Investigation of the reward cycle associated with play behaviour in lambs

Undersökning av belöningscykeln kopplad till lekbeteende hos lamm

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1. Summary

The aim of this study was to investigate if behaviour, ear positions and cortisol in lamb which were given access to play followed the different phases of the reward cycle of anticipation, consumption and relaxation. The study was done on 8 Dorset and 12 Dorset x Fine Wool uncastrated male lambs with an average age of 10 weeks. The lambs were housed pairwise in litter pens (2 x 3 m). Pairs of lambs were taken to a play arena (5.9 x 5.5 m) three times a week during five weeks (first week for learning). They were then first kept in a holding pen where they could look into the play arena for 5 minutes and thereafter released for 20 minutes in the play arena which had a ball, two chains and a tunnel. They walked back to their home pen and were observed for 6 minutes thereafter. Observations of behaviours were made as frequency per minute in all three places. Four different ear postures were recorded in the three places to study emotional valence in lambs. During the last week saliva sampling was done for cortisol analyses during the last week; one sample in the home pen before play and another in the home pen after play. Lambs showed significantly more anticipatory behaviour than non-anticipatory behaviour in the holding pen ($p<0.01$). Anticipatory behaviour did not change over the five minutes, and was higher during week 2-3 than week 1 and 4. In the play arena, play behaviour was significantly higher than other behaviours ($p<0.01$). Social play was higher than locomotor play and object play. Play behaviour was higher during the first ten minutes than the last ten minutes in the play arena ($p<0.0001$) and was highest during the second week of study ($p<0.0001$). The level of behaviours indicating relaxation was not higher than behaviours indicating no relaxation in the home pen after having been in the play arena (N.S.). Raised ear postures were most frequently recorded in the holding pen, whereas backward ear postures were more frequently recorded in the play arena and plane ear postures were most frequently recorded in the home pen both after play and on days without play. Saliva cortisol level increased after play in lambs during the first day, but not during the second and third day of sampling during the fourth week of play. It is concluded that lambs seem to show behaviours indicating anticipation to play and they do play in a known play arena, but relaxation after having played is less obvious during the first minutes. When given access to play the lambs seemed to pass through all three phases of the reward cycle expressing different anticipation, consumption and relaxation behaviours.

Sammanfattning

Syftet med denna studie var att undersöka om beteende, öronställningar och kortisol hos lamm som gavs lekmöjligheter följde belöningscykelns olika faser för förväntan, konsumtion och avslappning. Studien gjordes på 8 Dorset och 12 Dorset x Finull okastrerade bagglamm med en genomsnittlig ålder på 10 veckor. Lammen inhystes parvis i halmförsedda boxar (2 x 3 m). Varje par togs till en lekarena (5,9 x 5,5 m) tre gånger i veckan under fem veckor (första veckan för inlärning). De hölls först i en väntebox där de kunde se in i lekarenan under 5 minuter och därefter släpptes de ut i lekarenan, som hade en boll, två kedjor och en tunnel, och fick vistas där under 20 minuter. De fick gå tillbaka till sin hembox och observerades där under 6 minuter. Observationer av beteenden gjordes som frekvens per minut på alla tre ställen. Fyra olika öronställningar registrerades på de tre platserna för att studera emotionell nivå hos lammen. Under den sista veckan togs salivprov för kortisolanalyser, ett prov i hemboxen innan lek och det andra i hemboxen efter lek. Lamm visade signifikant mer beteenden som indikerar förväntan än beteenden som indikerar icke-förväntan i vänteboxen utanför lekarenan ($p<0,01$). Förväntansbeteendena förändrades inte under de fem minuterna, och var högre under vecka 2-3 än vecka 1 och 4. I lekarenan var lekbeteenden signifikant högre än andra beteenden ($p<0,01$). Social lek registrerades mer än motorisk lek och objektlek. Lekbeteenden var högre under de första tio minuterna än de sista tio minuterna i lekarenan ($p<0,0001$) och var högst under den andra veckan av studien ($p<0,0001$). Beteenden som tyder på avslappning var inte högre än beteenden som tyder på ingen avslappning i hemboxen efter att ha varit i lekarenan (icke signifikant). Resta öronställningar var mest frekvent i vänteboxen, medan bakåtvända öronställningar oftare registrerades i lekarenan och plana öronställningar var mest frekvent i hemboxen både efter lek och på dagar utan lek. Salivkortisol ökade efter lek hos lamm under den första dagen, men inte under den andra och tredje dagen efter provtagning under den fjärde veckan med lek. Slutsatsen är att lamm tycks visa beteenden som indikerar förväntan på att få leka och de leker i en känd lekarena, men avslappning efter att ha lekt är mindre uppenbart under de första minuterna. När lamm gavs möjlighet att lek verkade de passera igenom alla tre faser av belöningscykeln, vilket visades i olika förväntans-, konsumtions- och avslappningsbeteenden.

2. Introduction

2.1 Background

Animal welfare concern originates from the social and legal acknowledgement that animals are sentient beings capable of subjectively experiencing pain or pleasure (Boissy et al., 2011). They can experience both negative and positive aspects of life and feel a broad spectrum of emotions (Balcombe, 2009). There are certain situations that create risk of survival for the organisms. During evolution, the organism found a ‘trick’ to avoid these situations which are related to negative emotion. On the other hand, some experiences evoke positive emotions that are rewarding and guide the organism to acquire resources that can have immediate benefits or experiences that may promote survival, health and reproduction in their later life (Held and Spinka, 2011). The concept of welfare is determined by the balance between positive and negative experiences or affective states which may range from positive (good welfare) to negative (poor welfare) (Spruijt et al., 2001).

The vast majority of concern for animal welfare appears to be centered upon negative concepts such as abnormal behaviours and physiological ‘stress’ responses (Burman et al., 2011). However, in recent years, there has been increased focus in the assessment of good welfare in a scientific way including positive outcomes (Yeates and Main, 2008). Reviews of positive emotions, pleasure and rewards have addressed play behaviour, affiliative behaviours and some vocalizations as the three most promising indicators for assessing positive affective states in farm animals (Boissy et al., 2007; Balcombe, 2009; Held and Spinka, 2011). In relation to experiencing positive affective states which are evoked by rewards; anticipation, consumption and post-consumption of reward has been identified (Burman et al., 2011). The animal which passes through these phases of reward possession and consumption is likely to experience positive affective states (Burman et al., 2011). Since play is suggested by Held and Spinka (2011) to be a rewarding activity, acquisition of play may invoke a broad spectrum of sensations and can lead to that the animal experience a pleasurable emotion (Balcombe, 2009). Therefore, play appears to be very important behaviour in assessing and enhancing behavioural expressions of positive emotions (Boissy et al., 2007).

2.2 Play behaviour

Play has been considered as a behavioural need (Held and Spinka, 2011). Jensen and Toates (1993) have defined behavioural need as a specific behavioural pattern that the animal performs irrespective of the environment or of the animal's physiological needs. The motivation to perform these behaviours is governed by the display of behaviour itself rather than to meet short-term physiological needs (Boissy et al., 2007). Play is a behaviour for which animals have high internal motivation and is ready to work and invest energy in (Jensen et al., 2004). Play is expressed when an animal's basic needs for food, shelter, safety etc. are met (Fraser and Duncan, 1998). When the animal is playing, it appears to be excited and gives the impression that it is having "fun" (Donaldson et al., 2002) and shows that it is free from sickness, hunger, injury, predation risk, thermal stress or other challenges to its fitness (Held and Spinka, 2011).

There are numerous behavioural and neurobiological evidences that suggest that play works as a reward and may be accompanied by the pleasure like emotional state that leads to reward consumption (Held and Spinka, 2011). Many studies have confirmed the central role of brain opioids in the regulation of social play in rats (Vanderschuren et al. 1995b, cited by Held and Spinka, 2011). Animals actively seek out play partners and solicit play behaviour if they are allowed to play (Boissy et al., 2007). Various conditioning studies have used play as a strong reinforcement. In the study done by Mason et al. (1963), experimental subjects were trained to press different levers for access to food or to a social interaction (either play or petting), and levels of hunger and types of food were varied (Held and Spinka, 2011). The results from the study showed play to be a stronger reinforcer than petting or a nonfavoured food and as strong reinforcer as the most highly valued food (Held and Spinka, 2011). So an opportunity to play may be used as a reward which shows that play may be a rewarding activity (Held and Spinka, 2011).

The motivation to perform play decreases in conditions intuitively associated with bad welfare such as poor housing, poor nutrition, disease or unfavourable climatic conditions such as during cold wet weather (Brownlee, 1984; Donaldson et al., 2002). Play is easily interrupted when there are immediate threats to an animal's fitness (Held and Spinka, 2011). There are various studies which emphasize how environmental and physiological factors can impact an animal's internal motivation to perform play. Insufficient food supply suppresses play behaviour in domestic pigs

(Donaldson et al., 2002), castration reduces play behaviour in lambs (Thornton and Waterman-Pearson, 2002) and domestic pigs in a semi-natural environment did not play during periods of cold weather (Newberry et al., 1988). Berger (1980) reported that when playing lambs experienced physical pain by stepping on cacti, the play behaviour ceased immediately. New or novel situations and the addition of fresh bedding will invoke a play response (Donaldson et al., 2002; Jensen, 2001). Therefore, Boissy et al. (2007) suggests that occurrence of play behaviour indicates that the proximate needs of the animals have been met and that the animals are not deprived of important sources of pleasure.

2.2.1 Functions of play

Play is a wide spread behaviour seen in many species like reptiles, birds and mammals (Balcombe, 2009). Play is an activity which has become prominent in the behavioural repertoire of younger animals especially infants and juveniles. It has been hypothesized that play, in early ontogeny, functions as a means of exercise, allows self assessment, facilitates neuromuscular development and motor performance, and enhances cerebellar synaptogensis and skeletal muscle performance (Donaldson et al., 2002; Spinka et al., 2001; Thompson, 1996). The function of play behaviour in earlier studies was thought to be exclusively long-term benefits (Boissy et al., 2007). However, in recent studies it has been proposed that along with long term benefits, an immediately rewarding, pleasurable positive emotion mediated by brain opioids may also accompany play (Held and Spinka, 2011). Therefore, current theories addressing functions of play mainly highlights short-term benefits to the juvenile and focuses less on long-term benefits (Boissy et al., 2007). Long term benefits include any enhanced skills, competencies and somatic qualities of the animal resulting from playing whose effects may persist for a limited or long time, or even for life (Held and Spinka, 2011). Play may lead to the forging of long-lasting social bonds and may aid in the development of cognitive abilities (Dugatkin, 2009). Play fighting in juveniles may serve as a means of developing skills, and increasing fitness for real fighting later in life. Role reversal during play fighting, that is playing the subordinate and dominant role and change may teach individuals how to be a balanced fighter (Dugatkin, 2009). This may result in individuals experiencing fewer injuries during real aggressive interactions as they have experienced both submissive and dominant fighting action patterns (Dugatkin, 2009).

Expression of play behaviour has more immediate benefits in the situations like feeding, aggression or sexual behaviour as play behaviour often precedes, accompanies or follows behavioural patterns that are important in these situations (Held and Spinka, 2011). Play helps animals to obtain useful information about the environment, about group members or about itself. It may enable the animal to provide information about its development or current status. The current social situation of the animal can be influenced through play, for example, by reinforcing its dominance status, reducing tension around feeding or turning a stranger into a familiar animal by showing play markers (Held and Spinka, 2011).

Adult animals may use play to stimulate their brains endogenously when external stimulation is low (i.e. when they are bored) (Held and Spinka, 2011). Domestic sows when moved from a barren to a strawed pen of equal size or sheep when released from the shearer display exaggerated play (Boissy et al., 2007). Since play provides the individual with valuable experiences that aid the organism in achieving survival goals, a proximate reward mechanism must be involved to ensure that animals engage in play at appropriate times rather than performing adaptive behaviours like resting and conserving energy (Linda Keeling- personal communication).

2.2.2 Types of play

Animals generally perform three different types of play; social play, locomotor play and object play (Dugatkin, 2009). Social play refers to playing with conspecifics (Dugatkin, 2009). Actions such as leaps, runs, jumps, twists, shakes, whirls and somersaults e.t.c. are included in locomotor play. Object play is the play that focuses on the use of inanimate objects (Dugatkin, 2009).

2.2.3 Play behaviour in domestic mammals

Play behaviour in animals is characteristically flexible and variable within and between species (Boissy et al., 2007). A highest frequency of play occurring during infancy and the juvenile period shows that play has an inverted U-shaped distribution over ontogeny (Held and Spinka, 2011). Domestic pigs are especially playful around 2-6 weeks of age and then decrease in frequency of play is observed from six to 14 weeks (Newberry et al., 1988). In a study by Blackshaw et al. (1997) social, object and locomotor play began at 3-5 days of age and peaked at

21-25 days in piglets when the play behaviours were recorded for 28 days. Play fighting in piglets is generally seen from 3 to 9 weeks of age (Worsaae and Schmidt, 1980). Locomotor play in dairy calves has been shown to develop rapidly after birth, peaking at two weeks of age, then declining with increasing age (Jensen et al., 1998), whereas social play develops at two weeks of age and peaks at approximately six months of age (Napolitano et al., 2009). Research on play in dairy calves found that play decreases with age and is positively affected by increase in space allotment and by group housing (Jensen and Kyhn, 2000). Some closely related species like rats and mice can vary considerably in their play behaviour: mice show much locomotor play but little play fighting, whereas rats show more play fighting than locomotor play (Held and Spinka, 2011).

2.2.4 Play behaviour in sheep

The ontogeny of play in lambs follows a bimodal course, and little play is observed in the first week of life (Sachs and Harris, 1978). From the age of 2 weeks, the lamb makes frequent contacts with his/her peers and shows a peak of play around 2 to 3 weeks, then low amount of play is seen between 4 to 6 weeks and again a rise in play around 7 to 9 weeks of age (Sachs and Harris, 1978). Decline in play behaviour around 4 weeks time is explained by the energy cost of parent-offspring conflict over access to milk (Fagen, 1980). Milk production in lactating ewes decrease after the fourth week, and then she starts rejecting her lambs sucking attempts, which increases survivorship cost in lambs, thus leading to decreased time and energy available for play at this age (Fagen, 1980). Besides this temporarily increased cost of play, phylogenetic constraints and demographic changes might also be responsible for the bimodal distribution of play in lambs (Fagen, 1980). Play results in the utilization of different kinds of behaviour patterns in the sheep and the expression of these behaviours in adulthood is determined by how lambs used to perform these behaviours during their juvenile stage (Berger, 1979). Orgeur (1995) reported that lambs display social play during 4 to 8 weeks and mostly they consist of male sexual patterns. The most commonly seen components of play behaviour are ano-genital sniffing, nudging, mounting, flehmen, patterns of aggressive behaviour (butts and threats), running and gamboling (Orgeur, 1995). A study done by Sachs and Harris (1978) reflected that motor patterns mostly observed during play are butting, mounting and gamboling. Pawing the ground and repeated head lowering are seen before butting. Male lambs are involved in more butting and mounting whereas female lambs are more often involved in showing gamboling behaviour (Sachs and Harris, 1978).

2.3 Reward Cycle

A theoretical framework for the study of positive affective states in animals has been proposed by Keeling et al. (2008). The framework has been created by integrating a functional approach which describes the emotional process as proximate mechanisms that aid in achieving survival goals and the phenomenal approach which states that emotions have valence and arousal dimensions (Keeling et al., 2008). Affective states are states elicited by rewards and punishment. Rewards are ‘anything for which an animal will work’ (Burman et al., 2011). Behaviours such as social, reproductive, play, foraging, auto-grooming and dust-bathing are supposed to have rewarding properties (Boissy et al., 2007).

Positive affective states can be presented as a multipurpose reward cycle (Fig. 1) consisting of three phases that can occur repeatedly when animals are exposed to different forms of reward (feeding, drinking, sexual activity, play, enriched cages, social contact etc.) (Burman et al., 2011; Keeling et al., 2008). The first phase comprises the appetitive phase where the animal anticipates the reward. This is followed by the consummatory phase where the reward is acquired. The third phase is the post consummatory phase where the animal attains satisfaction once the reward is acquired. Keeling et al. (2008) have hypothesized that successful acquisition of the reward i.e. the animal passes through both appetitive, consummatory and post consummatory phases of the reward cycle, induces the strongest positive affective states

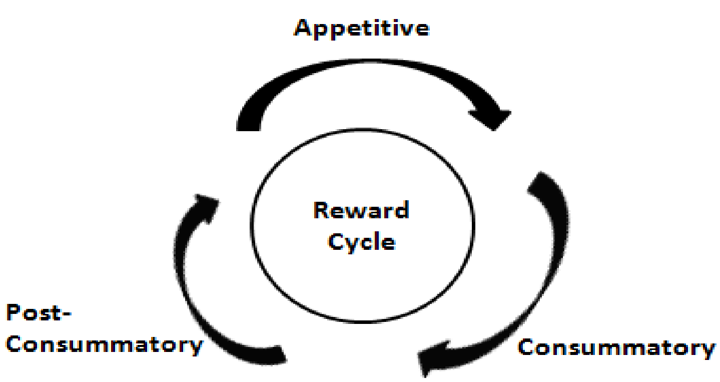

Figure 1. Model of the reward cycle and its three phases (after Keeling et al., 2008).

The mechanisms in the brain which is involved in mediating rewards have been functionally related to motivational aspects of natural behaviours and behaviours of addiction (Boissy et al., 2007). High arousal positive emotions (excitement, anticipation and desire) are suggested to be associated with an appetitive motivational state (Fig. 2, Keeling et al., 2008). A subsequent consummatory motivational state is linked with pleasure or liking induced by innate or acquired positive reinforcement from the resource/reward. Then, post consummatory motivational state is associated with low arousal positive emotions such as satisfaction or relaxation which functions to aid recovery or restoration once the resource/reward is acquired. The variations in the arousal of positive emotions form the starting point of the conceptual "Reward Cycle" model in animals which is hypothesized to result in the strongest positive affective state (Keeling et al., 2008). Strong support to this view is given by Boissy et al. (2007) who has proposed that classically motivated behaviours can be divided into two components: appetitive and consummatory behaviours and thus positive emotions can be separated into three temporal categories: i) past (e.g. post-consummatory satisfaction), ii) present (e.g. pleasant sensory activity), and iii) future (e.g. positive expectation, anticipatory joy).

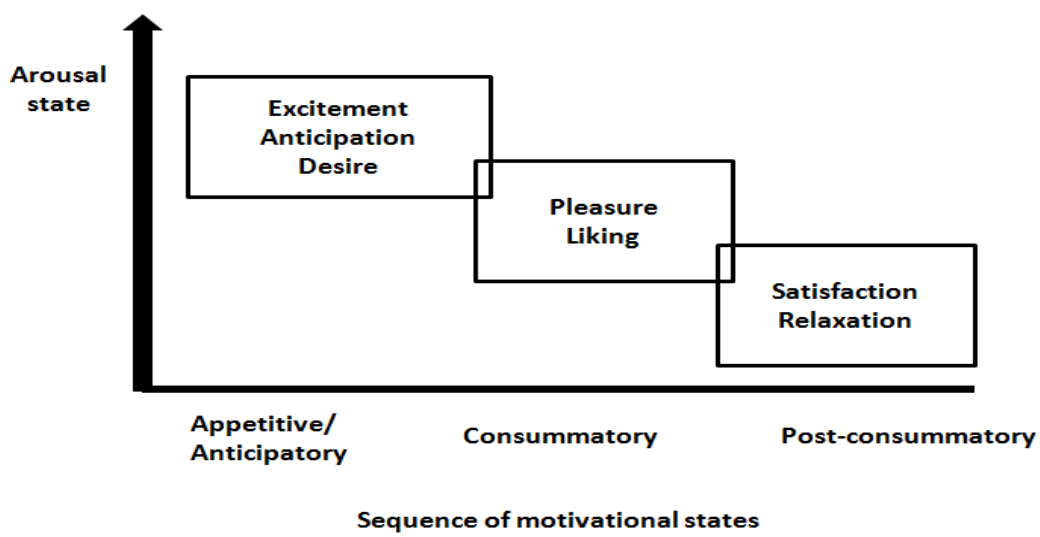

Figure 1. Representation of the relationship between arousal and different positive emotional states in the 'Reward Cycle' conceptual model (after Keeling et al., 2008).

2.3.1 Appetitive/Anticipatory phase

A high arousal positive emotion such as anticipation of a stimulus of high hedonic value is hypothesized to represent an appetitive type of positive affective states (Moe et al., 2009). Anticipatory behaviour occurs during the appetitive phase which is elicited by rewarding stimuli and prepares an animal for a forthcoming change and leads to and facilitates the consummatory phase (Van Der Harst and Spruijt, 2007). Anticipation requires the ability to internally represent expectations of a forthcoming reward or other event (Spruijt et al., 2001). Anticipatory behaviour can be quantified by the level of activity measured by the total frequency of all behavioural elements displayed during the anticipatory phase (Van Der Harst et al., 2003). Anticipatory behaviour is expressed as frequency of transitions of goal-directed behavioural patterns, such as locomotor and investigatory behaviour (Van Der Harst and Spruijt, 2007).

Behavioural activation in the anticipation of arrival of a reward represents the activation of reward centres in the brain (Spruijt et al., 2001). During this appetitive phase, dopaminergic activity is increased in the ventral striatum of the brain and thus dopamine appears to be involved in prediction of rewards (Spruijt et al., 2001). Dopamine is important for activational aspects of motivation, resource allocation and responsiveness to conditioned rewarding stimuli and action (Spruijt et al., 2001; Boissy et al., 2007).

Various types of conditioning paradigm are applied for the induction of anticipatory behaviour in animals such as classical/pavlovian conditioning, operant conditioning and trace conditioning (Van Den Bos et al., 2003, Moe et al., 2009, Trezza et al., 2011). The types of behaviour that are displayed during anticipation of a reward or positive event differ among different species and conditions (Van Den Bos et al., 2003; Vinke et al., 2004; Dudnik et al., 2006). Depending on species and test conditions, frequency of changes either increase or decrease during the anticipatory phase. In rats, *Rattus Norvegicus*, increased general activity, increased locomotion, increased investigatory behaviour, gnawing and hopping is seen as anticipation of a positive event such as food stimuli, sexual contact, enriched cages and social contact (Van Den Bos et al., 2003, 2004; Van Der Harst et al., 2003). When anticipating aversive stimuli such as play deprivation, social defeat and social isolation, rats decrease their locomotor activity (Van Den Bos et al., 2004; Van Der Harst et al., 2003). Mink show their anticipatory activity in the same way as rats by an increase in behavioural transitions and increased activity (Vinke et al., 2004).

When anticipating a positive reward, silver foxes become more active, perform more stereotypical behaviour, have more erected ears and spend more time in front of the cage compared to anticipating aversive stimuli (Moe et al., 2006). In contrast, cats decrease their activity during anticipation of a food reward (Van Den Bos et al., 2003). In the anticipation of a positive event, hens show more comfort behaviours such as preening and wing flapping whereas they show more head movements and locomotion in the anticipation of a negative event (Zimmerman et al., 2011). Standing alert with head and neck stretched has also been shown as anticipatory behaviour in hens (Moe et al., 2009). Announcement of a reward to piglets caused an increase in play behaviour (Dudnik et al., 2006). Positive emotional vocalizations have been exhibited during anticipation of rewards in primates and rodents (Burgdorf and Panksepp, 2006).

Regular announcement and presentation of rewards or regular induction of anticipation to a positive rewarding stimulus has welfare benefits to the animals and has been shown as a measure of positive affective states (Van Der Harst et al., 2005; Moe et al., 2009). After announcement and presentation of rewards to rats and pigs, stress-reducing effects, decreased frequency of aggressive behaviour, decreased aggression after weaning and reduced weaning stress has been found (Dudnik et al., 2006; Van Der Harst et al., 2005).

2.3.2 Consummatory phase

Presentation of a reward or positive stimuli is done in the consummatory phase of the reward cycle. The animal's correct prediction of a reward in the appetitive phase leads to successful reward acquisition and consumption in the consummatory phase (Boissy et al., 2007). The consummatory motivational state is linked with pleasure or liking induced by innate or acquired positive reinforcement from the resource/reward (Keeling et al., 2008). Pleasure and liking are characterized by high arousal positive emotions (Keeling et al., 2008). The consummatory component has been neurobiologically dissociated from the appetitive component and Boissy et al. (2007) suggest that the opioid system in the brain is related to the consummatory, liking aspect of motivation. Consummatory behaviour includes too many different types of behaviour to be changed coherently by a single brain manipulation (Berridge and Robinson, 1998). Consummatory behaviours may vary according to different types of reward and also by incentive value and characteristics of the reward (Boissy et al., 2007). Presentation of rewarding stimuli could lead to the successful consumption of these rewards by displaying various behavioural and physiological signs of emotions, thus reflecting positive affective states (Boissy et al., 2007).

2.3.3 Post consummatory phase

The post consummatory phase has been suggested by Boissy et al. (2007) to be a state of fulfilled needs and cessation of drive for further rewarding sensations characterized by reduced activities in a complex network of forebrain structures including the hypothalamus, amygdala, and frontal and insular cortex. In contrast to anticipatory and consummatory motivational states where reward is sought and eventually gained; post consummatory motivational state has been suggested to be associated with low arousal positive emotions such as satisfaction or relaxation which functions to aid recovery or restoration once the resource/reward is acquired (Keeling et al., 2008). Dopamine and opiods have been shown to be the neuronal basis of anticipation and consumption of reward (Spruijt et al., 2001). In contrast to this, very little is known about the neuronal basis of satisfaction. Ventral tegmental dopamine has been found to link the specific requirement of a particular need/reward with an expected satisfaction of that need (Boissy et al., 2007). Unlike the anticipatory and consummatory phases of reward acquisition, little research has been directed towards the positive affective states that might be experienced during the post consummatory phase (Burman et al., 2011).

2.4 Ear postures

Apart from behavioural and physiological measures used for the assessment of positive affective states in animals, observation of ear postures may also be useful in assessing emotional valence in sheep. Physiological measures generally provide a quantitative assessment of the emotional activation (i.e. the arousal or the intensity of the emotional response) without clearly defining the exact nature of the emotion (e.g. the positive or negative valence of the emotional experience) (Boissy et al., 2011). Behavioural measures consisting of fixed action patterns, such as startle, offensive or defensive postures, freezing, or approach only provide information about the intensity of the underlying emotion. Behavioural and physiological indicators along with identification of different ear postures allow a convenient and reliable assessment of emotion in sheep (Boissy et al., 2011).

In humans, expression of emotions has been extensively studied through changes in facial expression. But sheep have a limited superficial facial muscles network so they do not have a wide array of facial expressions and they are also hard to interpret from a human point of view (Reefmann et al., 2009). Ruminants have high mobility of the neck offering various head postures and have several muscles for rotating their ears (Reefmann et al., 2009). Although some of the studies have provided the indications that ear postures may be useful in assessing emotional valence in animals, very few studies have measured the ear postures in farm animals. In the study by Schmied et al. (2008) high occurrence of pendulous ear postures was used as an indicator of the animal's positive rating of their favourite grooming sites in cattle.

Boissy et al. (2011) identified four discrete ear postures from a comprehensive approach which distinguish all theoretically measurable ear postures without a priori interpretations. The ear postures are (See Fig. 3) horizontal ears or plane ears (P posture) which is noted when two ears are in the frontal plane and auricles are concealed from frontal view. It includes combination pr1pv1 and pr2pv1 from figure 3. Second one is ears raised up (R posture). 'R' posture of ears is noted when two ears are either ahead or aligned and auricles are visible from the front view (pr2pv2). Third ear posture is ears pointed backward (B posture). B' posture of ears is noted when two ears are behind the frontal plane and auricles are concealed from the front view (pr3pv3). Fourth ear posture is asymmetric posture (A posture) which is noted when two ears are in two distinct positions relative to the frontal and visibility of auricles is asymmetrical (pr4pv4). These different ear postures are linked with different positive and negative emotional states in sheep in the studies done by Boissy et al. (2011) and Reefmann et al. (2009) which clearly give the idea that ear postures can be related to specific affective states in animals.

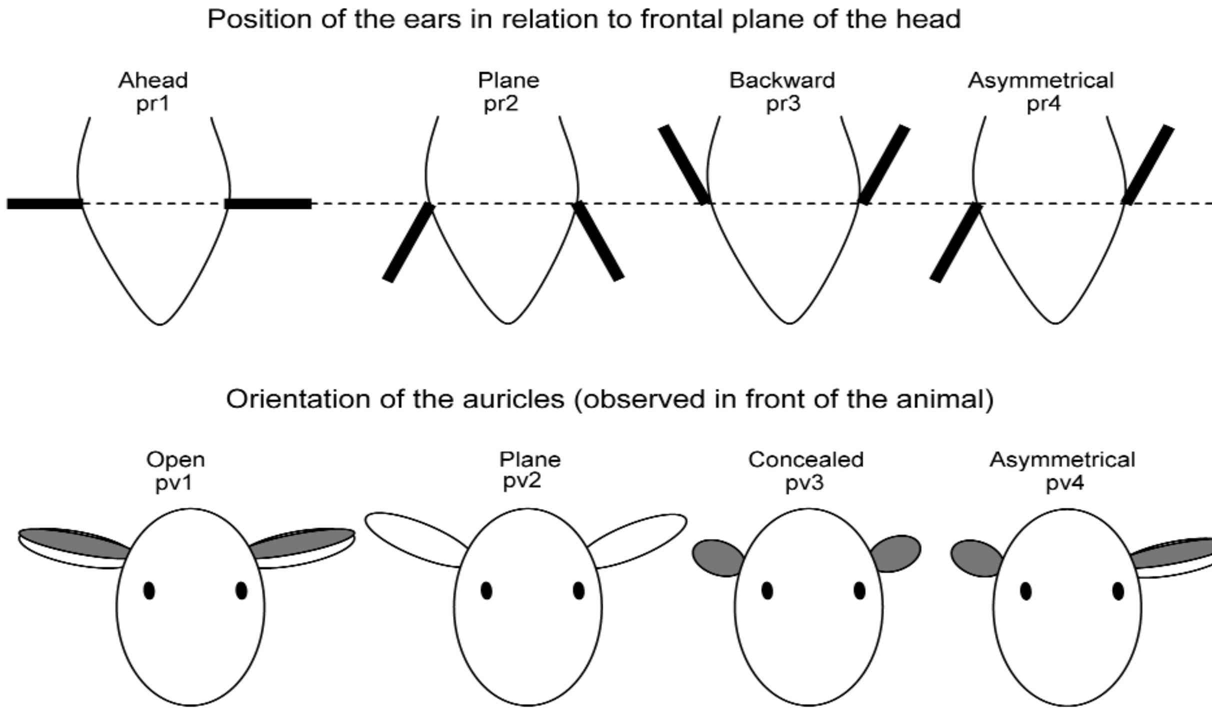

Figure 2. Position of ears according to two criteria: position of the ears in relation to frontal plane of the head (top) and orientation of the auricles observed in front of the animal (bottom). (Boissy et al., 2011).

2.5 Physiological responses

Physiological measurement such as determination of heart rate, respiration rate, cortisol etc has been widely used in farm animal welfare assessments (Scott et al., 2009). There has been a lot of research in physiological measurements indicative of negative states in animals. Various studies demonstrate that the change of cortisol can have a significant effect on an animal's behaviour and welfare. Increase in cortisol levels has been found to be associated with decrease in rumination which is generally considered as state of relaxation for cows (Bristow and Holmes, 2007). Increase in cortisol concentration has also been reported in cattle after periods of confinement or social isolation (Bristow and Holmes, 2007). Fear of humans in pigs appears to lead to chronic stress effects such as sustained elevations of plasma corticosteroid concentrations (Hemsworth et al., 1986).

It has been proposed by Worsaae and Schmidt (1980) that positive emotions are integral to play performance and situations which evoke negative emotions, such as environmental stress can have an inhibitory effect on the expression of play behavior. However, there could also be an opposite relationship between play and stress. There is limited research in the area of play behaviour and cortisol concentrations. Some recent studies have reported that play activity may contribute to the reduction of stress (Horvath et al., 2008). It has been shown that play behaviour is negatively correlated with elevated plasma cortisol in piglets (Worsaae and Schmidt, 1980), elevated urinary cortisol in cats (Carlstead et al., 1993) and is negatively correlated with increase salivary cortisol in dogs (Horvath et al., 2008). Measurement of cortisol in our study could provide information about how cortisol and play activity is linked to positive states in lambs.

Determination of cortisol which is supposed to have an opposite effect on oxytocin could be an alternative way to find out about the physiological measurements indicative of positive states in animals. Oxytocin plays a critical role in the causes and effects of positive social interaction and influences consummatory as well as motivation and rewarding aspects of sexual behavior (IsHak et al., 2011). Uvnäs-Moberg (1998) has proposed that the release of oxytocin can become conditioned to emotional states. Therefore, the actions of oxytocin may provide an additional explanation for the long-term benefits of positive experiences (Uvnäs-Moberg, 1998).

There are various ways to detect metabolites of corticosteroids among which the most common method is to detect in the serum, plasma, urine or fecal matter. Collecting blood for serum or plasma analysis is invasive and handling and physical restraint associated with blood sampling can affect the hormone concentration dramatically if the blood sampling is not done within short time (Möstl et al., 2002; Saco et al., 2008). There are other alternatives to blood sampling which are less invasive which include collecting saliva, urine or fecal matter to analyse steroid and metabolite concentrations (McKenzie and Deane, 2005; Dreschel and Granger, 2009). Saliva sampling for cortisol measurement has many advantages; it is a non-invasive method, comparatively stress free and allows for frequent and rapid sampling. Trained staff and specialized equipment are not necessary and sampling can take place outside of a laboratory, in the field conditions and at several times throughout each day (Levine et al., 2007). Small volume of saliva sample (as little as 25 µl) is sufficient for cortisol measurement (Dreschel and Granger, 2009). Salivary cortisol is highly correlated with plasma cortisol in cattle (Hernandez et al., 2005). Generally up to 4 min could be taken to collect a saliva sample from a dog without the effect of handling being reflected in cortisol concentrations (Dreschel and Granger, 2009).

Therefore, salivary cortisol has been increasingly used as a measure of stress response in welfare studies, reaction to stress and human–animal interactions in dogs and other species (Dreschel and Granger, 2009).

2.6 Aim

Based on previous research, play seems to be a behaviour that animals have a high motivation to perform and that it is a rewarding activity, so presentation of play as a reward to the animal should induce positive anticipation and facilitate consummatory response. Also, acquisition of a reward (e.g. play) should induce relaxation in animals. To our knowledge, studies of complete reward cycles including play as a reward has not been studied till date. Therefore, the aim of the present study was to define, describe and quantitatively analyze play behaviour of lambs and to investigate whether the lambs show signs of anticipatory, consummatory and post consummatory behaviour before, during and after performing play.

The following questions were investigated:

1. Do lambs show any behaviour indicative of their expectation (i.e. excitement and wanting) to play in a play arena?
2. How do lambs use a play arena and are they more active in a play arena than in their home pen?
3. Do lambs show behaviour indicative of pleasure and liking during their stay in a play arena?
4. Do lambs show behaviour indicative of relaxation and satisfaction after having been in a play arena?
5. Do lambs show a decreased in the physiological stress response after having been in a play arena compared to before they were taken to the play arena?

We hypothesized that lambs would be more excited and show anticipation to play when they were released in a larger area with some playing objects, that they would be motivated to play and use the space and playing objects, and that they would show signs of being more relaxed and satisfied after having played for a certain duration in the play arena.

3. Materials and Methods

3.1 Animals

The study was approved by the Swedish Ethical Committee of Experimental Animals of Gothenburg (Dnr: 123-2011) prior to initiation. The study was carried out at Götala Research Farm in Skara; in the southwest of Sweden, between 21 June and 30 July in 2011. The research animals comprised of twenty uncastrated male lambs of Dorset breed (n=8) and Swedish Fine Wool and Dorset mixed breed (n=12). The lambs were leased from a nearby farmer. Lambs were taken from the pasture, weaned from their mother and transported to Götala research farm on the 21^{st} of June in a horse wagon by the farmer. The average age of the lambs was 10 weeks (Mean 70.6 ± 5.6 days) ranging from around 11 to 8 weeks of age. On the day of arrival, lambs were off-loaded to a temporary holding pen (8 x 3m) and identity and weight of all the lambs were recorded. The average weight of lambs was 24.84 kg (S.D. = 2.79), with lambs weighing maximum 29.2 kg and minimum 19 kg. The final average weight of the lambs at the end of the experiment was 40.5 kg (S.D. = 3.68).

3.2 Housing and management

3.2.1 Home pens

Ten pens measuring 2 x 3 m wide were built in the farm with galvanized steel gates. In between the pens, a play arena was built so that it divided ten pens into five pens on each side of the play arena (Fig. 4). The pens stood next to each other so that lambs could sniff at their neighbours through the gates. Straw was used as bedding material in the pens and fresh straw was added every third day depending on the wetness of the bedding. Lambs were randomly allocated as pairs to the pens on the arrival day after the identity and weight of lambs had been recorded. Lambs having approximately equal weight were allocated to the same pen and twin lambs were housed in different pens. Animals in each home pen were marked with blue and red paint across the hips and shoulders so that the animals would be easily identifiable. Paint was refreshed in the middle of the experiment.

3.2.2 Feeding

At the front of the home pens, lambs had access to a feed trough which contained standard mixed diet. An automatic water cup provided lambs with ad libitum access to water. Feed was given ad libitum and new feed was given in the morning around 7 to 7:30 every day. A standard feed ration (per kg DM) consisting of grass silage, distillers dried grains (DGG), barley and heat treated rapeseed (RSM) in standard proportion of 0.42, 0.20, 0.23 and 0.13 respectively was formulated before the start of the study. The proportion of forage NDF was 218 and concentrate was 148. The DM% of the feed was 67.4, ME 12 MJ, CP 202.3g, digestible CP 164g, AAT 102.9g, PBV 40.6g, NDF 366g, Starch 164.1g and EE 34.1g. Minerals added in the feed were Ca 7.5g, P 6.0g and K 15.4g. A small block of salt was placed in the trough inside the pen so that lambs could lick at it to get the required sodium chloride (NaCl) content.

3.3 Experimental design

3.3.1 Play arena and holding pen

A play arena of 5.9 x 5.5 m was built with galvanized steel gates. A solid wood wall was placed on two sides to hinder the lambs in home pens from looking into the play arena. The play arena was enriched with different playing objects like a tunnel that they could pass through, two hanging chains that they could chew on and pull at and a volley ball that they could roll (Fig.4). Two holding pens measuring 1.95m long and 1.30 m wide were built on each side of the play arena (Fig. 4). One of the sides of the holding pen was facing the play arena so that the lambs were able to look into it. A thin layer of sawdust was spread on the floor of the play arena and holding pen. Urine and faeces were removed everyday from the holding pen and play arena and fresh saw dust was added.

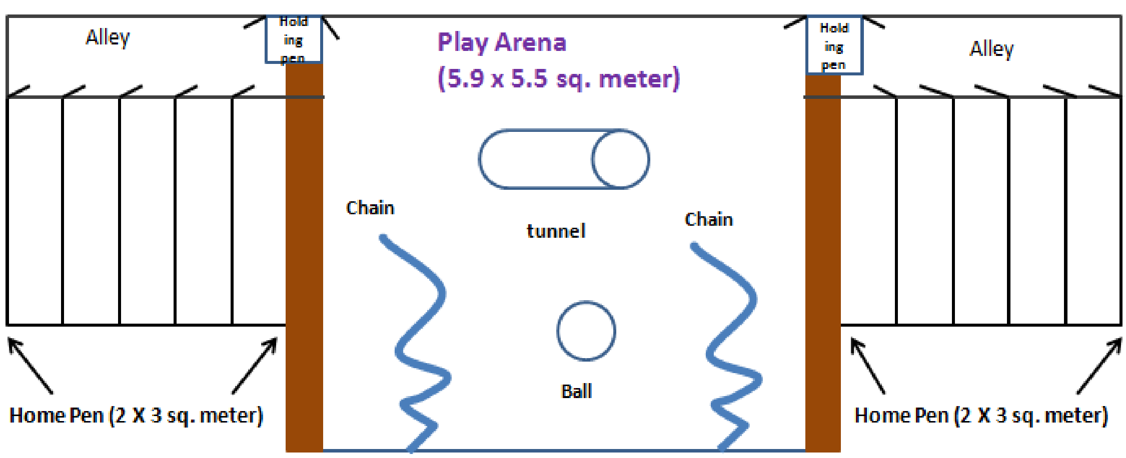

Figure 4: Drawing of the play arena, two holding pens and 10 home pens for the tested 10 pairs of male lambs. Play arena is enriched with two chains, volleyball and a tunnel.

3.3.2 Pre –Experimental period

After arrival, lambs were habituated to their pen and human handling for five days. During this period, one person went inside the pen, stayed there for 15 minutes and tried to approach the lambs gently, keeping some distance to the lambs. After 15 minutes, the person left the pen and approached the lambs in another pen. This was done for all ten pens for five days.

A pre-experimental period started one week before the start of the actual experiment and lasted for seven days. Lambs were assisted in learning to stay in the holding pen for 5 minutes which was gradually increased from 1 minute to 5 minutes during seven days. They were conditioned to anticipate playing in the play arena by being kept in the holding pen for 5 minutes. During the learning session, each pair of lambs separately were released from their home pen and walked freely to the holding pen. After 5 minutes of stay in the holding pen, the gate of the holding pen was opened and the lambs were free to walk into the play arena. If the lambs did not walk voluntarily, they were gently pushed to the play arena. After playing for 20 minutes in the play arena, the gate to the holding pen was opened and the lambs could walk freely back to their home pen. Within the training period, pilot observations of different behaviours of the lambs were done to identify and define the most important behaviours. Protocols for recording different behaviour shown by the lambs in the holding pen, the play arena, the home pen and the home pens (no play) (i.e. when lambs were in their home pen and not playing that day) were formulated.

3.3.3 Experimental period

The experimental period started one day after the end of the pre-experimental period and lasted four weeks. Lambs always played between 9.00 and 13.00 of the day. On the first day of the experimental period, random division of the ten pairs of lambs was done to form two groups which consisted of five pairs in each. Pairs were randomly selected from both sides of the play arena so that two pairs from one side and three pairs from the other side of the play arena were in each group. Five pairs were given the opportunity to play in the play arena on the first day, and the day after, the remaining five pairs were allowed to play. This playing routine was maintained for the whole four weeks of the experimental period which lead to playing in all the ten pairs of lambs three times in a week and 12 times in the whole experimental period. There were no play sessions on Sundays.

On the first test day of every week, time of play for five pairs of lambs was randomly allocated so that the order of letting the lambs play in the play arena was set and kept for that week. The same procedure was applied for lambs that played on the day after. A new playing schedule was randomly selected for the second, third and fourth week following the same procedure. Consideration was taken regarding the random allocation of time so that on the whole four weeks period, all lambs got the tentative opportunity to play at five observation times (9, 10, 11, 12 and 13).

3.3.4 Test procedure

In each test day, the door of the home pen was gently opened so that the two lambs left their home pen and walked through an alley to the holding pen, whose door was shut and then they stayed there for 5 minutes. After that they were released into the play arena and were left undisturbed to play for 20 minutes. Both the lambs behaviour was recorded for 5 minutes in the holding pen prior to the release in the play arena (anticipatory phase) and a 20 minute period in the play arena (consummatory phase). Thereafter, they walked back to their home pen and the behaviour was recorded for 6 minutes in the home pen (post consummatory phase).

3.3.5 Behavioural observations

Direct observation of lamb behaviour was done and the behaviours were recorded on protocol made for the behaviour observed in the holding pen, the play arena and the home pen. The pair of lambs was observed simultaneously but recordings were done individually as focal sampling. The frequency of behaviours per minute was recorded in all recording situations i.e holding pen, play arena and home pens. A clock was set to give a signal every minute. Ethogram of the behaviours that were observed is presented in Table 1 and 2.

For the home pen (no play) observation, behaviour of all the lambs was monitored and recorded on the day when they were not allowed to play in the play arena (three times in a week). So the recording of five pairs of lambs who played on Monday, Wednesday and Friday were done on Tuesday, Thursday and Saturday; and remaining five pairs of lambs who played on Tuesday, Thursday and Saturday were done on Monday, Wednesday and Friday. Lambs were always observed between 13.00 and 14.00. An observer passed each home pen and recorded the behaviour of lambs during a two minute period (frequency per minute) making three successive turns around the home pen for half an hour. Each pairs of lambs were observed for six minutes in total. Recording of behaviour was done only for three weeks. Ethogram of the behaviours that were observed is presented in Table 1 and 2.

Table 1. Ethogram of play behaviour recorded in lambs in the holding pen (HOP), play arena (PA), home pen (HP) and home pen (no play)(CP)

Behaviour	Description	Place
Running	Fast movement across pen or any gait faster than a walk, motion in forwards or sideway direction.	PA
Jumping	The two forelegs are lifted from the ground; the forepart of the body is elevated. Movement upward.	PA
Gamboling	Stiff legged jumping motions during which the body and head are commonly twisted repeatedly.	PA
Butting	Two animals push or run into each other with their heads. Can be in a unidirectional fashion, but is generally bidirectional. Distinct lowering of head in attempts to butt other animal within 2 cm is included. Butting body from side, front and back is also included.	HOP,PA HP,CP
Pawing	Using front leg to strike ground. Every extension on paw is not counted. But if the animal changes the leg and starts pawing then it is counted as new pawing.	HOP, PA, CP
Mounting	Animal stands on back two legs and raises front two legs on top of another animal (mounts another lambs' head or body from front, side, or back). Unsuccessful attempts to mounts are also included.	HOP,PA,HP,CP
Sniffing objects	Nose within 5 cm of the ball, tunnel or chain and inhaling air.	PA
Pass through tunnel	Two forelegs and half of the body inside tunnel or entire body passing through the tunnel. Includes walking, sniffing and jumping inside tunnel.	PA
Roll ball	Head or foot of lamb touching volley ball with slight movement or heavy movement of volleyball. Each rolling is counted as separate roll.	PA
Pull / chew chain	Using head to play with chain or chain in the mouth with distinct chewing of chain. Every single attempt to play is counted.	PA

Table 2. Ethogram of general behaviour recorded in lambs in the holding pen (HOP), play arena (PA), home pen (HP) and home pen (no play) (CP)

Behaviour	Description	Place
Lying	Belly and flanks of lamb in contact with the ground. No weight is	PA,HP,CP

	supported by any of the hooves. Eyes are open or closed.	
Lying while ruminating	Lying with open or closed eyes and ruminating.	HP,CP
Standing	All standing while not eating, drinking or exhibiting other motor behaviours. Standing with rumination is also included.	PA,HP,CP
Walking	Slow, leisurely movement, two or three hoofs touch the ground at any time, at least 2 steps forward.	HOP
Walking	Slow, leisurely movement, two or three hoofs touch the ground at any time, at least 3 steps forward.	PA,HP, CP
Sniffing pen mate	Nose within 5 cm of any part of the body of a pen mate and inhaling air.	PA,HP,CP
Sniffing pen	Nose within 5 cm of the floor, walls or substrate and inhaling air. Walking with sniffing is also included.	HOP, PA,HP,CP
Rubbing and Scratching	Rubbing body against the pen or objects, scratching body with hind leg or stretching whole or part of the body.	HOP, PA,HP,CP
Licking	Using tongue to lick any part of the body.	HOP, PA,HP,CP
Feeding	Lowering the head with muzzle in feed trough. Eating straw of pen is also included.	HP,CP
Drinking	Muzzle in contact with water and ingesting water.	PA,HP,CP
Vocalization	Long bleat of lambs.	HOP, PA
Chew tunnel, ball, pen	Licking or chewing tunnel, ball and pen.	PA
Pushing	One lamb push with the head or body another lamb while eating from the feed trough.	HP,CP
Defecation	Excretion of faeces in holding pen.	HOP
Standing HP	Standing facing holding pen within distance of 5 cm, continuous looking towards the holding pen for 10 seconds. Includes biting bar of holding pen.	PA
Standing facing home pen	Lambs standing looking towards home pen from holding pen.	HOP
Standing facing play arena	Lambs standing looking towards play arena from holding pen	HOP
Standing still	Lambs standing in middle or side of holding pen without looking at play arena or home pen.	HOP
Biting bar facing play arena	Mouth in the bar of holding pen facing towards play arena.	HOP
Biting bar facing home pen	Mouth in the bar of holding pen facing towards home pen.	HOP

3.4 Ear postures

Four different ear postures as defined by Boissy et al. (2011) were used as a reference for categorizing different ear postures in lambs. The recorded ear postures were; horizontal or plane ears (P posture), ears raised up (R posture), ears pointed backward (B posture) and asymmetric posture (A posture). Instantaneous recording every 15 seconds during three minutes (12 sample points) was performed in all four places. In the holding pen, ear postures were recorded during the second, third and fourth minute. In the play arena, ear postures for two lambs were recorded separately; lamb one was recorded during the 2nd, 9th and 18th minutes and the second lamb was recorded during the 3rd, 10th and 19th minutes. In the home pen, ear postures were recorded during the second, third and fourth minute after the lambs came back from the play arena. Ear postures for the experimental groups were recorded all four weeks of the study period (144 recordings/ lamb/place). For the home pen (no play), ear postures were recorded during the first minute of behavioural observation as described above. This makes in total 3 minutes observation for each pen. The ear postures were recorded for three weeks in home pen (no play) (108 recordings/ lamb).

3.5 Physiological responses

Saliva sampling was done for the measurement of cortisol and was carried out during the fourth week for all ten pairs of lambs. The saliva samples were taken according to the same schedule as recording of behaviour i.e. five pairs of lambs were sampled on Monday, Wednesday and Friday and the other five pairs on Tuesday, Thursday and Saturday.

On the day of saliva collection, two samples were taken for each lamb; first in the home pen before the lambs were released in the holding pen and then after they had been in the play arena and came back to the home pen. In total there were 120 saliva samples from 20 lambs during the fourth week. The lambs were restrained gently by a staff standing with the lambs in the corner and petting them to calm them down. A cotton swab held by a surgical tongue was placed in the mouth of the lamb for 1-2 minutes. As the lamb chewed on the swab it became wet and then enough saliva had been collected. After this, the cotton swab was taken out from the mouth and put in labeled sample tubes. The sample tubes were placed in an icebox and centrifuged immediately in the farm after sampling from each pen had been completed. The saliva was extracted by centrifuging the cotton swab in 3000 rpm for 15 minutes. The sample was immediately frozen at -20^{0}C for later analysis of cortisol in saliva. A specific protocol was used for recording the identity of lambs, time of sample collection and number of saliva sampling.

After the completion of the experiment, the saliva samples were sent to a laboratory at Skövde University, Sweden for analysing cortisol. ”High sensitivity salivary cortisol enzyme immunoassay kit” manufactured by the company Salimatrics was used for the analysis. A standard manual was followed for the analysis of saliva samples by a trained laboratory technician.

3.6 Statistical analysis

Data were analyzed with the statistical software SAS (SAS Institute Inc., Cary, USA) version 9.2. Before analyzing, the different behaviours recorded in the holding pen, play arena, home pen and home pen (no play) were grouped into different categories to meet the aim of the experiment. The behaviours were categorized based on previous research (Van Den Bos et al., 2003; Vinke et al., 2004; Dudnik et al., 2006; Moe et al., 2006; Zimmerman et al., 2011; Donaldson et al., 2002; Blackshaw et al., 1997; Dobao et al., 1984; Newberry et al., 1988; Jensen and Kyhn, 2000; Sachs and Harris, 1978). The behaviours included in each category were:

<u>Behaviour in holding pen before playing in a play arena</u>

Anticipation of play: Standing facing play arena, walking, mounting, butting, pawing, sniffing pen, biting bar facing play arena

No anticipation of play: Standing facing home pen, standing still, biting bar facing home pen, defecation, vocalization, licking/scratching

<u>Behaviour in a play arena</u>

Social play: Mounting, butting, sniffing pen mate

Locomotor play: Running, gamboling, jumping, pawing

Object play: Roll ball, pass through tunnel, pull / chew or play with chain, sniffing object

Total play: social, locomotor and object play

Other behaviours: Walking, sniffing pen, standing, lying, drinking, standing facing holding pen, vocalization, chew tunnel/ball/ pen, rubbing/ scratching/licking

Behaviour in home pen after being in play arena

Relaxation: Lying, lying while ruminating, feeding, drinking, scratching body, licking

No relaxation: Walking, standing, mounting, butting, pushing, sniffing pen, sniffing pen mate

Behaviour in home pen (no play)

Relaxation: Lying, lying while ruminating, feeding, drinking, scratching body, licking

Other behaviours: Walking, standing, pushing, sniffing pen, sniffing pen mate

Play: Mounting, butting, pawing

3.6.1 Behaviour

Data followed a Poisson distribution. Mean number of recordings ± SE (Proc means) of each behaviour and each category of behaviour was calculated on pairs (n=10). Week wise calculation of mean number of recordings of behaviour was done. Mean number of recordings of the behavioural elements in each category were analysed per min for 5 minutes in holding pen and 6 minutes in home pen. Mean number of recordings of behaviour in first 10 minutes and last 10 minutes in the play arena was also calculated.

Wilcoxon signed rank test was used for comparison between anticipation and no anticipation; total play and other behaviours; relaxation and no relaxation. Generalized linear model (Proc Genmode) was applied with repeated subject as animal (pen) to determine the effect of time and week for anticipation and relaxation. It was also used to determine effect of period and week for total play, social play, locomotor play and object play in the play arena. Because individual in a pen could not be considered to be independent in the analysis, we calculated mean values per pen for each test and behaviour. Paired Chi2-test was used as a post hoc test to analyse if there were significant differences between the four weeks and different recording minutes.

A Spearman's rank correlation was conducted (Proc corr, Spearman) to test for correlations between anticipation, total play and relaxation. Differences were considered to be significant if $p < 0.05$; a trend was considered were $0.05 \leq p \leq 0.1$. Results were non-significant when $p > 0.10$ (N.S.).

3.6.2 Ear postures

Mean percentage of the four ear postures ± SE in holding pen, play arena, home pen and home pen (no play) was calculated (Proc means, n=10). The effect of week on the recorded ear postures of lambs was also calculated.

3.6.3 Cortisol Measurement

Mean ± SD was calculated for each test day for cortisol before play and cortisol after play (n=20). Paired t-test of difference in cortisol before and after play was carried out to test if there was any significant difference between cortisol before and after play (Proc ttest). Pearson correlation test between cortisol before and after play with anticipation, total play and relaxation was carried out (Proc corr). Differences were considered to be significant if $p < 0.05$, and non-significant when $p > 0.05$ (N.S.).

4. Results

4.1 Behaviour

4.1.1 Holding pen (Before play)

Walking and sniffing the pen had the highest mean number of recordings among all the behaviours shown by lambs during their stay in the holding pen (Table 3). Standing facing the play arena, butting, standing facing home pen, standing still and licking/scratching were also frequently shown behaviours. The least recorded behaviours were defecation and vocalization. There were large individual differences in some behaviours as can be seen in minimum and maximum values. Lambs from some pens never showed mounting, vocalization and biting bar facing play arena. Also, the minimum recorded defecation and biting bar facing home pen was less compared to other behaviours. Large individual variation in the butting was recorded, where lambs of some pens frequently showed butting whereas some lambs showed butting only during certain occasions. Chewing the wall of holding pen was also frequently seen but it was not recorded as it had not been defined in the ethogram.

Table 3. Mean number of recordings of behaviour of male lambs in the holding pen during 5 minutes of observation three times per week for four weeks (n=10 pairs)

Behaviour	Mean	SE	Minimum	Maximum
Anticipation				
Standing facing play arena	0.62	0.046	0.42	0.81
Biting bar facing play arena	0.10	0.024	0	0.25
Sniffing pen	1.17	0.069	0.85	1.46
Walking	1.18	0.070	0.93	1.74
Mounting	0.09	0.018	0	0.20
Butting	0.50	0.104	0.07	1.16
Pawing	0.32	0.072	0.1	0.10
No anticipation				
Standing facing home pen	0.44	0.043	0.2	0.64
Standing still	0.42	0.026	0.23	0.54
Biting bar facing home pen	0.13	0.028	0.008	0.26
Defecation	0.05	0.008	0.01	0.1
Vocalization	0.006	0.002	0	0.01
Licking/scratching	0.39	0.056	0.23	0.82

Behaviour indicative of anticipation to play was expressed significantly ($S= 27.5$, $p<0.01$) more than behaviour indicative of no anticipation to play during the five minutes in the holding pen (Fig. 5).

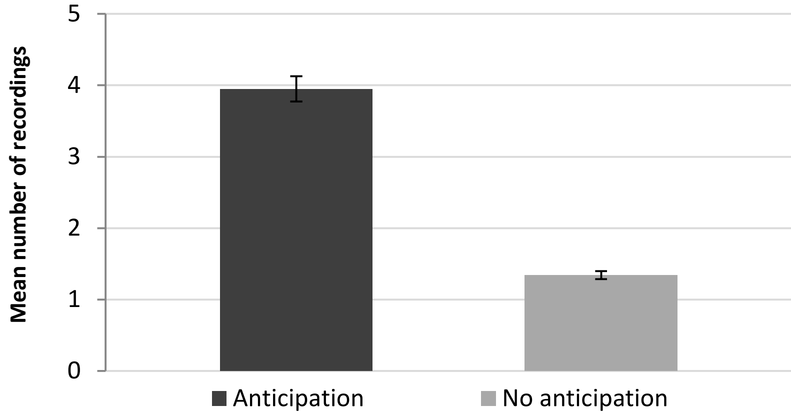

Figure 5. Mean number (± SE) of recorded anticipation and no anticipation in pairs of male lambs in the holding pen during five minutes of observation three times per week for four weeks (n=10 pairs).

Behaviours indicating anticipation to play did not change over the five minutes that the lambs stayed in the holding pen (χ^2=6.77, p=N.S., Fig. 6). Behaviours indicating no anticipation to play also did not change over the five minutes in the holding pen.

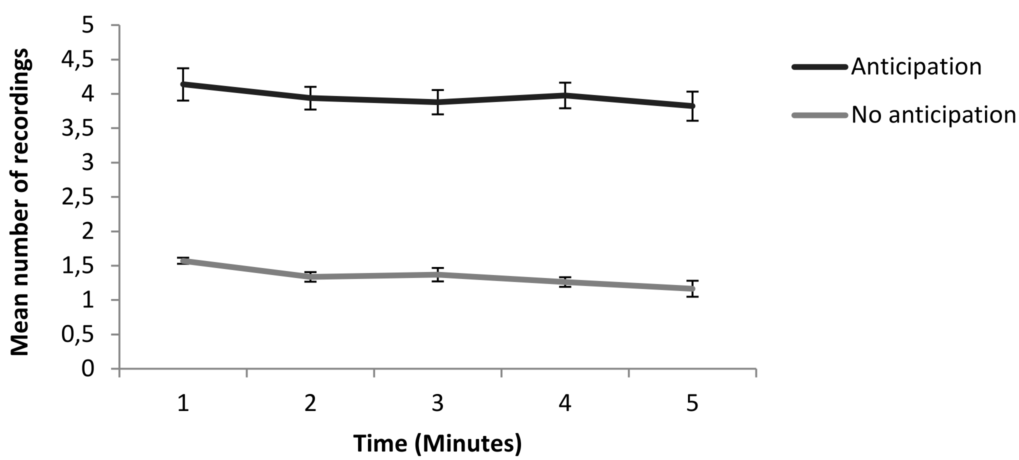

Figure 6. Mean number (± SE) of recorded anticipation and no anticipation every minute in pairs of male lambs in the holding pen during the five minutes of observation three times per week for four weeks (n=10 pairs).

There was significant difference between the weeks in the observed anticipatory behaviour in the holding pen (χ^2=14.54, p=0.0023, Fig. 7). Anticipatory behaviour increased significantly ($p<0.001$) from the first to the second week and was almost similar in the third week. The decrease in anticipatory behaviour from the third week to the fourth week was significant ($p<0.05$).

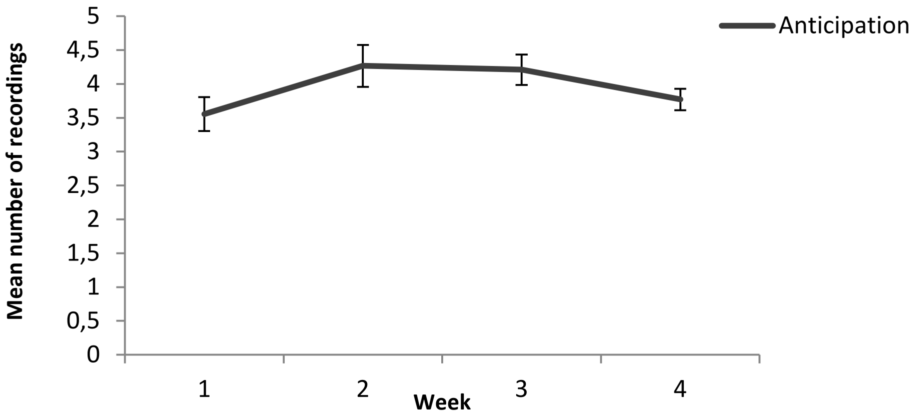

Figure 7. Mean number (± SE) of recorded anticipation in pairs of male lambs in the holding pen during the four weeks of experimental period (n=10 pairs).

4.1.2 Play arena (During play)

Butting and walking had the highest mean number of recordings among all the behaviours shown by lambs in the play arena (Table 4). Running, roll ball, sniffing pen, mounting, pass through tunnel, pull chain and pawing were the other more common behaviours. Lying was the least recorded behaviour. Large individual variations in running, roll ball and pass through tunnel was recorded. Lambs of some pens were running, rolling ball and passing through tunnel in all the playing occasions whereas some lambs performed these play behaviours only during certain play occasions. Vocalization was recorded only in the lambs of three pens. The highest number of recorded vocalization was from one lamb who vocalized in almost all the play occasions. Due to this, the vocalizing lamb had to be taken out of the play arena after 10 minutes and 11 minutes of the play in the first two playing occasions during the experimental period since we were confused whether the lamb was stressed due to being in the play arena. It was noted that lambs also performed flehmen in the play arena but this behaviour was not recorded as it was not part of the ethogram.

Table. 4. Mean number of recordings of behaviour of male lambs in the play arena during the 20 minutes of observation three times per week for four weeks (n=10 pairs)

Behaviour	Mean	SE	Minimum	Maximum
Social play				
Butting	1.58	0.205	0.70	2.65
Mounting	0.26	0.033	0.12	0.44

Sniffing pen mate	0.04	0.006	0.02	0.08
Locomotor play				
Running	0.56	0.123	0.07	1.60
Gamboling	0.14	0.031	0.03	0.30
Jumping	0.13	0.024	0.04	0.25
Pawing	0.21	0.030	0.11	0.44
Object play				
Roll ball	0.48	0.094	0.08	0.96
Pull/chew chain	0.24	0.021	0.10	0.34
Pass through tunnel	0.25	0.066	0.01	0.78
Sniffing object	0.11	0.009	0.08	0.16
Other behaviours				
Walking	1.20	0.047	0.98	1.39
Standing	0.21	0.020	0.12	0.36
Sniffing pen	0.44	0.028	0.28	0.56
Lying	0.01	0.007	0	0.08
Rubbing/Scratching/Licking	0.23	0.027	0.13	0.43
Chew tunnel, pen	0.19	0.028	0.09	0.39
Drinking	0.10	0.011	0.05	0.15
Standing facing holding pen	0.07	0.009	0.03	0.13
Vocalization	0.12	0.116	0	1.17

Total play was expressed significantly ($S=25.5$, $p<0.01$) more than the other behaviours (Fig. 8). Lambs were active in the play arena and used the play arena for performing different types of play rather than showing other behaviours. Lambs performed total play significantly more during the first 1-10 minutes of their stay in the play arena than during the 11-20 minutes ($\chi^2=76.67$, $p<0.0001$, Fig. 9).

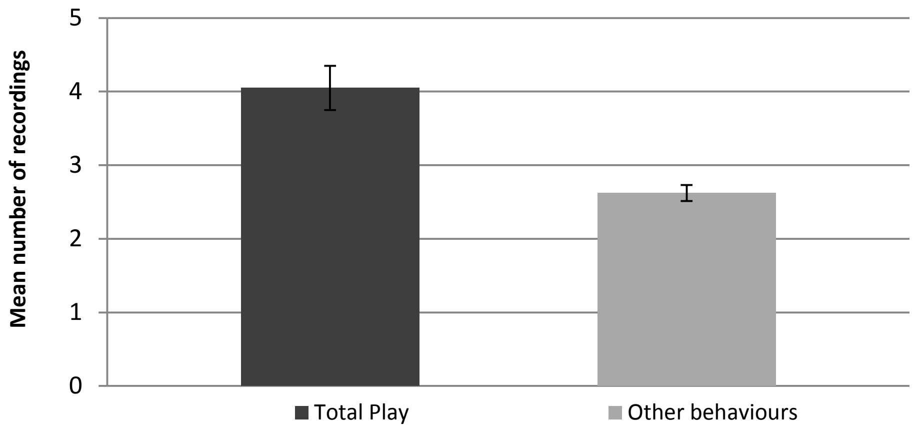

Figure 8. Mean number (± SE) of recorded total play and other behaviours in pairs of male lambs in the play arena during 20 minutes of observation three times per week for four weeks (n=10 pairs).

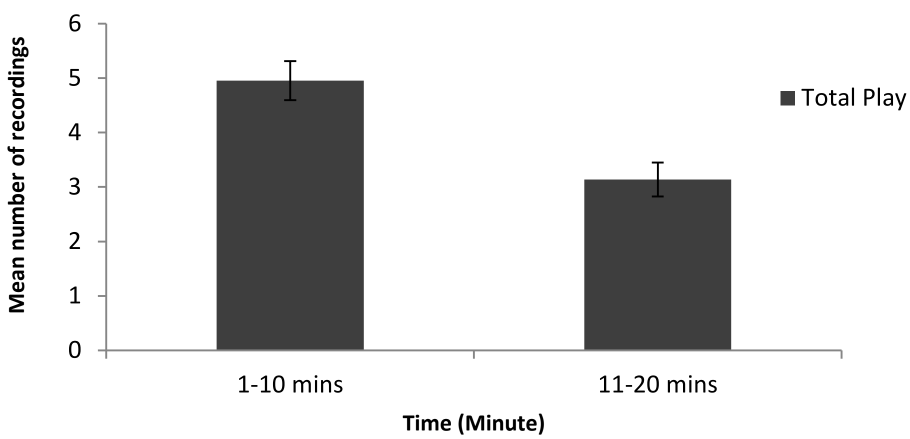

Figure 9. Mean number of recorded (± SE) total play in pairs of male lambs in the play arena during 1-10 minutes and 11-20 minutes of the 20 minutes of observation three times per week for four weeks (n=10 pairs).

Among the three different types of play, lambs performed significantly ($p<0.0001$) more social play during 1-10 minutes than 11-20 minutes (Fig. 10). Also, the performance of locomotor play ($p<0.0001$) and object play ($p<0.001$) was significantly higher during 1-10 minutes than during 11-20 minutes.

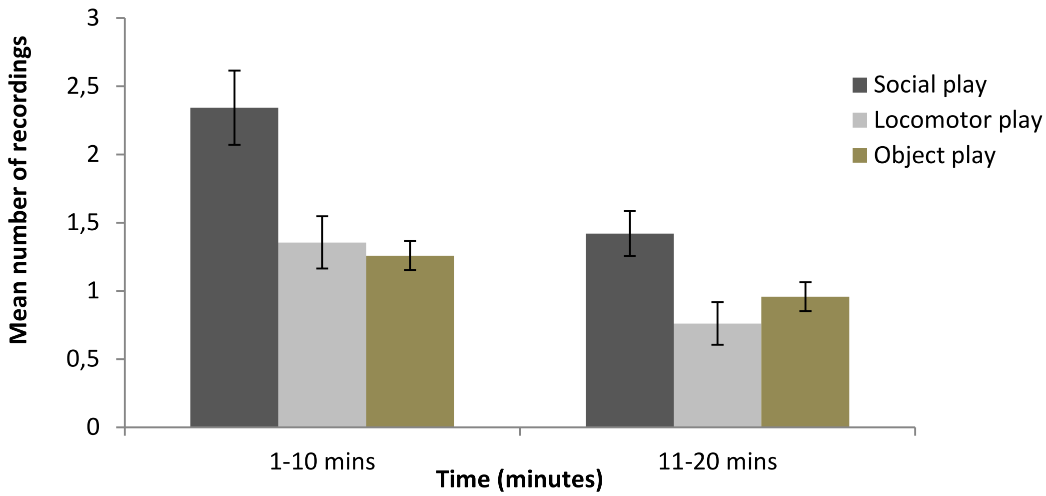

Figure 10. Mean number of recorded (± SE) social play, locomotor play and object play in pairs of male lambs in a play arena during the 1-10 minutes and the 11-20 minutes of 20 minutes of observation three times per week for four weeks (n=10 pairs).

There were significant differences between the weeks in the observed total play in the play arena (χ^2 =24.40, p<0.0001, Fig. 11). Lambs performed total play more in the second week compared to the other weeks. Total play increased significantly (p<0.0001) from the first to the second week and gradually decreased in the third and fourth week. Decrease of total play in the third week was not significant (N.S.) but the decline from the third to the fourth week was significant (p<0.05).

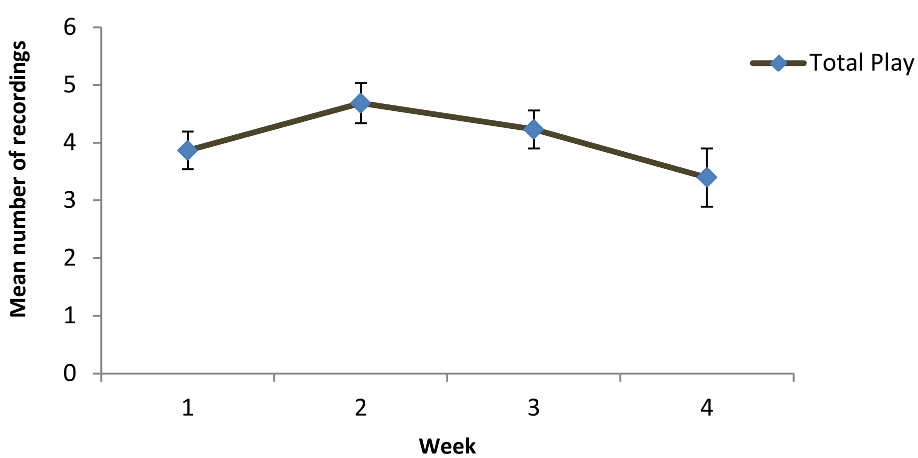

Figure 11. Mean number (± SE) of recorded total play in pairs of male lambs in a play arena during the four weeks of experimental period (n=10 pairs).

There were significant differences between the weeks in the observed social play (χ^2 =12.14, $p<0.01$), locomotor play (χ^2 =11.79, $p<0.01$) and object play (χ^2 =20.89, $p<0.001$) in the play arena (Fig. 12). Lambs expressed significantly ($p<0.01$) more social play in the second week and then a tendency of decreased ($p<0.1$) social play in the third week and a significant decrease ($p<0.001$) of social play from the third week to the fourth week. Locomotor play increased significantly ($p<0.05$) from the first to the second week. Significant decrease ($p<0.05$) of locomotor play was observed in the third week which continued till the fourth week (N.S.). There were no significant changes between the weeks in object play (N.S.).

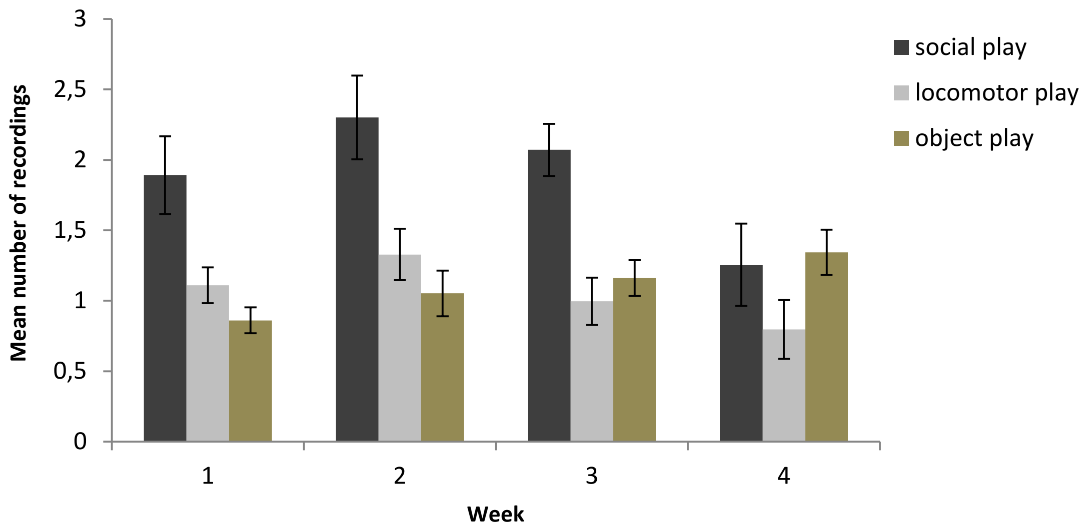

Figure 12. Mean number (± SE) of recorded social play, locomotor play and object play in pairs of male lambs in the play arena during the four weeks of experimental period (n=10 pairs).

4.1.3 Home pen (After play)

Feeding, walking and standing had the highest mean number of recordings among all the behaviours shown by lambs in the home pen (Table 5). Mounting was the least recorded behaviour. There were lambs who never showed lying, lying while ruminating, licking, mounting and butting. Lambs were mostly recorded as standing near the gate of home pen facing towards the observer until the second minute of returning back from the play arena, but after that they walked towards the feed trough and started eating the feed.

Table 5. Mean number of recordings of behaviour of male lambs in the home pen during 6 minutes of observation three times per week for four weeks (n=10 pairs)

Behaviour	Mean	SE	Minimum	Maximum
Relaxation				
Feeding	0.91	0.063	0.61	1.12
Lying	0.05	0.015	0	0.14
Lying while ruminating	0.02	0.010	0	0.07
Drinking	0.04	0.009	0.006	0.11
Scratching body	0.01	0.005	0.006	0.06
Licking	0.03	0.008	0	0.08
No relaxation				
Walking	0.41	0.039	0.24	0.64
Standing	0.35	0.056	0.16	0.75
Sniffing pen	0.08	0.014	0.02	0.16
Sniffing pen mate	0.03	0.004	0.006	0.05
Mounting	0.001	0.00	0	0.006
Butting	0.03	0.010	0	0.10
Pushing	0.03	0.010	0.006	0.09

Behaviours indicative of relaxation after play were not significantly different from behaviours indicative of no relaxation in the home pen after play (N.S., Fig. 13).

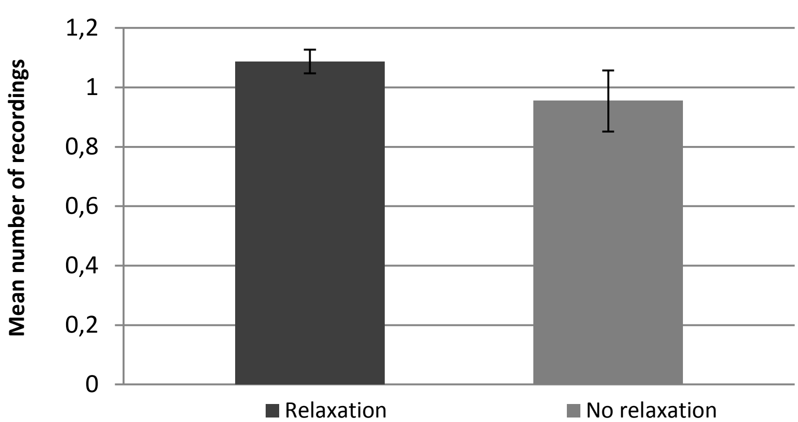

Figure 13. Mean number (± SE) of recorded relaxation and no relaxation in pairs of male lambs in the home pen after play during 6 minutes of observation three times per week for four weeks (n=10 pairs).

Lambs did not show relaxation during the first minutes after they came back from the play arena to the home pen (Fig. 14). They were standing near the gate of the home pen and looking towards the observer until the second minute. After the third minute, lambs started to show some behaviour indicative of relaxation and it continued till the sixth minute. Gradual decrease of no relaxation was seen after the third minute.

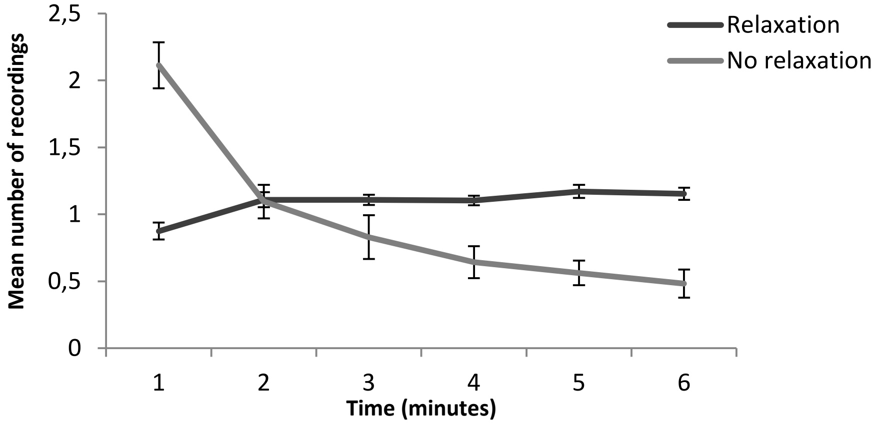

Figure 14. Mean number (± SE) of recorded relaxation and no relaxation every minute in pairs of male lambs in the home pen during six minutes of observation three times per week for four weeks (n=10 pairs).

There was a significant interaction between weeks and time for the behaviour indicative of relaxation in the home pen after play (χ^2=202.84, p<0.0001, Fig. 15). Lambs were recorded to show more relaxed behaviours during the second minute at the second week, but during the fourth week there were fewer relaxed behaviours during 4-6 minutes.

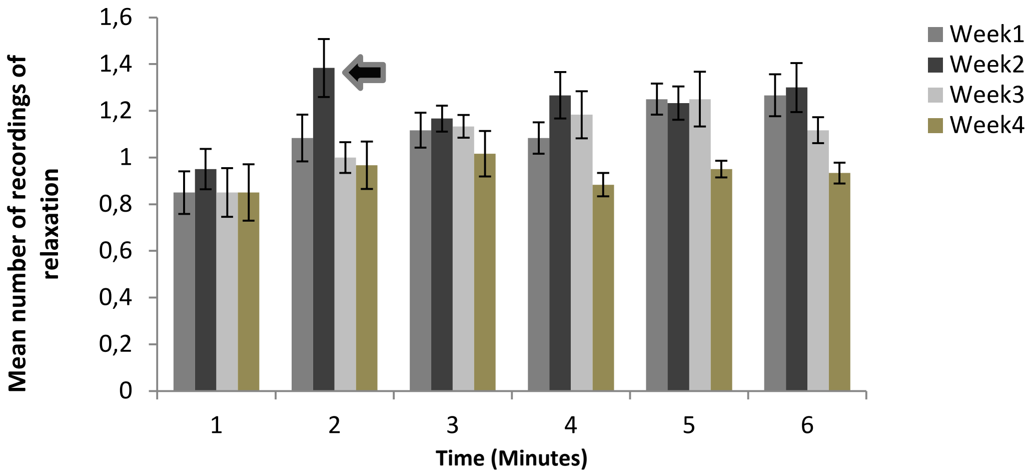

Figure 15. Mean number (± SE) of recorded relaxation every minute in pairs of male lambs in the home pen after play during four weeks of experimental period (n=10 pairs).

4.1.4 Home pen (no play)

Lambs mostly showed behaviour indicative of relaxation in the home pen when they were not provided the opportunity to play (Fig. 16). Feeding, lying, lying while ruminating and standing were the commonly recorded behaviours. Play behaviour was the least expressed behaviour by the lambs.

Figure 16. Mean number (± SE) of recorded relaxation, other behaviours and play in pairs of male lambs in home pens (no play) during 6 minutes of observation three times per week for three weeks (n=10 pairs).

There were no clear differences between the weeks in the observed behavioural categories in the home pens (no play) (Fig. 17). A slight increase of relaxation was seen in the second week. However, statistical analysis was not done for this result.

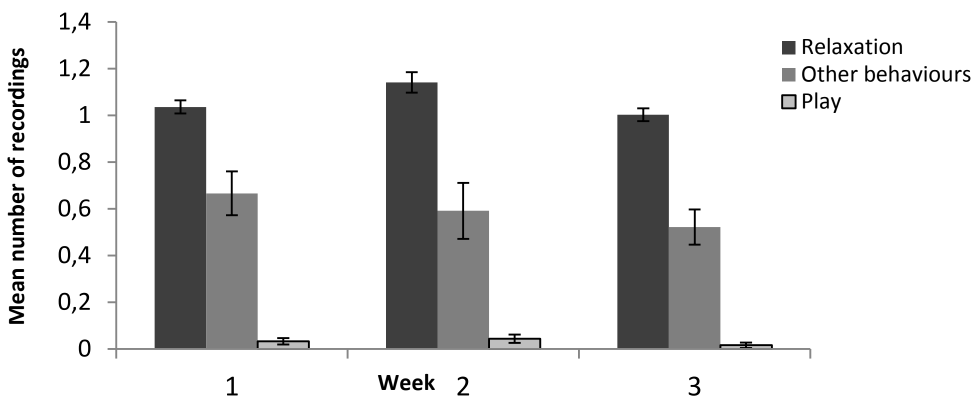

Figure 17. Mean number (± SE) of recorded relaxation , other behaviours and play in pairs of male lambs in home pens (no play) during 6 minutes of observation three times per week during three weeks (n=10 pairs).

4.1.5 Correlations

There was no significant correlation between behaviours indicative of anticipation in the holding pen and total play in the play arena (r = 0.25, N.S.). Also, no correlation was found for total play and behaviours indicative of relaxation in the home pen after play (r = -0.25, N.S.).

4.2 Ear postures

Raised and backward ear postures had the highest recordings in the holding pen and play arena (Fig. 18). In the home pen after play and the home pen (no play), plane ear postures were most common. In the holding pen, the highest recording of raised ear posture was found in the second week (Fig. 18A). The second highest recorded ear posture in the holding pen was backward ear posture which was found to peak at the third week. In the play arena, the highest recorded backward ear posture was recorded in the third week (Fig. 18B). Raised ear posture gradually increased from the second week till the fourth week. In the home pen after play; the low number of raised and backward ear postures did not change much between the weeks (Fig. 18C). Asymmetric ear posture was negligible compared to other ear postures. In the home pen (no play), plane ear posture decreased in the second and third week compared to the first week (Fig. 18D). The second highest recorded ear posture was backward ear posture which gradually increased from the second week till third week. Asymmetric ear posture was similar during all the three weeks.

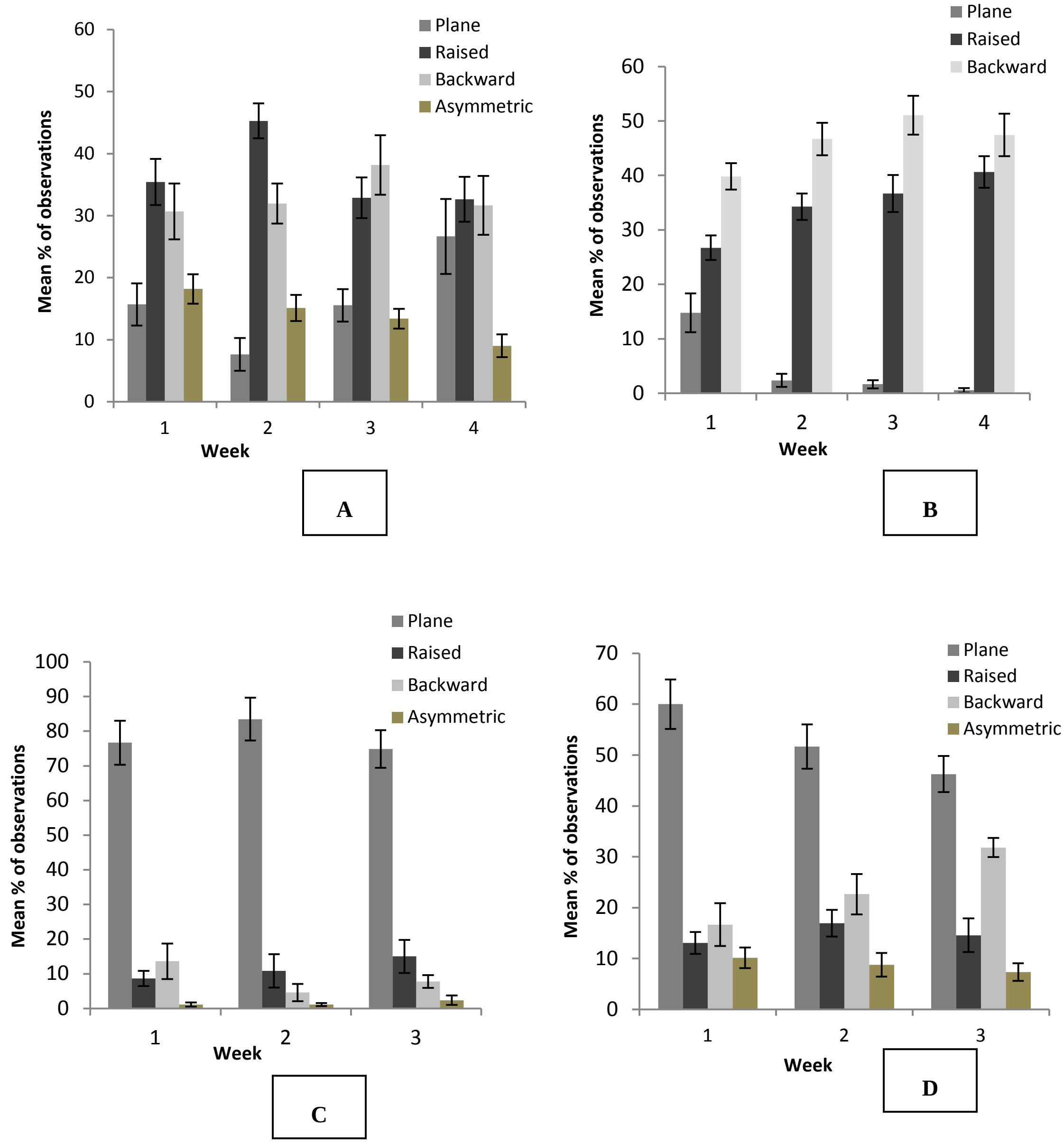

Figure 18. Mean % (± SE) of recordings of plane, raised, backward and asymmetric ear postures in pairs of male lambs in holding pen (A), play arena (B), home pen after play (C) and home pen no play (D) during 3 minutes of observation three times per week for four weeks (n=10 pairs).

4.3 Saliva cortisol

Cortisol level increased significantly ($p<0.05$) after play in lambs during the first day of sampling (Fig. 19). Changes in cortisol level before and after play during the second and third day of sampling were not significant. The four lambs that had the highest cortisol level before play had a decrease in cortisol level after play (0.11-0.10, 0.10-0.07, 0.10-0.08 and 0.10-0.09).

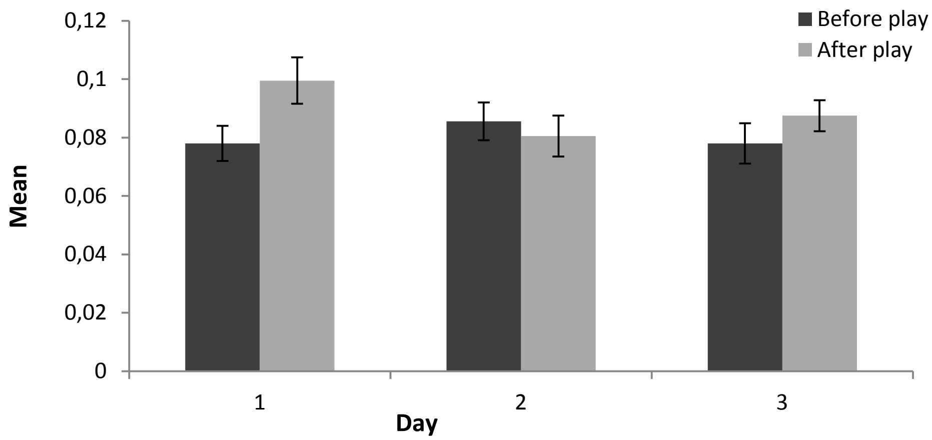

Figure 19. Mean cortisol (µg/dl) in male lambs before play in the home pen and after play in the home pen sampled during three times in the fourth week of experiment (n=20).

4.4 Correlations

There was no significant correlation between the level of cortisol after play and the amount of play behaviours (r = -0.08, N.S.) However, there was a significant negative correlation between cortisol before play and relaxation after play (r = - 0.48, $p<0.05$).

5. Discussion

The major findings of this study were that the lambs showed behaviours indicative of anticipation to play in a holding pen where they had learned that they shortly afterwards would get access to a play arena. Successively the lambs performed large amounts of play behaviours in the play arena. However, after coming back to the home pen lambs did not show behaviours indicative of relaxation during the first minutes.

5.1 Anticipatory phase

Various studies have addressed the rewarding value of play and proposed play to be associated with positive emotions (Boissy et al., 2007; Balcombe 2009; Held and Spinka, 2011; Trezza et al., 2011). Wanting as suggested by Trezza et al. (2011) is a motivation for a reward which promotes approach behaviours and makes subjects work to get a certain reward. In our study, lambs did not have to perform any operant work to acquire the reward. However, waiting in the holding pen for 5 minutes to go into the play arena seemed to be sufficient to induce wanting/anticipatory behaviour and probably made the lambs prepared to play in the play arena. Lambs were showing more active and investigatory behaviours like walking, butting, mounting and sniffing pen in the holding pen which is interpreted as their expression of anticipation to play. This result is in accordance with anticipation studies done by Van Den Bos et al. (2003), Van Der Harst et al. (2003), Vinke et al. (2004) and Moe et al. (2006) who found increase in activity and investigatory behaviour in rats, mink and silver foxes anticipating various forms of positive reward. However, it is contrary to earlier findings in cats (Van Den Bos et al., 2003) and laying hens (Zimmerman et al., 2011) who found decrease in locomotor activity and behavioural transition during anticipation of food reward.

The lambs in this study were standing facing the play arena which indicates that they were anticipating play which has also been shown in previous research. Foxes were found to spend most of the time in front of the cage when anticipating positive rewards (Moe et al., 2006) and lambs were standing in front of the door of the test pen and sniffed the door for longer time when expecting food reward (Desire et al., 2004). When the gate of the home pen was opened in this study, lambs easily came out and walked to the holding pen. The lambs in adjacent pens were seen approaching the door of the home pen and showing play behaviours in their pen when the test lambs were released from their home pen. This suggests that opening the gate of the home pen might have acted as a signal of play for both the lambs who walked to the holding pen and those who were still in their home pen. Therefore, additional waiting in the holding pen for five minutes might have acted as a strong signal for the expression of anticipatory behaviour in lambs.

Since social play has been found to be highly rewarding for rats (Trezza et al., 2011) and anticipatory response is related to the positive nature of the stimulus (Van Der Harst et al., 2003), announcing the reward e.g. play in our study might have enabled the lambs to express their motivation to play by showing different behaviours in the holding pen. Spruijt et al. (2001) have argued that behavioural activation in anticipation of the arrival of a reward represents the activation of reward centres in the brain and the level of activation depends on the incentive value of the reward. The incentive value of play might be high for the lambs in our study which was shown by the expression of anticipatory behaviour in the holding pen. However, there was no change in anticipation during the five minutes.

Anticipatory behaviour did not change over the five minutes in this study. Waiting for this time in the holding pen for the reward seems to be enough for the lambs to show significant anticipatory behaviour. Longer stay in the holding pen might have been stressful as studies have shown that passive waiting for an anticipated reward for long time can be stressful due to loss of control (Manteuffel et al., 2009). However, lambs were more active in the holding pen and we do not know what could have happened if the lambs were kept in the holding pen for more than five minutes. Various studies that have tried to estimate anticipatory behaviour have used the interval of certain seconds to 10 minutes between the offset of prediction of reward and onset of reward depending upon the nature of experiment and species of animals used (Zimmerman et al., 2011; Moe et al., 2009; Van Der Harst et al., 2003). Anticipation of a reward that is obtained after a short time while the animal is active in a goal-directed manner can increase the appreciation of the following consummatory act (Manteuffel et al., 2009). This seems to have happened in the present study as the lambs showed high amounts of play behaviours in the play arena.

The increase of anticipatory behaviour in the second week of this study may be due to that the lambs learned to effectively associate their presence in the holding with the rewarding value of play. Trezza et al. (2011) have postulated that learning allows animals to choose what they really like, and to make associations, representations, and predictions about future rewards based on past experiences and to adapt their behaviour accordingly. So lambs in our study might have gradually learned to better predict the upcoming possibility to play and showed increased anticipatory behaviour in response to it. The decrease of anticipatory behaviour in the fourth week can be the result of habituation to the same reward which might have decreased the incentive value of play for the lambs. Also, on the fourth week, the lambs were released to the holding pen immediately after taking saliva samples in their home pen which might have affected the result. Anticipation of a stimulus of high hedonic value (eg. play in our study) is hypothesized to represent an appetitive type of positive affective state associated with mesolimbic dopaminergic activation. The study of anticipation in our study might therefore give indirect information about the mechanisms underlying the display of positive emotions (Moe et al., 2009) in lambs.

5.2 Consummatory phase

During the second phase i.e. the consummatory phase when the lambs got the reward in the play arena, play behaviours shown by the lambs were significantly higher than other behaviours. Lambs explored the play arena, used the arena and were active in the play arena performing different play behaviours. Higher frequency of play behaviours in the play arena might be due to an increased internal buildup of motivation to perform these behaviours. Also, the activity might have been elicited by the mere release in a new enriched environment (Jensen and Kyhn, 2000) or the high incentive value of play seemed to cause spontaneous play performance in lambs in the play arena. It seems plausible that both internal and external factors may stimulate play in lambs if the lambs are released in an enriched play arena.

Among the play behaviours shown by the lambs, they were mostly recorded to be engaged in social play like butting and mounting which was also shown by Sachs and Harris (1978). Lambs are strongly attracted to other lambs which might have promoted the social play (Hass and Jenni, 1993). Dobao et al. (1984) also found more social play like pushing, butting and mounting in a group of weaned male piglets.

Some lambs showed flehmen during play which could be due to that the lambs were growing and becoming more sexually mature during the study period. Orgeur (1994) also reported that play behaviour in male lambs mostly consists of male sexual patterns like butting, mounting, ano-genital sniffing, nudging, running and gamboling, most of which were also observed in this study. Hass and Jenni (1993) also reflected on that the behaviour patterns used by lambs in social play were the same as those used by adults in intrasexual conflict and courtship. Sexual play is also a more prominent feature of social play among young males of ground squirrels (Nunes et al., 2003).

Locomotor play and object play also comprised a large part of the total play in our study and lambs were frequently recorded to run, jump, gambol and paw. In juvenile big horn sheep, young lambs were often observed to be engaged in locomotor play (Hass and Jenni, 1993) which supports our results. Jensen and Kyhn (2000) also reported that locomotor play is the most prominent form of play in young calves and is mainly expressed as galloping, bucking and kicking. Lambs in our study were using the objects i.e. tunnel, chain and ball for playing which was also found by Jensen et al. (1998) in young calves directing their play towards the objects in pen. Young piglets show object play along with social play (Newberry et al., 1988). Mink kits have been found to show more social and solitary manipulation and play with objects instead of social and solitary play without objects when they are reared and housed in an environmentally enrichment system (Vinke et al., 2004).

Lambs in our study were engaged more in playing during the first ten minutes of their release in the play arena than during the next ten minutes. Hedonic activation is maximum after presentation of a reward and is reflected as the liking or pleasure with the gradual consumption of reward (Berridge and Robinson, 1998). Since the lambs were anticipating in the holding pen to play in the play arena, they might have been more excited to attain the pleasure by grabbing the playing opportunity immediately after their release in the play arena from the holding pen. This might have enabled the lambs to show more play behaviours in the play arena during the first ten minutes. The gap of one day between the playing sessions of lambs might also have created some rebound effect on total play. This gap might have created building up of motivation to perform play behaviour. The rebound effect of play has been shown in calves confined for weeks in the study of Jensen (1999) and she also suggests that the internal motivation to perform locomotor play may increase within a few days or hours. As proposed by Keeling et al. (2008) an anticipatory act is followed by a subsequent consummatory act and the result of this study show that lambs were consuming the reward immediately after showing anticipation for the reward in the holding pen. Thus the two phases of the reward cycle seemed to occur subsequently one after the other.

The level of play behaviours shown by the lambs decreased after having a peak the second week. The decrease in play behaviours over weeks is more likely to reflect a general effect of age. Our result is supported by previous studies (Jensen et al., 1998; Jensen and Kyhn, 2000; Krachun et al., 2010). It could also be the result of habituation to the same reward which might have decreased the incentive value of play for the lambs after the second week. The peak in play behaviours at the second week could be the result of the lambs being more excited and because of increased interest in the play arena with the pen mates and playing objects.

Social play was found to peak in the second week of the study when the lambs were around 14 weeks of age which contrasts the findings of Hass and Jenni (1993) who found that social play of big horn lambs decreased rapidly after 12 weeks of age and was seldom observed after that. However, in this study the lambs were well fed and stimulated to play in a play arena with objects, whereas in the study by Hass and Jenni (1993) lambs were living under natural circumstances where food could be scarce at times. Play is easily interrupted when there are immediate threats to an animal's fitness and if the cost of playing is higher than the benefit (Held and Spinka, 2011). Bighorn lambs received most of their nutrition from available vegetation after seven weeks of age which was not enough according to their nutritional need which lead to suppress the play (Hass and Jenni, 1993). Krachun et al. (2010) also found that running by calves was reduced by a low milk allowance.

Locomotor play was also highest during the second week and decreased during the third and fourth week. This might be explained by the age factor which caused decrease in locomotor play in lambs which is also supported by the findings of Jensen and Kyhn (2000) who reported that locomotor play decreases over the weeks in calves after having a peak at 4-5 weeks of age. Releasing calves in a large arena enabled them to show higher motivation to perform locomotor play even when they were ten weeks old (Jensen and Kyhn, 2000). This shows that if the animals are provided with larger space to play then age might not be the only factor responsible for a decrease in play activity. Ensuring larger space enabled lambs to perform significant amount of locomotor play in this study even though they were quite older.

Object play in lambs increased over the whole four weeks period. Using the objects more often over the weeks might have increased their motivation to play with those objects and also the lambs were becoming more familiar and habituated with the playing objects. However, habituation to unchanged toys lead to a decline in the intensity of play by the third session in both pet and cattery cats in the study by Hall et al. (2002). Play response was disinhibited in cats when a new toy both contrasting in colour and odour was presented in the fourth session (Hall et al., 2002). In this study, play objects were the same for all four weeks but the lambs played on alternate days so this might have reduced the habituation to the play objects.

5.3 Post consummatory phase

During the post consummatory phase of the reward cycle i.e. when the lambs came back from the play arena to their home pen, they were not showing signs of relaxation during the first few minutes. They were standing near the door of their pen and looking towards the observer from their home pen. The lambs looked as if they wanted to come out of their home pen rather than to relax. The reason for this could be that the lambs might still be in an activated state during the first few minutes after they came back from the play arena. However, after the third minute, lambs started to show behaviour indicative of relaxation. This indicates that it may take some time for the lambs to calm down after having been in an excited stage. This suggests that if the relaxation behaviours had been recorded for longer time then we could have shown more relaxation behaviours.

The other reason could be that the lambs might still have a high play motivation since the frequency of total play was not zero during the last minute of play. The play rate was gradually going down after 10 minutes but it was still present in the 20th minute. This persistent play motivation might have taken some time to suppress and shift over to relaxation in lambs. Haskell et al. (1996) who aimed to determine the relationship between food consumption and persistence of post-feeding foraging behaviour in sows found positive feedback effect of food on the underlying feeding motivation in sows but these effects were transitory and could be reduced by experience. Similar to this study, lambs in our study also might have shown some motivation for playing immediately after they came back from the play arena but this effect was found to be transitory as the lambs were seen involved in other relaxing behaviours like feeding after the third minute. Lambs showed more relaxation behaviours during the second week when both anticipation in the holding pen and total play in the play arena was high. Since the lambs in our study seemed to pass through the anticipatory, consummatory and post consummatory phases, providing the environment with possibilities to play could be a way to induce positive emotions in lambs, as these three phases of reward cycle are proposed to be associated with positive affective states.

When the lambs were in their home pen and not given the opportunity to play, they mostly expressed general behaviours like lying and feeding. They were not active in their home pen and the play behaviour was recorded in very rare occasions. This suggests that the space might not be large enough for the lambs to express their motivation to play. Also, there were no enrichment materials in the pen except straw bedding. Suppress in play activity has been shown in various studies of pigs, cattle and other farm animals in small pens and barren environment where there is no opportunity to explore (Jensen et al, 1998).

5.4 Ear postures

It was difficult to record the ear postures of lambs because they changed their ears a lot. Also the interpretation of different ear postures is very hard to do because there are only two studies that have recorded and described ear postures according to different affective states in lambs. This study show that raised and backward ear posture were most common in the holding pen when the lambs were waiting for the reward. Lambs were trained for one week to stay in the holding pen for five minutes before being released in the play arena which might have enabled them to learn to predict the reward when they were in the holding pen. Raised ears might show that the lambs were more alert and attentive during the anticipation phase. However, silver foxes in the study by Moe et al. (2006) showed flat and backward-rotated ears during the anticipation of both positive unpredictable and negative predictable situations.

Boissy et al. (2011) have proposed that sheep point their ears up when facing negative situations but controllable, hence likely to elicit anger. Reefmann et al. (2009) showed that the proportion of forward ears was highest during separation of sheep from other group members, hence likely to elicit negative experience. However, the lambs in our study were believed to anticipate positive reward (e.g. play) and might therefore have a positive experience. Play involves a lot of locomotion which activates the lambs and the physiological reaction is similar to when animals go through a stressful situation. However, play is supposed to be positive for the lambs compared to other negative stressful conditions. So the occurrence of raised and backward ear posture in the holding pen is difficult to compare with negative situations as the expression of anticipation for a reward has also been shown to correlate with positive states in previous studies (Van Der Harst et al., 2005; Moe et al., 2009). Asymmetric ear posture was less recorded than other ear postures which contrast the results of Reefmann et al. (2009) where lambs when anticipating food reward showed highest proportion of asymmetric ear postures.

Occurrence of backward ear postures in the play arena might also be related with the positive experience since the lambs when playing (especially butting) were pointing their ears backward.

This is in accordance with the results of Reefmann et al. (2009) where backward ear posture was observed during hay feeding which is supposed to be associated with positive emotional valence. However, the result contrasts the findings of Boissy et al. (2011) who have proposed that sheep point their ears backward when they face unfamiliar and unpleasant uncontrollable situations, hence likely to elicit fear. Lambs seemed to be more excited while playing with each other and also the play arena was predictable for the lambs so it is rather doubtful to relate the situation of the lambs in our study to a fearful situation.

Plane ear posture was mostly recorded in the home pen during feeding which was also shown by the ewes in the study of Boissy et al. (2011) during feeding. Boissy et al. (2011) proposed that the horizontal plane ear posture corresponds to a neutral state so the feeding situation might be regarded as a neutral state for the lambs in our study. Although, we did not calculate the correlation between different ear postures and behaviour of lambs in our study, the ear postures recorded and the behaviour observed in the experimental condition showed that the lambs showed backward ear posture while butting, lying and lying while ruminating and plane ear posture during feeding.

5.5 Saliva cortisol

Cortisol level in the saliva increased after play in the lambs during the first test day of fourth week which was against our hypothesis that the lambs will have decreased physiological stress response after having been in the play arena. It might be that the first test day for saliva sampling was stressful for the lambs since the lambs were habituated to only going into the play arena for three weeks plus the training week. Therefore, sudden exposure to handling inside the pen and taking saliva sample might be stressful for the lambs. However, Dreschel and Granger (2009) have reported that generally up to 4 minutes could be taken to collect a saliva sample from a dog without the effect of handling being reflected in cortisol concentrations. In our study, lambs were only handled for one to two minutes for saliva collection.

Increase in cortisol during and subsequent to exercise has been found in sheep by Cook (2002). In humans, increase in cortisol has been reported after a marathon run (Dessypris et al., 1976). Mülleder et al. (2007) also found higher cortisol in cows that spent more time in gentle play. Although we did not find any significant correlation between total play and cortisol before and after play, the lambs in our study were running, butting and showing different play behaviours in the play arena so we suspect that the higher activity level involved in total play might have caused the increase in saliva cortisol level after play. There are some studies on play and cortisol which contrasts our results. In young pigs, higher plasma cortisol concentrations have been correlated with a decrease in play fighting and running (Worsaae and Schmidt, 1980). In cats, higher urinary cortisol was negatively correlated with active exploratory and play behaviour (Carlstead et al., 1993). Play behaviour was positively correlated with decrease salivary cortisol in dogs in the study of Horvath et al. (2008).

Cortisol level after play decreased in four lambs that had the highest cortisol level before play among the twenty lambs. In the study done by Horvath et al. (2008), border guard dogs with high baseline cortisol were more motivated to play with the handler and started to play immediately. Lambs having high cortisol level before play in our study might have shown more play behaviours than other lambs. Hence, decreased cortisol level after play in those lambs supports previous research (Worsaae and Schmidt, 1980; Carlstead et al., 1993) and supports the view that play behaviour is positively correlated with low cortisol level. The lamb who vocalized a lot had medium cortisol level before play and decreased cortisol level after play. Although he was vocalizing during almost all the play occasions, he was showing more play behaviour compared to other lambs.

Negative correlation between cortisol before play and relaxation suggests that lambs having low saliva cortisol before play were seen to show more relaxation after play. Our result is in accordance with the findings of Bristow and Holmes (2007) where they have reported that in a non-stressful situation (pasture) in cattle, high levels of cortisol was correlated with decrease in rumination which is generally considered as state of relaxation for cows. In this study we measured cortisol immediately after play in the home pen when the lambs came back from the play arena. This means that the values of cortisol reflected more what they did in the play arena, as cortisol in saliva can have up to 10 minutes delay compared to plasma levels of cortisol (Hernandez et al., 2005). So the values of cortisol after play could not be correlated to relaxation after play. If we had managed to take the saliva sample 30 minutes after play then we could have got better cortisol value for evaluating whether the lambs were relaxed or not after having been in the play arena.

6. Conclusion

The results of this study showed that lambs seem to show behaviours indicating anticipation to play when they were in a holding pen and expecting to play in a play arena**.** Anticipatory behaviour did not change during the five minutes, and was higher in week 2-3 than week one and four. In the play arena, play behaviour was significantly higher than other behaviours. Social play was higher than locomotor play and object play. Play behaviours were seen more during the first ten minutes and was highest during the second week of the study. The level of behaviours indicating relaxation was not higher than behaviours indicating no relaxation in the home pen after having been in a play arena. Relaxation after having played seems to be less obvious during the first minutes but it increased after the third minute. The study of three phases of the reward cycle (appetitive, consummatory and post consummatory) seem to provide useful information about behaviour of lambs during each phase. Since the lambs in our study seem to pass through these three phases of the reward cycle where play was provided as a reward, they are supposed to have experienced positive affective states during each phase of the reward cycle. It is difficult to identify the definite ear posture which was signaling the positive valence in lambs because all three ear postures; raised, backward and plane ear postures were recorded in the holding pen, play arena and home pens respectively where lambs experienced positive affective states. Saliva cortisol level increased after play in lambs during the first sampling day. Although further validation is required, along with behavioural measures, observing ear postures and measuring saliva cortisol may be a useful approach for assessing emotional reactions of sheep during different phases of the reward cycle when play is provided as a reward.

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