaning (Fig. 7). The asymmetrical ear posture was shown more in the period during weaning than the period before weaning (Fig. 7). After weaning it was below the level of the period before weaning. The percentage of observations that a backward ear posture was shown was not affected by weaning period (38.4±2.5%).

The tail positioned zero degrees from perpendicular to the backbone was shown more in the period after weaning than in the period before and during weaning (86.6±1.1%, P=0.005). The tail positioned ten degrees from perpendicular to the backbone was shown less in the period after weaning than in the period before and during weaning (11.0±0.9, P=0.027). The tail positioned ninety degrees from perpendicular to the backbone was shown less in the period during and after weaning than before weaning (Fig. 7).

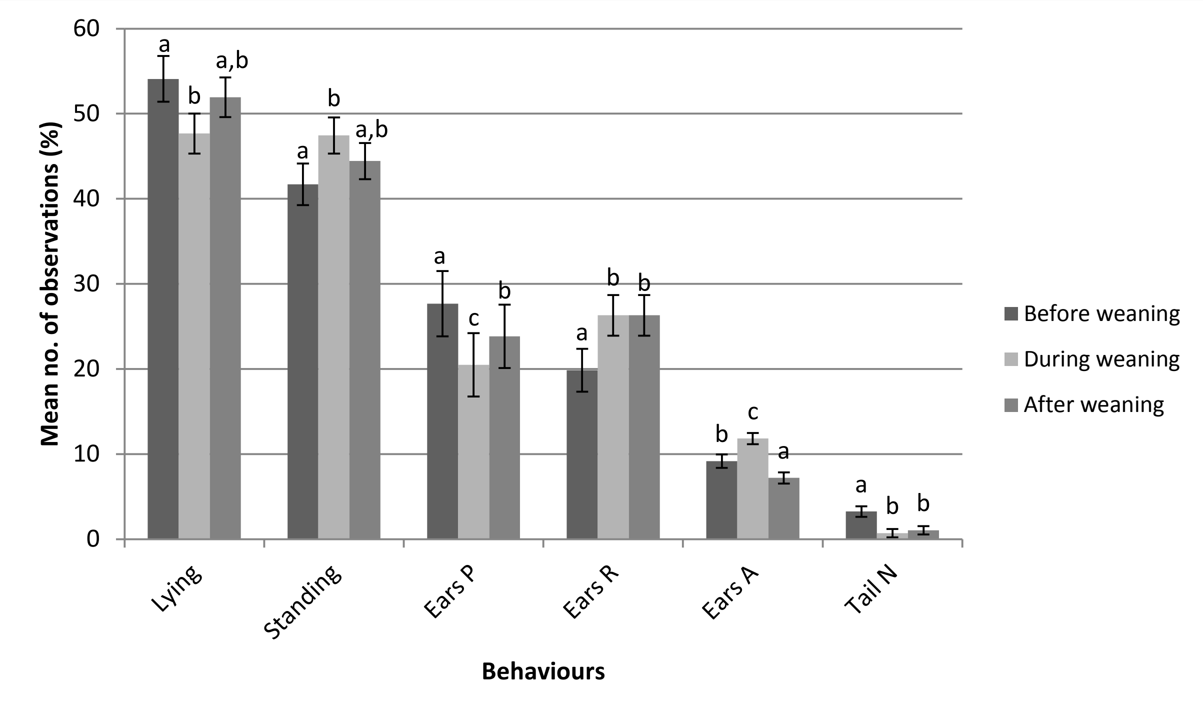

Figure 7. Predicted mean percentage of observations (± s.e.) that body, ear and tail postures are shown over the weaning periods, based on 18720 records on 15 twins. Letters denote differences of a behaviour between weaning periods. Ears P: ears in plane position, Ears R: ears in raised position, Ears A: ears in asymmetrical position, Tail N: tail ninety degrees from perpendicular to the backbone. (Wald tests, Lying: X^2_2=6.89, P=0.032; Standing: X^2_2=5.95, P=0.051; Ear in plane position: X^2_2=15.97, P<0.001; Ears in raised position: X^2_2=19.33, P<0.001; Ears in asymmetrical position X^2_2=42.41, P<0.001; Tail ninety degrees from perpendicular to the backbone: X^2_2=10.86, P=0.004).

The percentage of observations that the lambs were eating concentrate increased over the periods (4.4±0.8%, 7.2±0.7% and 9.0±0.7% for before, during and after weaning respectively) (P<0.001).

There was a significant effect of time in weeks on weight gain (X^2_{11}=59.41, P<0.001), however reductions in weight gain did not occur directly after the mother was removed from the home pen.

Sex effect

The mean number of recordings per 2 minutes of total play and social play were larger for males than for females (Fig. 8). Males tended to show more social play with the twin lamb than females did (Fig. 8).

The interaction effect of weaning period and sex on social play with other lambs (P<0.001) and other ewes (P=0.013) were in fact main effects of sex, because play with the lambs and ewes was very rare during and after weaning. It was shown that only the males showed social play with the other lambs in the period before weaning (0.103±0.017/2 min.) and it (almost) ceased after weaning step one; it was rarely shown trough the bars during or after weaning. The numbers of recordings on social play with the other ewes were however too low to analyse for sex differences. Females performed less social play than males, but the play they did perform was mainly directed to their twin lamb and less to their mother; they barely played with the other ewes or other lambs. Males played as much with other lambs as with their twin lamb, and less with the mother. They barely played with the other ewes.

Males tended to perform more social contact than females (Fig. 8). When it comes to the direction of the contact, males performed significantly more social contact with the twin lamb than females (Fig. 8). Other forms of social contact did not differ between the sexes. The number of recordings of vocalisations was larger for females than for males (Fig. 8).

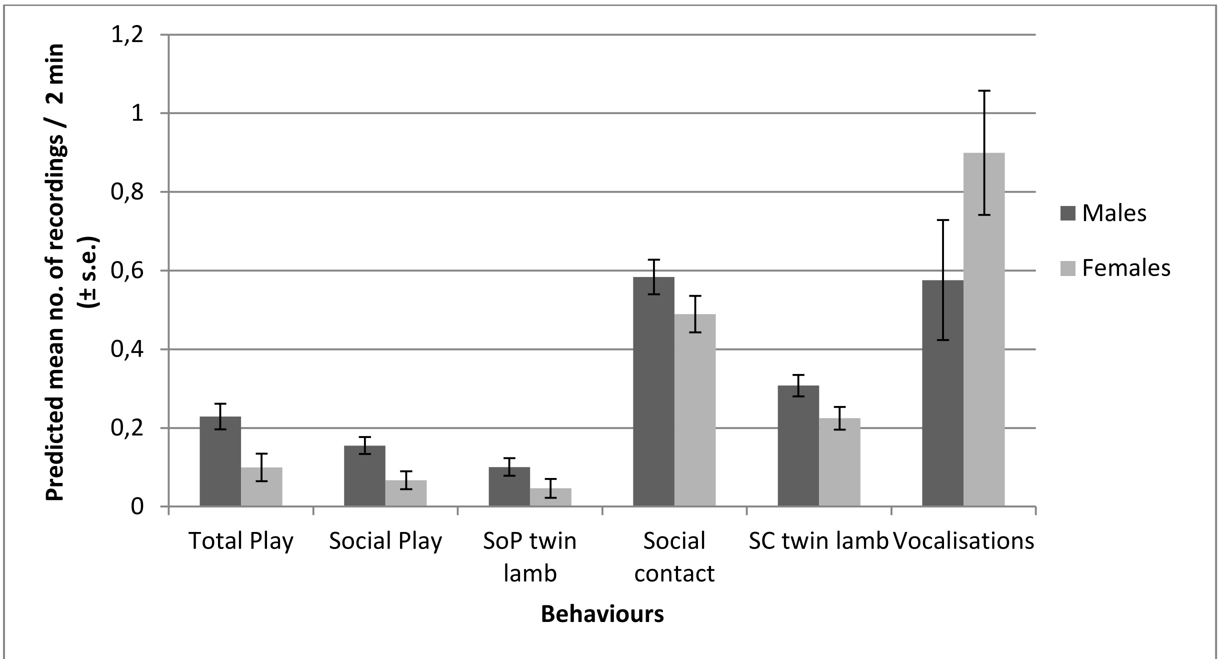

Figure 8. Predicted mean number of recordings (± s.e.) per 2 minutes per sex over all weaning periods, based on 2340 records on 15 twins. (Wald tests, total play X^2_2=6.06, P=0.014; social play X^2_2=7.11, P=0.008; social play with the twin lamb X^2_2=3.71, P=0.054; social contact X^2_2=3.70, P=0.054; social contact with the twin lamb X^2_2=6.92, P=0.009 and vocalisations X^2_2=5.03, P=0.025).

There were no significant effects of sex on the % of observations of body postures, ear postures, tail postures or eating concentrate.

Potentially confounding subject and situational variables

As several variables could not be controlled in the present study (Table 2), tests were carried out to investigate whether they had had any effect on the results through dependence with the distribution of sexes (See Appendix B for cross tabulations and Fisher exact tests on various subject and situational variables). The variable weaning period could not be affected by any of the variables in Table 2, as all the lambs went through all the weaning periods. However, sex could possibly have been balanced unevenly with these subject and situational variables, resulting in sex effects that could actually have been caused by the other variables.

The only variable that was distributed not independently of sex over the sample was playground size. The sum of the probability of the observed sequence of scores in the cross tabulation and scores that are less likely to occur by chance is 0.03278 (i.e. 3%) and therefore it significantly deviates from expected values when they would be independent. Males were mainly placed on playgrounds of $12m^2$, while females were more evenly spread over the playgrounds of different sizes.

The sex of the lamb was independent of age at weaning (P=0.25079), playground size per lamb (P=0.2167), the number of playmates on the playground in the week before weaning (P=0.06448) (although there is a tendency, no clear pattern is visible), the maximum number of playmates on the playground during the lamb's life (P=0.18924), the sex ratio of the playmates on the playground in the week before weaning step one (P=0.38894) and the mean age of the playmates on the playground in the week before weaning step one (P=0.35285).

Silage additive effect

The lambs fed the silage treated with the salt-based additive showed more social play with the lambs than the control group, while the group fed the silage treated with the acid-based additive showed a level in between the other groups not differing significantly from either level (P=0.024). The group fed silage treated with the acid-based additive ate concentrate for a higher % of observations than the group fed silage treated with the salt-based additive (P=0.017), while the control group was in between both treatment groups not significantly differing from either level. The group fed silage treated with the acid-based additive ate silage for a higher % of observations than the control group (P=0.011), the group fed silage treated with the salt-based additive did not differ from either level.

Effects of other variables and confounding factors

During the observations, there were several subject and situational variables that could not be controlled and could have had an effect on the behaviours measured (Table 2). Moreover, an effect of one variable might in fact be an effect of (one or more) other variables or a joint effect of two or more variables. From Chi square tests on the cross tabulations of the variables (Table 2) in Appendix C, it appears that the number of playmates on the playground in the week before weaning step one, maximum number of playmates on the playground during the lamb's life, playground size before weaning and potentially sex ratio of the playmates on the playground in the week before weaning were positively confounding. However, counts were very low, often below 5. Therefore, mentioned confounding effects should be handled with caution.

To investigate whether subject and situational variables (Table 2) could have had an effect on the behaviours, tests were carried out to analyse the effect of each variable and its potential interaction effect with weaning period on all the behaviours. Silage additive treatment was again added to the model to correct for it. The detailed results are shown in Appendix D.

Total play (P=0.010) and social play (P=0.014) were affected by playground size in the period before weaning (when the data were log transformed for a better fit). A larger playground in the period before weaning caused more social play (0.05±0.04/2 min., 0.12±0.02/2 min. and 0.19±0.04/2 min. for the playground sizes 8, 12 and 16 m^2 respectively), and thus more total play overall (0.08± 0.06/2 min., 0.17±0.03/2 min. and 0.27±0.07/2 min. for the playground sizes 8, 12 and 16 m^2 respectively). The increase in social play was again the result of an increase in social play with the twin lamb (when the data were log transformed for a better fit) (P=0.028) by lambs that were on larger playgrounds in the period before weaning (0.00±0.04/2 min., 0.08±0.02/2 min. and 0.16±0.04/2 min. for the playground sizes 8, 12 and 16 m^2 respectively).

Locomotor play (P=0.013) and thereby total play (P=0.037) were affected by sex ratio. Lambs that had playmates with a higher sex ratio, (i.e. increasingly more males than females as playmates in the week before weaning step one) showed more total play (0.06±0.06/2 min., 0.10±0.04/2 min., 0.15±0.03/2 min., 0.20±0.03/2 min. and 0.24±0.04/2 min. for a sex ratio of -1, -0.5, 0, 0.5 and 1.0 respectively), because of an increase in locomotor play (-0.01±0.03/2 min., 0.02±0.02/2 min., 0.04±0.01/2 min., 0.07±0.01/2 min. and 0.10±0.02/2 min. for -1, -0.5, 0, 0.5 and 1.0 respectively).

There was an interaction effect of playground size before weaning and weaning period on social play with the mother (P<0.001). However, this interaction effect was actually a main effect of playground size, as the mother was removed during and after weaning. Lambs that were on smaller playgrounds in the period before weaning showed more social play with the mother than lambs on larger playgrounds (0.07±0.01/2 min., 0.03±0.01/2 min. and -0.01±0.01/2 min. for the playground sizes 8, 12 and 16 m^2 respectively). In fact, it was not or rarely shown by lambs that had access to the largest playground of 16m^2. Therefore, removing the mother at weaning step one only caused a decrease in social play with the mother on playgrounds of 8 and 12 m^2. Moreover, there was an interaction effect of sex ratio of the playmates in the week before weaning and weaning period on social play with the mother (P<0.001). This interaction effect was also a main effect of sex ratio of the playmates in the week before weaning, as the mother was removed during and after weaning. Lambs on playgrounds with playmates that had a lower sex ratio showed more social play with the mother than lambs with playmates with a higher sex ratio (0.07±0.01/2 min., 0.05±0.01/2 min., 0.04±0.01/2 min., 0.02±0.01/2 min. and 0.01±0.01/2 min. for a sex ratio of -1, -0.5, 0, 0.5 and 1.0 respectively). The lambs that had a very high sex ratio barely showed any social play with the mother and therefore no reduction when she was removed from the pen. Therefore, there was only a decrease during weaning in the lambs with playmates that had a sex ratio of -1, -0.5 or 0. It is difficult to explain the actual mechanism that caused a reduction of social play with the mother in some lambs, due to the potential confounding of playground size and sex ratio of the playmates before weaning.

Lambs with playmates that had a higher sex ratio performed more vocalisations overall (0.34±0.24/2 min., 0.50±0.18/2 min., 0.66±0.15/2 min., 0.82±0.15/2 min. and 0.97±0.19/2 min. for a sex ratio of -1, -0.5, 0, 0.5 and 1.0 respectively) (P=0.037).

Recovery time

To investigate how the behaviours varied over the days around weaning and how many days it took until the behaviours reached levels of before weaning again (recovery time), tests were performed on behaviours recorded on 2 days before weaning and 10 days after weaning on the same 15 lamb twins using the same test as used above.

A number of behaviours varied over the days around weaning (Fig. 9 and 10). Total play was first analysed using the raw data, which did not give a significant effect of the day around weaning (Wald test, X^2_1= 0.08, P= 0.778). However, as total play did not have a high variation, sums were calculated to get values per lamb per 12 min (i.e. a session or day). This did not result in a significant effect of days around weaning either (Wald test, X^2_1= 0.10, P=0.753).

There was however a significant effect of days around weaning on the frequency that the lambs showed social contact with the twin lamb (P<0.001) (Fig. 9). Day -1 (the day before weaning with the larger frequency of social contact with the twin lamb of the 2 days before weaning) was taken as reference for comparison with the other days. Not only did it increase after weaning, it also regained the level of before weaning on the second day after weaning.

There was also a significant effect of days around weaning on the number of vocalisations (P<0.001) (Fig. 9). Vocalisations started when the mother was removed from the home pen. With day -1 (the day before weaning with the larger frequency of vocalisations of the 2 days before weaning) as reference, the increase in vocalisations regained the level of before weaning after the second day after weaning step one, but in any case on the fourth day. Probably the level was reached on the third day; however no measurements were done on this day.

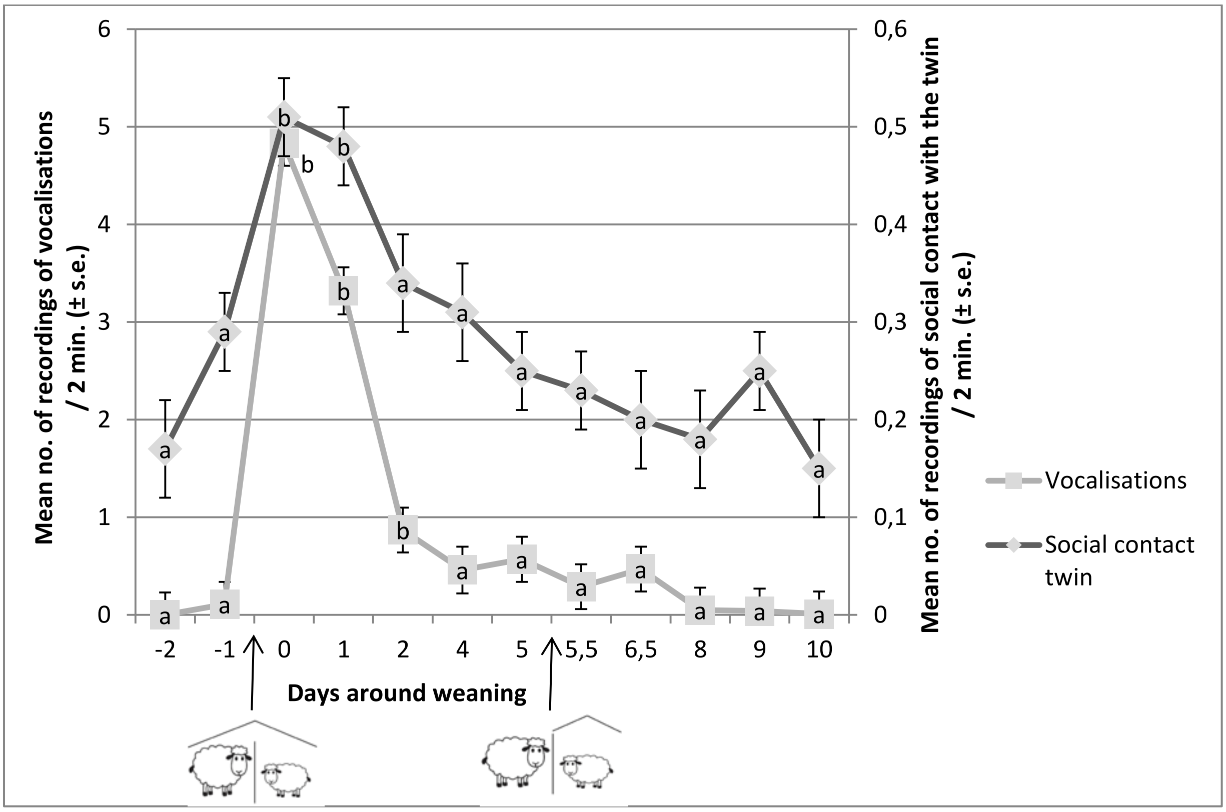

Figure 9. Predicted mean number of recordings (± s.e.) per 2 minutes of social contact with the twin lamb and vocalisations in the days around weaning, based on 2160 records on 15 twins. (Wald tests, social contact with the twin lamb X^2_1=16.40, P<0.001; vocalisations X^2_1=134.45, P<0.001).

Although there was an effect of weaning period on lying, there was only a tendency of days around weaning on this behaviour (X^2_1=3.44, P=0.064). However, a decrease in percentage of observations lying was shown directly after weaning. There was no effect on percentage of observations standing (X^2_1=0.49, P=0.482). Although there was an effect on moving (X^2_1=11.65, P<0.001), there was no difference between the moment before and after weaning step one. Only the day after weaning the percentage of observations moving was increased compared with the days before weaning. Already on the second day after weaning levels were normal again.

There was no effect of days around weaning on percentage of observations ruminating (X^2_1=0.38, P=0.536) and percentage of observations eating silage (X^2_1=0.23, P=0.631). There was a significant effect on eating concentrate (X^2_1=30.47, P<0.001), however, differences were large between all days, not specifically between the moment before and after weaning step one. Again there was a significant effect on comfort behaviour (X^2_1=5.78, P=0.016), however there was no difference between the moment before and after weaning step one.

The percentage of observations of raised ears increased after the mother was removed from the home pen and regained its previous level on day three or four after weaning (Fig. 10). The exact day cannot be determined as no observations were done on day three after weaning. On day 9 and 10 levels rose again. Day -1 (the day before weaning with the larger mean percentage of observations of raised ears of the 2 days before weaning) was taken as reference. The percentage of observations that asymmetrical ears were shown increased as well after the mother was removed from the home pen (Fig. 10). On day two after weaning it regained its previous level. On day 10 the level is lower than before weaning. When values were larger than the value at Day -1, then this day was taken as a reference to determine deviations. When values were smaller than Day -2, then this day was taken as a reference. The percentage of observations of backward ears was steady over all days of observation (Wald test, X^2_1 = 0.32, P= 0.572).

There was an effect of days around weaning on the percentage of observations that the lambs had their tail positioned zero degrees from perpendicular to the backbone (X^2_1= 9.25, P=0.002), however, there were only differences between other days than the day before and after weaning. The same holds for the percentage of observations that the lambs had their tail positioned ten degrees from perpendicular to the backbone (X^2_1= 10.93, P<0.001). And although there was a significant effect of weaning period on the time that the tail was positioned ninety degrees from perpendicular to the backbone, there was no significant effect of days around weaning on this tail posture (X^2_1= 2.45, P=0.118).

There was an interaction effect of sex and days around weaning on the ear posture plane ears. However, the percentages of observations that the plane ear posture was shown per sex did not vary at all over the days (Fig. 10).

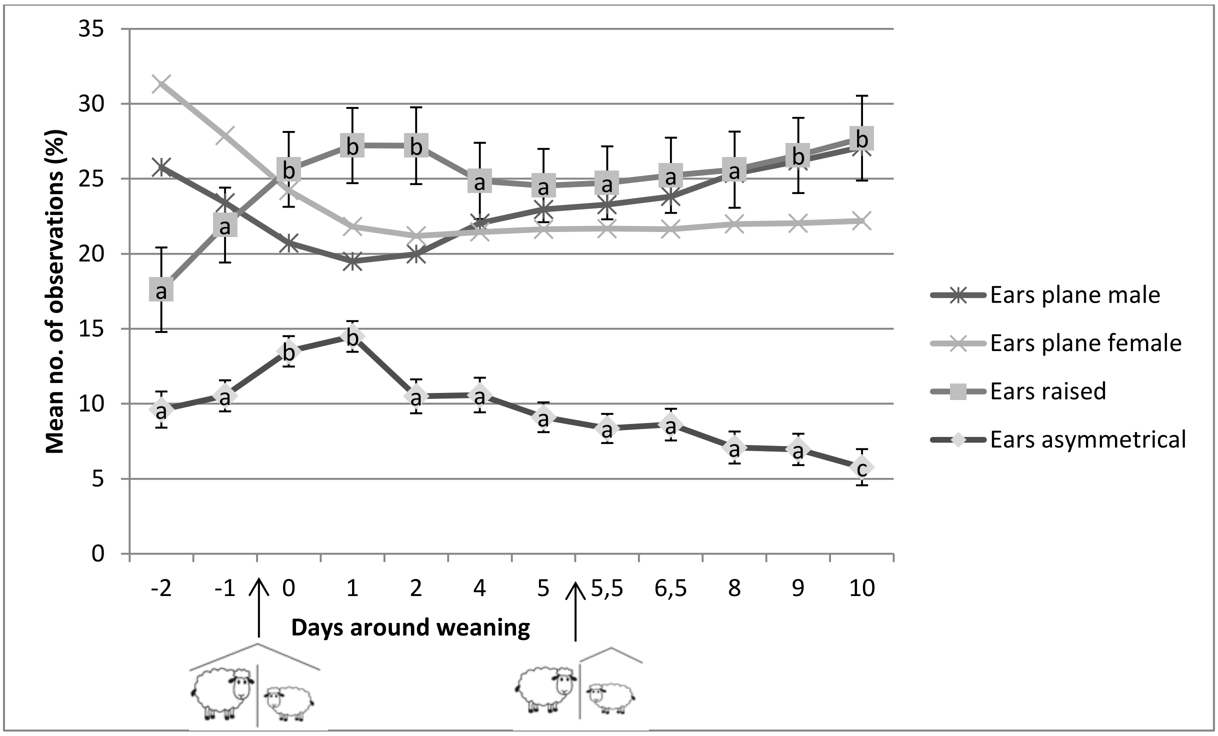

Figure 10. Predicted mean percentage of time that ear postures are shown in the days around weaning, based on 17280 records on 15 twins. Of the plane ear posture, only the lines are given. (Wald tests, Ears plane, interaction effect between sex and days around weaning X^2_1=5.58, P=0.018; Ears raised X^2_1=5.98, P=0.014; Ears asymmetrical X^2_1=35.40, P<0.001).

(2) Diurnal play cycle: Indirect observations

Total play, and both its components social play and locomotor play, were separately analysed over the hours of the day in the pre-weaned lambs (Fig. 11). There appeared to be a diurnal cycle in the play of the lambs. Three peaks of play overall (total play) can be distinguished, at the 7th hour (7:00-8:00), one at the 9th hour (9:00-10:00) and one at the 19th hour (19:00-20:00).

The hour of the day has an effect on both social play (Wald test, X^2_{23} = 40.77, P=0.013) and locomotor play (Wald test, X^2_{23} = 45.91, P=0.003). Social play behaviour levels differ from the lowest values at the 7th (7:00-8:00), 9th (9:00-10:00) and 19th (19:00-20:00) hour. The same holds for locomotor play, which levels were also elevated at the 7th, 9th hour and 19th hour.

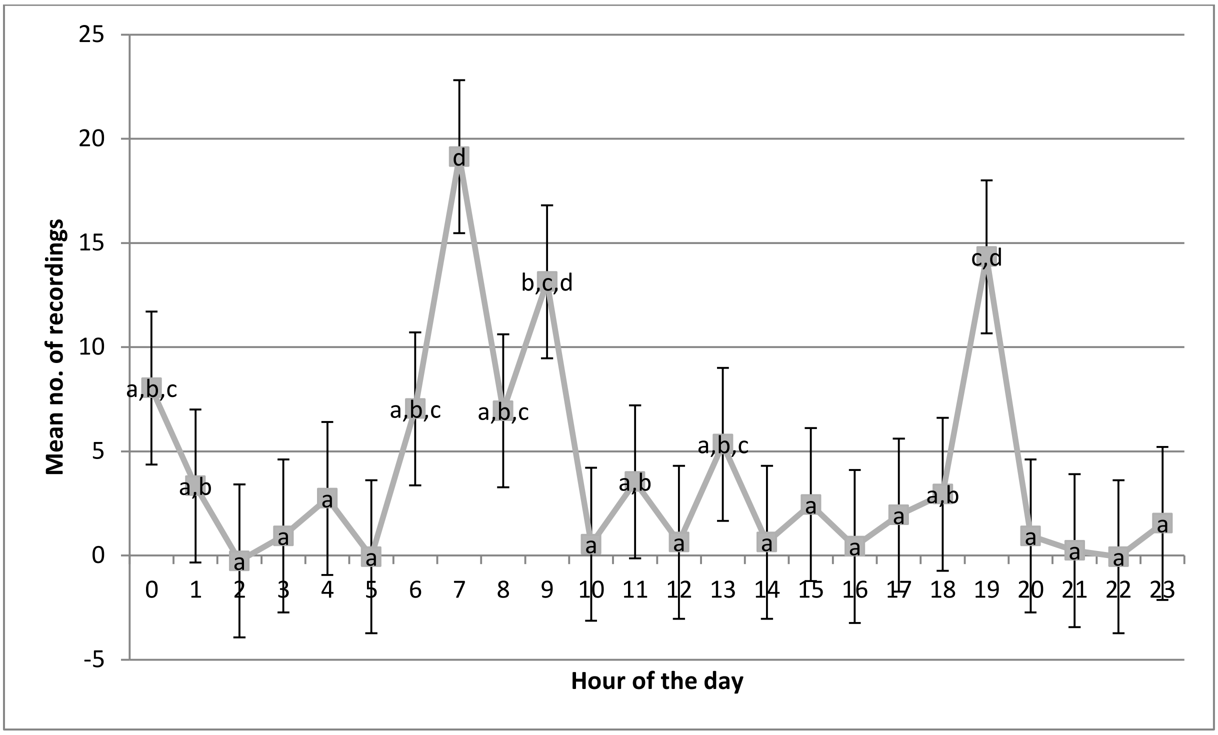

Figure 11. Predicted mean number of recordings (± s.e.) of total play per hour in pre-weaned lambs, based on 240 records on 15 twins. (Wald tests, total play X^2_{23}=45.21, P=0.004**).

Discussion

The present study assessed if and for how long the welfare of lambs is affected by artificial weaning, meaning separation from the ewe and additional changes in social environment and pen size, at around 7-8 weeks of age. Also, the diurnal cycle of play in lambs was investigated. In accordance with the study of Orgeur *et al.* (1998) on lambs of 3 months of age, impairment of welfare was found in the presently weaned lambs of 50-65 days of age. Removal of the mother from the home pen to another pen in the same barn, within hearing distance but out of sight of her lambs (and the concurrent reduction of space and separation of playmates by bars; during weaning), evoked changes in behavioural parameters used as indicators of affective state that lasted 2 to 4 days. The removal of the mother from the barn after another 5 days (after weaning) did not seem to have an effect on the behavioural parameters and weight gain. Parameters indicative of negative affect in the study lambs seemed to be increased social contact with the twin, increased frequency of vocalisations and decreased proportion of time lying, whereas play was assumed a parameter indicative of positive affect. Moreover, ear postures appeared to be a promising indicator of affective state in lambs.

In line with literature, play behaviour during weaning was initially reduced and subsequently normalized to the original level after weaning. This was true for both social and locomotor play. Surprisingly, there was no effect of days around weaning on play, i.e. there was no abrupt reduction in play following the moment after weaning; the reduction was rather gradual and formed a reduction over several days during weaning. Differences may be more profound in dairy sheep (Gorecki & Kieltyka, 2012), and more research is needed to determine breed differences.
The decrease in play during weaning might have been (partly) caused by a reduction in area and playmates, as they lost playground area at weaning step one. However, an increase in play was shown in the period after weaning, to equal the level of before weaning. This might be because the lambs were getting used to a smaller area, however more likely is that the increase in play is a recovery of play after weaning. The finding that locomotor play frequency after weaning was in between the levels of before and during weaning and did not differ from either one, may be caused by the reduction in playground, which probably makes it harder to perform locomotor play. The limited space before, as well as during and after, weaning might also be a reason for suppressed play behaviour. Perhaps more play behaviour is shown in a larger area, and consequently a larger reduction in play after weaning. The time related changes in play behaviour were not shown for any of the individual social behaviours, i.e. defined as the sheep at which the social behaviour was directed (twin lamb, mother, other ewes or other lambs). This may be due to the method of weaning, that is to say a complete separation of the mother, a partial separation from other ewes and lambs by means of bars and no separation from the twin lamb. Therefore, the decrease in total social play in the period during weaning is explained by a cessation of play with the mother and a near cessation of play of the males with the other lambs (only some butting and pawing through the bars), as only the males play with the other lambs (see below).

Although play frequency with the twin lamb was equal before and during weaning (and only increased after weaning), two opposing processes might be occurring during weaning. Considering the increase in play frequency with the twin lamb after weaning, it appears that the twin lamb functions as a substitute playmate for the mother and the other lambs. Play frequency with the twin lamb increases only after weaning, possibly when the lambs recovered from the negative affective state they experienced during weaning. The idea that the twin lamb functions as a substitute playmate is clearly shown by the time course of overall social play frequency. After weaning, the social play frequency equals the level of before weaning. This means that the lambs show as much social play after weaning as before, only the play partner in part changed from the lamb's mother to its sibling. It cannot be ruled out that lambs also were comforted by playing with their sibling during weaning.
Moreover, the result that the twin lamb compensates for play with the mother and other lambs after weaning, does not automatically mean it completely fulfils the lamb's needs for social play, i.e. although the frequency of social play after weaning equals the level before weaning, it does not say the change in playmates has no effect on welfare. It can be said that welfare impairment due to changed penmates is not likely to be major, as after weaning other behaviours indicative of affective state also regain levels of before weaning.

The same reasoning can be applied to social contact. The frequency of this behaviour did not change during weaning, but did decrease after weaning. The steady number of recordings of social contact over the period before weaning and during weaning is explained by an increase in social contact with the twin lamb, a cessation of social contact with the mother and a reduction in social contact with the other ewes. Therefore, it can be concluded that the loss of social contact with the mother was (partly) compensated by contact with the twin lamb, i.e. the twin again functions as a substitute. After weaning, social contact with the twin decreased again to the level of before weaning. Possibly, the lambs have a transient need for increased social contact with the twin during weaning. After weaning, there might have been no need to seek comfort anymore, as it is no longer in a negative affective state. When looking at the effect of days around weaning, it appears that the level of social contact with the twin recovers to its normal level already on the second day. Increased social contact seems to be a good indicator of a negative affective state induced by weaning.

As shown by among other researchers Porter *et al*. (1995) and Orgeur *et al*. (1998), vocalisation frequencies increased during weaning indicating a negative affective state. Like play behaviour, levels before weaning were regained after weaning. When looking at the effect of days around weaning, it appears that the level of vocalisation frequency recovers to the level before weaning on the third or fourth day. This could not be determined exactly, as there were no observations done on the third day. Bleats were rarely heard before weaning and made a steep rise in frequency during weaning. This established indicator of negative affect constituted a very strong base to infer the meaning of play behaviour in terms of affect.

Other behaviour indicative of a negative affective state was lying, and indeed the percentage of observations that this behaviour was performed decreased during weaning. However, days around weaning only tended to have an effect on the behaviour. Nonetheless, a decrease in time lying was shown directly after weaning step one. Standing behaviour tended to increase during weaning and moving did only increase after weaning. Only the day after weaning the percentage of observations of moving was higher compared with the days before. This was not yet shown during the afternoon directly after weaning. There were no differences in body postures the moment before and after weaning step one. And it seems that body posture is not a very good indicator for affective state, or that display time of body postures only changes when affective state is affected more intensely.

Feeding time was expected to decrease during weaning; however, this was surprisingly not the case. Lying and ruminating are associated in dairy cows and relate negatively with feeding (Schirmann *et al.* 2012), but here the time the lambs ate silage or ruminated was constant over the weaning periods and the time they ate concentrate increased over the periods. It seems that feeding is not a good parameter for measuring affective state, because it is a primary need for animals and is fulfilled irrespective of affective state. Also, no decrease in weight gain was found around weaning step one. The steady time lambs spent on comfort behaviour was a surprise, as a decrease in percentage of observations was expected following literature. There were no significant differences in time that the behaviour was performed around weaning.

Weaning period had a strong significant effect on three out of four ear postures, in line with expectations based on the study of Boissy *et al.* (2011). Surprisingly, the time that the backward ear posture was shown during weaning did not increase, unlike we expected based on literature (Reefmann *et al.*, 2009a,b; Boissy *et al.* 2011). Boissy *et al.* (2011) reported that the plane ear posture is shown when the sheep are in a neutral affective state, the raised ear posture in negative controllable situations and asymmetrical ear posture in response to sudden events. The studies of Reefmann *et al.* (2009a,b) used other ear descriptions, however they are roughly comparable and showing the same results, besides that the plane ear posture (described as the passive ear posture) is also found indicating a positive affective state. Weaning is a negative sudden uncontrollable situation. The observation of the increase in time that the asymmetrical ear posture was shown is in line with Boissy *et al.* (2011), indicating stress. The increased time of raised ear posture might mean there were certain aspects that the lamb could control, e.g. it could (partly) compensate the loss of social play and contact with the lambs and other ewes, by social play and contact with the twin lamb. Another explanation would be that raised ears are also shown in uncontrollable situations.
After weaning, the time that the different ear postures were displayed did not regain levels of before weaning. The time that raised ears were displayed stayed the same as during weaning, and the time that asymmetrical ears were displayed reached a level even below the level before weaning. It can be argued that the lambs experience a negative affective state still more often than before weaning, however they are recovering and more often feel they can control the situation again (decrease in time displaying an asymmetrical ear posture). They still might feel a negative affective state because of the changed environment, i.e. the reduction in playmates and the loss of playground (steady display of raised ear posture during and after weaning). Alternatively, the increase in age may have played a role.

The only extensive study carried out so far to my knowledge that tries to link tail posture to affective state is the study of Reefmann *et al.* (2009a). Although this study found a high proportion of sheep's tails being raised during separation of group members, they did not find it useful for determining affective state. They argue that, based on their study, literature on suckling and qualitative observations, a raised tail (described as higher than 10 degrees from perpendicular to the backbone) indicates a strong emotional activation, being either positive or negative. Therefore, the lambs were expected to display a tail positioned 10-90 degrees or >90 degrees for a longer period of time during weaning than before weaning. After eliminating observations in which the lamb was lying, defecating or urinating, it turned out not to be true. In fact, the posture more than 90 degrees from perpendicular to the backbone was shown less often during and after weaning than before weaning. There was an effect of days around weaning on this posture, however there were no differences between the days before weaning and the days thereafter. The other tail postures only differed in the period after weaning. Again there was an effect of days around weaning on the posture 10-90 degrees from perpendicular to the backbone, however there were no differences between the day before weaning and the days thereafter. Days around weaning did not affect the posture >90 degrees from perpendicular to the backbone at all. These results are difficult to explain. Sheep do not appear to use the tails for communication (Dwyer 2008), as dogs do. Therefore, the change in social environment over the weaning periods did probably not cause the decrease in time of the display of the posture >90 degrees from perpendicular to the backbone. Maybe this weaning method did not cause strong emotional activation, as they still had their twin and could see and contact with other ewes and sheep through the bars, i.e. in contrast with the study of Reefmann *et al.* (2009), where they could not see any sheep. One can argue that if observations of raised tails during defecation and urination would have been included in the analyses, the results would have been different because of their possible relation with affective state. This is however unlikely, as a difference in time urinating and defecating is probably minor, observations of defecation and urination were fairly spread over time. Another explanation for the results would be that tail posture is not a very reliable indicator for affective state.

Effect of sex

As expected, we found differences in the behaviour of male lambs and females, especially regarding play. Surprisingly, locomotor play did not differ between the sexes, while several studies found that females engage in locomotor play more often than males. Based on literature (Sachs and Harris 1978), social play was expected to be performed more by males than females. Social play with other lambs was found almost exclusively in males in this study, while females mainly played with their twin lamb. They both played with the mother, but less than with the twin lamb, other lambs or other ewes. Social play with the ewes was barely shown. There were two interesting findings; males performed significantly more social contact with the twin than females, and females performed significantly more vocalisations than males. A possible explanation for both findings could be the difference between the sexes in the relationship with the mother. Females tend to stay close to their mothers for a longer period of time than males (Guilhem *et al.* 2006). Therefore, it might be more stressful for the female than for the male to lose its mother. The males are more often at a distance from their mother, and may therefore have more opportunity to play with their twin.

Due to the finding that the study subjects took part in another experiment (a silage additive experiment), different variables may have affected the present outcomes and tests were carried out to find out whether there had been any confounding variables. The only variable that could have been confounding with sex was playground size. The other subject and situational variables were balanced well across sexes.

Effect of silage treatment

Silage treatment had an effect on some behaviours, although not strongly. The group fed silage treated with the acid-based additive spent more time eating silage than the group fed silage treated with the salt-based additive. To infer whether they were just trifling with their silage or actually preferred it over silage treated with the salt-based additive, it should be compared with feed intake. Feed intake was, however, not measured in a precise way and therefore not suitable for the analysis. The time spent eating concentrate was equal between the groups fed the additive-treated silages, only the control group spent less time eating concentrate than the group fed the silage treated with the acid-based additive.

Effects of subject or situational variables

In order to find out how behaviours may have been affected by any of the subject or situational variables, additional tests were carried out. Confounding effects were found for the number of playmates on the playground in the week before weaning step one, maximum number of playmates on the playground during the lamb's life, playground size before weaning and sex ratio of the playmates on the playground in the week before weaning. However, p-values for the effect of weaning period on behaviour did not vary much when such variables were put into the statistical model. This suggests that the model that was used to examine the weaning effect, sex effect, treatment effect and interaction effect of weaning period and sex was valid.

Worthwhile mentioning regarding the confounding variables is that social play with the twin, and therefore social play overall, increased with increasing playground size in the period before weaning. Lambs that were on smaller playgrounds showed more social play with the mother than lambs on larger playgrounds and it was not or rarely shown by lambs that had access to the largest playground of $16m^2$. There might be some confounding here with sex ratio, as this effect was also significant. A male biased sex ratio resulted in less social play with the mother. The reason might be that with more playful males, there is no need to play with the mother anymore. Locomotor play, and therefore total play, increased with increasing (i.e. male biased) sex ration the week before weaning step one. Another finding was that lambs in male biased groups, before weaning, performed more vocalisations overall.

There seems to be a diurnal play cycle in pre-weaned lambs with three peaks; at the 7^{th} hour (7:00-8:00), the 9^{th} hour (9:00-10:00) and the 19^{th} hour (19:00-20:00). These peaks were also seen in the components of play, i.e. social and locomotor play. However, these peaks are probably not set for all lambs in all situations; the timing of the peaks were probably affected by the management of the caretakers, but could also coincide with a natural rhythm for play behaviour in lambs. Especially the play peak in the evening may have been released by other factors, as the caretakers went home at around 16:00.

Experimental design

For practical reasons, all sheep were placed and observed in the same barn. As a result, recordings of e.g. play behaviour are not independent of each other. If a lamb invited its twin lamb to play, the twin lamb would probably start playing as well or the whole group may have started playing. Moreover, if there was excitement in one pen, this may have excited lambs in other pens. A small incidence can thus be amplified in the data. Assumingly, such effects did not affect the weaning study much, but the diurnal play study more so. For reasons to uphold a rule of lambs weighing 25 kg at weaning, all lambs were not weaned at the same day. The group was divided into three groups, each group weaned with a period of one week in between. Also, the weaning groups were not of the same size. This could have had an effect on behaviours like vocalisations.

Different environmental factor were confounded with weaning period, like that the period during weaning included loss of playmates and playground. Although these changes were part of weaning as described in this study, it is important to make the distinction between different aspects of the changes following weaning. Presumably, effects of the different changes after weaning worked in the same direction on welfare, i.e. negatively. This means that behavioural effects were amplified and / or prolonged, like the percentage of time lying due to reduced space after weaning. However, behavioural scores after weaning often returned to levels before weaning. Therefore the behavioural changes are likely mainly due to actual weaning, i.e. withdrawal from milk and separation from the mother. However, some potential effects, e.g. by loss of playmates, cannot be distinguished from the loss of the mother.

Obviously, age of the lamb was confounding with weaning period as the lambs grew older when going through the three periods of weaning. The age of the lambs at weaning ranged from 50 up to 65 days. This means age varied a lot over the weaning periods and exceeds the ages studied by Sachs and Harris. Peaks and troughs in the frequency of play behaviour, as mentioned by Sachs and Harris, were possibly overruled by the effect of affective state caused by weaning; some lambs were observed before weaning at an age that falls within the trough of play frequency at 5 and 6 weeks old described by Sachs and Harris (1978). As play behaviour in this period was shown more often than in the period during weaning, this potential effect likely was of minor importance. Thus, the age related changes in play frequencies, as reported by Sachs & Harris (1978), did not seem to have major effects in the present study.

Practical implications of the findings for farmers

It is important to keep in mind that the results of this study only describe the lambs' responses to the specific weaning process described in the Material and Methods. It does not represent a commonly used procedure and for example the time that the ewes spent in the barn before they were removed might have changed the effect of weaning on the lambs' welfare. A welfare compromising effect could run via frustration as the mother was still in the barn and within hearing distance, but out of sight and touch. Arnold *et al*. (1975) have shown that auditory signals from the ewe are increasingly important for a lamb as it gets older to find its mother. By the time they are 7 days old, they recognize the sound of the mother and depend on it to find her when they have lost her. Over this period, visual cues become less important. Hearing the ewe vocalize in the barn after weaning and the consequent frustration due to the inability of the lamb to go and find her, might therefore have a stronger negative effect on the affective state of the lambs than when she would have been moved from the barn at once. Moreover, Napolitano *et al*. (2003) found a detrimental effect of weaning on behavioural and endocrine indicators of affect in lambs of one day of age when they were only prevented from suckling, compared with complete separation from the mother. They concluded that the presence of mothers unable to give what the lambs need is worse than complete absence of the mothers. They suggest this is due to frustration because they are unable to suckle or general frustration about not reaching a food source. Therefore, sudden weaning might be preferred over the weaning method used in the present study, i.e. keeping the mother in the barn for 5 days before removing her. Moreover, on the condition that the farmer can move the ewes to a place out of hearing distance for the lambs, sudden weaning is less effort for the farmer, since he does not need to move the ewes twice.

For the farmer it is important to reduce valence and duration of a negative affective state by weaning to a minimum (and increase valence and duration of a positive affective state to a maximum) for good health and performance of the lambs (and ewes). Therefore, it is important that a negative affective state can be readily noticed. Although play frequency is reduced after weaning, it is not a good indicator on the farm as it happens gradually and occurs infrequently. However, seeing lambs play frequently is a good indicator of good welfare. Other parameters, like frequency of social contact and display time of certain ear postures, are too time-consuming to record and require instruction to the farmer. Vocalisation frequency appears a useful indicator of a negative affective state in lambs. Number of vocalisations should reduce drastically in the days after weaning and can be noticed just by working on the farm. Leaving the environment and rations unchanged, removing the ewes from the lambs and not the other way around and leaving the lambs with their twin lambs or more lambs (more research needed to determine optimal number), are ways to achieve this. The sooner the vocalisation frequency returns to almost none, the less negative the affective state and thereby the better the welfare of the lambs.

Animal welfare implications of used indicators

To assess welfare of lambs during weaning on farms, play frequency appeared not to be a feasible indicator of affective state. The problem is, in order to be a fairly good indicator, it needs to be recorded before weaning and over a prolonged period of time after weaning, since it only shows a gradual reduction in occurrence after weaning. Besides, it is not performed often by the lambs and is probably too time-consuming for assessment on farm. A parameter that increases directly after weaning, is frequency of social contact with the twin, but like play behaviour it is time-consuming to record since it is not shown regularly. For research on welfare implications it seems a promising indicator of good welfare though. Vocalisation frequency appears to be the most useful indicator on farm found up to now. Before weaning there are hardly any vocalisations and it increases excessively immediately after weaning. Moreover, ear postures show to be a reliable indicator of negative affective state. Ear posture is easy to record reliably, e.g. relative to play behaviour, via scan sampling. The ear postures of interest, i.e. raised and asymmetrical ear postures, were shown during fair amounts of time and showed an increase in time displayed directly after weaning. Tail posture is not found to be a reliable indicator of affective state, but more research should be done to verify this. Lying seems a useful indicator in that it can be easily recorded with scan sampling, although this needs to be verified by other research. It seems useful for research on lamb welfare, not very useful for on farm assessment.

Besides finding more indicators to examine more accurately how welfare is impaired at weaning and how welfare of sheep can be assessed, the indicators identified here can be used in future research to find ways to improve welfare of lambs at weaning. For example, the support by different numbers of peers (twin lambs and familiar lambs) after weaning seems important. When finding the most optimal weaning method in terms of welfare, of both lambs and ewes, negative effects on health and growth rate can be prevented, and a rapid post-weaning regain of a positive affective state can be established.

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Appendix A

Table A-1. Predicted mean frequency of behaviours per 2 minutes over the complete period of observation and, where appropriate, over the period before weaning. Effects of silage treatment, weaning period, sex and interactions of weaning period and sex on each behaviour are shown, P values≥0.10 are deleted. Based on 2340 records on 15 twins.

Behaviour	Predicted mean of the constant ± s.e. per 2 minutes (before weaning)	P values			
		Silage additive treatment	Weaning Period	Sex	Weaning period * sex
Total play	0.164±0.024		0.006	0.014	
Locomotor play	0.053±0.012		0.019		
Social play	0.111±0.016		0.01	0.008	
with twin	0.074±0.018	0.078	0.019	0.054	
with mother[a]	0.011±0.004 (0.032±0.005)		<0.001		
with ewes	0.002±0.001				0.013
with lambs	0.026±0.006	0.024			<0.001
Social contact	0.537±0.035	0.056	<0.001	0.054	
with twin	0.266±0.022		<0.001	0.009	
with mother[a]	0.103±0.009 (0.308±0.017)		<0.001		
with ewes	0.041±0.016				
with lambs	0.126±0.012	0.090	0.021		
Vocalisations	0.738±0.134		<0.001	0.025	

[a] These behaviours could not be shown in the periods during and after weaning as the mother was removed from the pen during these periods.

Table A-2. Predicted mean duration of behaviours over the complete period of observation. Effects of silage treatment, weaning period, sex and interactions of weaning period and sex on each behaviour are shown, P values≥0.10 are deleted. Based on 18720 records on 15 twins.

Behaviour	Predicted mean of the constant ± s.e. in % of time	P values			
		Silage additive treatment	Weaning Period	Sex	Weaning period * sex
Lying	51.2±2.0		0.032		
Standing	44.5±1.8		0.051		
Moving	4.3±0.3		0.03		
Ruminating	20.8±0.8				
Eating concentrate	6.9±0.5	0.017	<0.001		
Eating silage	13.0±0.9	0.011			
Eating straw	2.0±0.6				0.024
Drinking	0.9±0.1			0.070	
Sniffing pen	3.7±0.3	0.098	0.056		
Comfort behaviour	2.4±0.2				
Ears plane	21.9±3.5		<0.001		
Ears raised	21.8±1.9	-	<0.001	0.098	
Ears backward	38.4±2.5				
Ears asymmetrical	8.6±0.5		<0.001		
Tail zero	86.6±1.1		0.005		
Tail ten	11.0±0.9		0.027	0.097	
Tail ninety	1.7±0.3		0.004		

Appendix B: Dependence of variables with sex

Cross tabulations of potentially confounding subject and situational variables on weaning period and sex

The null hypotheses described the situation in which the observed values did not differ from the expected values. All p-values of the same size or smaller than the point probability are added up to form the cumulative p-value that is given underneath each cross tabulation. Residuals are given in brackets in the tables. They were calculated as $Residual = \frac{(Observed - Expected)}{\sqrt{Expected}}$. The cell value deviates significantly from expected when the residuals are smaller than -2 or larger than 2. When the residual is smaller than -2, the observed cell value was expected to be larger. When the residual is larger than 2, the observed was expected to be smaller.

Sex-Age at weaning

Age at weaning	Male	Female
50-53	3 (-0.11)	3 (0.12)
54-57	7 (0.72)	3 (-0.77)
58-61	4 (0.45)	2 (-0.48)
62-65	2 (-1.10)	6 (1.18)

Two sided p-value: p(O>=E|O<=E): 0.25079 (i.e. 25%), independence.

Sex-Playground size before weaning

Playground size	Male	Female
8 m^2	1 (-1.23)	5 (1.31)
12 m^2	14 (1.02)	6 (-1.09)
16 m^2	1 (-0.77)	3 (0.83)

Two sided p-value: p(O>=E|O<=E): 0.03278 (i.e. 3%), no independence.

Sex and playground size are not independent. Males are mainly on playgrounds of 12m^2, while females are more evenly spread over the playgrounds of different sizes. This probably has not had an influence on the study results.

Sex-Playground size per lamb before weaning

Playground size per lamb	Male	Female
2.00-3.99	10 (-0.21)	10 (3.05)
4.00-5.99	1 (-0.77)	3 (0.83)
6.00-7.99	5 (1.01)	1 (-1.08)

Two sided p-values: p(O>=E|O<=E): 0.2167 (i.e. 22%), independence.

Sex-Number of playmates on playground in week before weaning

Number of playmates	Males	Females
1	5 (0.22)	3 (-0.25)
2	0 (0)	0 (0)
3	2 (-1.19)	6 (1.36)
4	1 (-0.12)	1 (0.14)
5	8 (0.98)	2 (-1.12)
6	1 (-0.12)	1 (0.14)

Two sided p-values: p(O>=E|O<=E): 0.06448, tendency not to be independent. However, no pattern of deviating cell values is visible.

Sex-maximum number of playmates on the playground during the lamb's life

Max. no. Of playmates	Males	Females
1-2	0 (-1.03)	2 (1.11)
3-4	3 (-0.11)	3 (0.12)
5-6	12 (0.77)	6 (-0.83)
7-8	0 (0)	0 (0)
9-10	1 (-0.77)	3 (0.83)

Two sided p-values: p(O>=E|O<=E): 0.18924 (i.e. 19 %), independence.

Sex-Sex ratio of the playmates on the playground in the week before weaning step one

Sexratio	Males	Females
(-1) - (-0.61)	2 (-0.09)	2 (0.10)
(-0.6)-(-0.21)	1 (-0.77)	3 (0.83)
(-0.2)-0.19	1 (-0.47)	2 (0.51)
0.2-0.59	3 (-0.38)	4 (0.40)
0.6-1	9 (1.03)	3 (-1.10)

Two sided p-values: p(O>=E|O<=E): 0.38894 (i.e. 39%), independence.

Sex-Mean age of playmates on the playground in week before weaning step one

Mean age	Males	Females
47-50.9	4 (0.45)	2 (-0.48)
51-54.9	1 (-0.77)	3 (0.83)
55-58.9	5 (-0.55)	7 (0.59)
59-62.9	6 (0.84)	2 (-0.90)

Two sided p-values: p(O>=E|O<=E): 0.35285 (i.e. 35%), independence.

Appendix C: Dependence of subject and situational variables

Cross tabulations of potentially confounding subject and situational variables on the same variables.

The null hypotheses described the situation in which the observed values did not differ from the expected values. Residuals are given in brackets. They were calculated as $Residual = \frac{(Observed - Expected)}{\sqrt{Expected}}$. The cell value deviates significantly from expected when the residuals are smaller than -2 or larger than 2. When the residual is smaller than -2, the observed cell value was expected to be larger. When the residual is larger than 2, the observed cell value was expected to be smaller.

Playground size before weaning - Age of the lamb at weaning step one

Weaning age	8 m^2	12 m^2	16 m^2
50-53	0 (-1.37)	4 (0.00)	2 (1.61)
54-57	2 (0.00)	8 (1.10)	0 (-1.52)
58-61	2 (0.91)	4 (0.00)	0 (-1.07)
62-65	2 (0.41)	4 (-1.17)	2 (1.13)

Pearson chi-square value is 7.18 with 6 degrees of freedom.
Probability level (under null hypothesis): p = 0.304.

Playground size before weaning - Playground size per lamb before weaning

Playground size per lamb	8 m^2	12 m^2	16 m^2
2.00-3.99	4 (0.00)	14 (0.55)	2 (-0.76)
4.00-5.99	2 (1.61)	0 (-3.04)	2 (2.32)
6.00-7.99	0 (-1.37)	6 (1.94)	0 (-1.07)

Pearson chi-square value is 11.70 with 4 degrees of freedom.
Probability level (under null hypothesis): p = 0.020.

Although playground size before weaning and playground size per lamb before weaning are not independent, no clear pattern is visible. These cell deviations probably have not had an impact on the study results.

Playground size before weaning - Number of playmates on the playground in the week before weaning step one

Number of playmates	8 m^2	12 m^2	16 m^2
1	2 (0.41)	6 (0.58)	0 (-1.30)
3	4 (2.48)	4 (-1.17)	0 (-1.30)
4	0 (-0.73)	0 (-2.07)	2 (3.73)
5	0 (-1.94)	10 (2.74)	0 (-1.52)
6	0 (-0.73)	0 (-2.07)	2 (3.73)

Pearson chi-square value is 37.25 with 8 degrees of freedom.
Probability level (under null hypothesis): p < 0.001.

Although differences are small, there seems to be a pattern; the larger the playground which the lambs had access to, the more playmates it had.

Playground size before weaning - Maximum number of playmates on the playground during the lamb's life

Max. no. playmates	8 m^2	12 m^2	16 m^2
1-2	2 (2.93)	0 (-2.07)	0 (-0.57)
3-4	4 (3.20)	2 (-1.94)	0 (-1.07)
5-6	0 (-3.35)	18 (4.74)	0 (-2.63)
7-8	0 (-0.01)	0 (-0.02)	0 (-0.01)
9-10	0 (-1.07)	0 (-3.04)	4 (5.48)

Pearson chi-square value is 51.33 with 8 degrees of freedom.
Probability level (under null hypothesis): $p < 0.001$.

Although differences are small, there seems to be a pattern; the larger the playground which the lambs had access to, the higher the maximum number of playmates it had during the lamb's life.

Playground size before weaning - Sex ratio of the playmates on the playground in the week before weaning

Sex ratio	8 m^2	12 m^2	16 m^2
(-1) - (-0.61)	3 (2.95)	1 (-1.90)	0 (-0.84)
(-0.6)-(-0.21)	3 (2.95)	1 (-1.90)	0 (-0.84)
(-0.2)-0.19	0 (-0.91)	0 (-2.57)	3 (4.65)
0.2-0.59	0 (-1.51)	6 (1.22)	1 (0.08)
0.6-1	0 (-2.23)	12 (3.16)	0 (-1.75)

Pearson chi-square value is 42.54 with 8 degrees of freedom.
Probability level (under null hypothesis): $p < 0.001$.

Lambs that had access to playgrounds of 8 m^2 had playmates with a lower sex ratio than expected, while lambs that had access to playgrounds of 12 m^2 had playmates with a higher sex ratio than expected. Lambs that had access to playgrounds of 16 m^2 had more lambs with a neutral ratio as expected. This might have had an impact on the results, but probably not a great impact.

Playground size before weaning - Mean age of the playmates on the playground in the week before weaning step one

Mean age	8 m^2	12 m^2	16 m^2
47-51	0 (-1.37)	6 (1.93)	0 (-1.07)
51-55	0 (-1.07)	2 (-0.76)	2 (2.32)
55-59	4 (1.49)	8 (0.00)	0 (-1.75)
59-63	2 (0.41)	4 (-1.17)	2 (1.13)

Pearson chi-square value is 11.92 with 6 degrees of freedom.

Probability level (under null hypothesis): p = 0.064.

Age of the lamb at weaning step one - Number of playmates on the playground in the week before weaning step one

Weaning age	1	3	4	5	6
50-53	0 (-1.65)	2 (0.41)	0 (-0.73)	2 (0.00)	2 (2.92)
54-57	2 (-0.58)	2 (-0.58)	0 (-1.03)	6 (2.19)	0 (-1.03)
58-61	6 (4.53)	0 (-1.65)	0 (-0.73)	0 (-1.93)	0 (-0.73)
62-65	0 (-1.99)	4 (1.74)	2 (2.42)	2 (-0.58)	0 (-0.88)

Pearson chi-square value is 37.30 with 12 degrees of freedom.
Probability level (under null hypothesis): p < 0.001.

Although age of the lamb at weaning step one and the number of playmates on the playground in the week before weaning step one are not independent, no clear pattern is visible.

Maximum number of playmates on the playground during the lamb's life - Number of playmates on the playground in the week before weaning step one

Max. no. of playmates	1	3	4	5	6
1-2	2 (2.43)	0 (-0.73)	0 (-0.39)	0 (-1.20)	0 (-0.39)
3-4	2 (-0.12)	4 (2.48)	0 (-0.88)	2 (-1.01)	0 (-0.88)
5-6	4 (-0.22)	2 (-1.10)	0 (-1.56)	10 (2.69)	0 (-1.56)
7-8	0 (-0.01)	0 (-0.01)	0 (0.00)	0 (-0.01)	0 (0.00)
9-10	0 (-1.30)	0 (-1.07)	2 (3.73)	0 (-1.75)	2 (3.73)

Pearson chi-square value is 41.25 with 16 degrees of freedom.
Probability level (under null hypothesis): p < 0.001.

Although differences are small, it seems that the higher the number of playmates on the playground in the week before weaning step one, the higher the maximum number of playmates on the playground during the lamb's life.

Number of playmates on the playground in the week before weaning step one - Sex ratio of the playmates on the playground in the week before weaning

Sex ratio	1	3	4	5	6
(-1) - (-0.61)	3 (2.35)	1 (-0.08)	0 (-0.57)	0 (-1.52)	0 (-0.57)
(-0.6)-(-0.21)	0 (-1.30)	4 (3.56)	0 (-0.57)	0 (-1.52)	0 (-0.57)
(-0.2)-0.19	0 (-1.10)	0 (-1.10)	1 (1.95)	0 (-1.29)	2 (4.39)
0.2-0.59	0 (-1.82)	3 (1.11)	1 (0.92)	3 (0.61)	0 (-0.81)
0.6-1	5 (1.52)	0 (-2.70)	0 (-1.20)	7 (2.37)	0 (-1.20)

Pearson chi-square value is 50.26 with 16 degrees of freedom.
Probability level (under null hypothesis): p < 0.001.

A pattern is vaguely visible; the higher the number of playmates on the playground in the week before weaning step one, the higher the sex ratio of the playmates on the playground in the week before weaning. However, this has probably barely affected the results.

Number of playmates on the playground in the week before weaning step one - Playground size per lamb before weaning

Playground size per lamb	1	3	4	5	6
2.00-3.99	0 (-4.63)	8 (2.33)	0 (-2.05)	10 (2.74)	2 (1.03)
4.00-5.99	2 (1.13)	0 (-1.30)	2 (3.73)	0 (-1.52)	0 (-0.57)
6.00-7.99	6 (4.53)	0 (-1.65)	0 (-0.73)	0 (-1.94)	0 (-0.73)

Pearson chi-square value is 41.25 with 8 degrees of freedom.
Probability level (under null hypothesis): $p < 0.001$

A pattern is vaguely visible; the smaller the number of playmates on the playground in the week before weaning step one, the larger the playground size per lamb before weaning. However, this mainly holds for the number of playmates of only one compared to the higher numbers of playmates. For the resulting counts, there is no clear pattern. Therefore, this has probably barely affected the results.

Appendix D. Effects of subject and situational variables.

Table D-1. Effects of the first part of the subject and situational variables and of their interactions with weaning period on the behaviours of which the frequencies were measured. P values≥0.10 are deleted. Based on 2340 records on 15 twins.

	P values													
	Silage + (weaning age *weaning period)		Silage + (playground size * weaning period)				Silage + (playground size / lamb * weaning period)				Silage + (no. of playmates * weaning period)			
Behaviour	Silage additive	Weaning period	Silage additive	Weaning period	Playground size	Playground size* weaning period	Silage additive	Weaning period	Playground size / lamb	Playground size / lamb * weaning	Silage additive	Weaning period	No. of playmates	No. of playmates * weaning period
Total play		0.006		log 0.003	log 0.010			0.006				0.006		
Locomotor play		0.019		log 0.002				0.018				0.018		
Social play		0.018		log 0.020	log 0.014			0.018				0.018		
with twin		0.019	0.078	0.019	0.028			0.019				0.019		
with mother[a]		<0.001		<0.001		<0.001		<0.001				<0.001		
with ewes		0.007		0.007				0.007		0.038		0.007		0.003
with lambs	0.024	0.002	0.024	0.002			0.024	0.002		0.010	0.024	0.002		0.001
Social contact	0.080	<0.001	0.093	<0.001			0.070	<0.001		0.070	0.035	<0.001	0.030	
with twin		<0.001		<0.001				<0.001				<0.001		
with mother[a]		<0.001		<0.001				<0.001				<0.001		
With ewes	-	-				0.046			0.007				0.037	
with lambs	0.082	0.021	0.085	0.021			0.074	0.021		0.096	0.059	0.021		0.065
Vocalisations		<0.001		<0.001				<0.001				<0.001		

[a] These behaviours could not be shown in the periods during and after weaning as the mother was removed from the pen during these periods.

Table D-2. Effects of the second part of the subject and situational variables and of their interactions with weaning period on the behaviours of which the frequencies were measured. P values≥0.10 are deleted. Based on 2340 records on 15 twins.

	P values									
	Silage + (max. no. of playmates * weaning period)				Silage + (sex ratio playmates * weaning period)				Silage + (mean age playmates * weaning period)	
Behaviour	Silage additive	Weaning period	Max. no. of playmates	Max. no. of playmates* weaning	Silage additive	Weaning period	Sex ratio playmates	Sex ratio playmates *weaning period	Silage additive	Weaning period
Total play		0.006				0.006	0.037			Log 0.003
Locomotor play		0.019				0.019	0.013			0.019
Social play		0.018				0.018				Log 0.019
with twin		0.019			0.069	0.019	0.088			Log 0.009
with mother[a]		<0.001		0.050			<0.001	<0.001		<0.001
with ewes		0.007	0.099			0.007				0.007
with lambs	0.024	0.002			0.024	0.002			0.024	0.002
Social contact	0.093	<0.001			0.083	<0.001			0.070	<0.001
with twin		<0.001				<0.001				<0.001
with mother[a]		<0.001				<0.001		log 0.017		<0.001
with ewes							0.091	0.014		
with lambs	0.085	0.021			0.094	0.021			0.074	0.021
Vocalisations		<0.001				<0.001	0.037			<0.001

[a] These behaviours could not be shown in the periods during and after weaning as the mother was removed from the pen during these periods.

Table D-3. Effects of the first part of the subject and situational variables and of their interactions with weaning period on the behaviours that were measured instantaneously. P values≥0.10 are deleted. Based on 18720 records on 15 twins.

	P values							
	Silage + (weaning age *weaning period)				Silage + (playground size * weaning period)			
Behaviour	Silage additive	Weaning period	Weaning age	Weaning age * weaning period	Silage additive	Weaning period	Playground size	Playground size* weaning period
Lying		0.031		0.083	0.071	0.032	0.038	
Standing		0.050		0.056	0.077	0.052	0.065	
Moving		0.036				0.037	0.053	
Ruminating								
Eating concentrate	0.005	<0.001	0.048		0.002	<0.001	0.014	
Eating silage	0.006				0.008			
Eating straw								0.060
Drinking								0.013
Sniffing pen		0.056			0.086	0.055	0.063	
Comfort behaviour								
Ears plane		<0.001	0.036			<0.001		0.069
Ears raised		<0.001	0.001			<0.001		
Ears backward								0.019
Ears asymmetrical		<0.001				<0.001		
Tail zero		0.005				0.005		0.080
Tail ten		0.027				0.027		
Tail ninety		0.004		0.049		0.004		0.016

Table D-4. Effects of the second part of the subject and situational variables and of their interactions with weaning period on the behaviours that were measured instantaneously. P values≥0.10 are deleted. Based on 18720 records on 15 twins.

	P values							
	Silage + (playground size / lamb * weaning period)				Silage + (no. of playmates * weaning period)			
Behaviour	Silage additive	Weaning period	Playground size per animal	Playground size per animal * weaning period	Silage additive	Weaning period	No. of playmates	No. of playmates * weaning period
Lying		0.030		0.004		0.031		0.091
Standing		0.048		0.003		0.050		0.088
Moving		0.037	0.049			0.037		
Ruminating								
Eating concentrate	0.012	<0.001			0.021	<0.001		
Eating silage	0.006				0.003			
Eating straw			<0.001				0.026	
Drinking				0.021				<0.001
Sniffing pen		0.055				0.055		
Comfort behaviour								
Ears plane		<0.001	0.014			<0.001		
Ears raised		<0.001				<0.001		
Ears backward			0.073					
Ears asymmetrical		<0.001				<0.001		
Tail zero		0.006	0.026			0.006	0.088	
Tail ten		0.028				0.028		
Tail ninety		0.004				0.004		

Table D-5. Effects of the third part of the subject and situational variables and of their interactions with weaning period on the behaviours that were measured instantaneously. P values≥0.10 are deleted. Based on 18720 records on 15 twins.

	P values										
	Silage + (max. no. of playmates * weaning period)				Silage + (sex ratio playmates * weaning period)				Silage + (mean age playmates * weaning period)		
Behaviour	Silage additive	Weaning period	Max. no. of playmates	Max. no. of playmates* weaning period	Silage additive	Weaning period	Sex ratio playmates	Sex ratio playmates *weaning period	Silage additive	Weaning period	Mean age playmates* weaning period
Lying		0.033			0.043	0.031	<0.001			0.032	
Standing		0.052			0.039	0.049	<0.001			0.051	
Moving		0.037				0.036				0.037	
Ruminating											
Eating concentrate	0.001	<0.001	0.005		0.017	<0.001			0.017	<0.001	0.020
Eating silage	0.006				0.007		0.030	0.067	0.006		
Eating straw											
Drinking				0.002							
Sniffing pen		0.056			0.054	0.056	0.067			0.053	0.026
Comfort behaviour											
Ears plane		<0.001				<0.001		0.095		<0.001	0.006
Ears raised		<0.001				<0.001				<0.001	0.024
Ears backward				0.011							
Ears asymmetrical		<0.001				<0.001		0.096		<0.001	
Tail zero		0.005				0.005		0.061		0.005	
Tail ten		0.027				0.026		0.040		0.027	
Tail ninety		0.004				0.004				0.004	

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