An enriched arena for lamb - anticipation behaviour before entering the arena, effects of skipping the anticipation phase and effects of being denied access to the arena

En berikad arena för lamm – förväntansbeteenden inför tillträde in i arenan, effekter av att hoppa över förväntansfasen samt effekter av att nekas tillträde till arenan

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1. Summary

This study aimed to investigate whether lambs show anticipatory behaviours in a holding pen prior to entering a known arena, whether the holding pen experience prior to the arena exposure affects their play expression in the arena and whether their behaviour (in home pen) is affected if arena access is denied. Twenty pair-housed male lambs (average 14 weeks old), were exposed, in chronological order, to five treatments. Holding pen treatment (HP-control): Each pair was led into a holding pen (2.7 m^2) and remained there for 5 minutes before returning to their home pen (6 m^2). Holding pen – arena treatment (HP-A): Each pair was led into a holding pen for 5 min and then was allowed access into the arena (22 m^2, containing two hanging chains, one ball and one platform) for 15 min before returning to their home pen. Arena treatment (A): Each pair was led into the arena for 15 min before returning to their home pen (without spending time in the holding pen). HP-A(2) treatment: Same as HP-A. Home pen treatment (H): Same as HP-control but under the assumption that lambs anticipated arena access. Prior to HP-control, lambs were habituated to enter the holding pen. Prior to HP-A, A, HP-A(2) and H, lambs were habituated to anticipate entering the arena. Observations were conducted over three days for HP-control, HP-A, HP-A(2) and A treatments and one day for H treatment. Behaviours were video recorded and analysed (Observer XT 11.5, Noldus Technology) for percentage of duration. Extracted behaviour data were analysed using Wilcoxon Signed ranks test. In the holding pen, no differences were found between HP-control and HP-A for total play, exploring, walking and number of behavioural transitions. In the enriched arena, significantly higher total play was expressed in treatment A compared to treatment HP-A ($P<0.05$). Similarly in treatment HP-A(2) higher total play was expressed compared to treatment HP-A ($P<0.05$). Comparing the first with the second half of the enriched arena sessions, a higher percentage of duration for total play was found in the first part for treatment HP-A($P<0.05$). No significant difference was found comparing duration of total play for the first and second half of treatment A and HP-A(2). In the home pen, lambs in H spent more time eating ($P<0.01$) and less time ruminating ($P<0.05$) and tended to lie less ($P<0.1$) compared to HP-control. Nevertheless holding pen exposure seems to affect when play is expressed as in treatment HP-A play was expessed more during the first part of the arena session compared to the second. No difference was found comparing the two parts for treatments A and HP-A(2). In the home pen, lambs in H were eating longer duration,ruminating shorter duration, and tended to lie and stand shorter duration ($p<0.1$) compared to HP-control. In conclusion, male lambs did not appear to express anticipatory behaviour before entering the play arena. Nevertheless holding pen exposure seemed to have an effect on when play was perfomed in the play arena (HP-A), and also had an effect on its total duration (A). There seemed to be no effect of holding pen exposure on when play was expressed in HP-A(2). Finally the denied access to the arena affected several behaviours as eating and ruminating that might have a connection with loss of arena access.

1. Sammanfattning

Syftet med denna studie var att undersöka om lamm visar förväntansbeteenden i en förväntansbox innan de fick tillgång till en bekant arena, om en förväntansperiod innan tillgång till arenan påverkade mängden lek i arenan samt om hur deras beteende påverkas (i deras hemmaboxar) av att nekas tillgång till den förväntade arenan. Tjugo bagglamm höllls i tio par (genomsnittlig ålder 14 veckor) och genomgick, i kronologisk ordning, fem behandlingar. Förväntansboxbehandling (HP-kontroll): Varje par leddes in i förväntansboxen (2,7 m^2) och stannade där i 5 minuter innan de återvände till sina hemboxar (6 m^2). Förväntansbox–arenabehandling (HP-A): Varje par leddes in i förväntansboxen där de stannade i 5 min och sedan fick de tillgång till arenan (22m^2, innehållande två hängande kedjor, en volleyboll och en plattform) i 15 min innan de återvände till sina hemboxar. Arenabehandling (A): Varje par leddes in i arenan där de fick stanna i 15 minuter innan de återvände till sina hemboxar (utan att spendera tid i förväntansboxen). HP-A(2) behandling: Samma som HP-A. Hemmaboxbehandling (H): Samma som HP-kontroll, men under antagandet att lammen förväntade sig tillgång till arenan. Innan HP-kontroll habituerades lammen till förväntansboxen. Innan behandlingarna HP-A, A, HP-A(2) och H tränades lammen att förvänta tillgång till arenan. Observationer utfördes under tre dagar för HP-kontroll, HP-A, HP-A(2) och A-behandlingarna och en dag för H-behandling. Beteenden videofilmades och analyseras (Observer XT 11.5, Noldus Technology) för procent av duration. Beteendedata analyserades sedan med Wilcoxon signed rank test. I förväntansboxen visades inga skillnader mellan HP-kontroll och HP-A för total lekbeteenden, utforskande, gå eller antal uppvisade beteenden. I den berikade arenan visades signifikant längre duration lekbeteenden i behandling A jämfört med behandling HP-A ($P<0,05$). I behandling HP-A(2) visades längre duration lekbeteenden jämfört med behandling HP-A ($P<0,05$). I en jämförelse mellan första halvan av tiden i arenan med den sista halvan tid i arenan visades längre duration lekbeteenden under den första halvan för behandling HP-A ($P<0,05$). Ingen sådan skillnad visade sig i behandling A och HP-A(2). I hemboxarna, spenderade lammen i H-behandlingen längre tid att äta ($P<0,01$), kortare tid att idissla ($P<0,05$) samt hade en tendens a ligga ner och stå kortare tid ($P<0,1$) jämfört med HP-kontroll. Slutsatsen är att bagglammen verkade inte uttrycka förväntansbeteenden innan de gavs tillgång till arenan. Ändå verkar förväntansboxen påverka när lekbeteenden uttrycks i arenan (HP-A), och även ha en effekt på dess totala längd (A). Förväntansboxen hade dock ingen påverkan på när lek uttrycks i HP-A(2). Slutligen, att nekas tillträde till arenan påverkade flera beteenden som att äta och idissla som kan ha ett samband med nekat tillträde till arenan.

2. Introduction

2.1 Background

Animals are referred to as sentient beings by the Council of the European Union (2004), yet there is no strong scientific knowledge on what their emotions are (Desire et al., 2002). Farm animal welfare research has mainly focused on behaviour and/or physiological responses to aversive stimuli (e.g. abnormal behaviours, physiological 'stress' responses) (Burman et al., 2011). Nowdays interest on a welfare science model that will promote the addition of positive outcomes on welfare assessment has risen. The ignorance of positive aspects of welfare disregards significant aspects of both ethological and physiological points in animal life outcomes (Yates and Main, 2009). It is essential to understand animals' emotional states since animal welfare is closely related to the feelings that animals experience, by the absence of negative affective states known as states of suffering and by the presence of positive affective states known as states of pleasure (Duncan, 2005). Therefore the promotion and acknowledgment of positive emotional experiences owes to be the objective (Boissy et al., 2007). Since measuring directly the conscious emotions in animals is not possible, their assessment has mostly relied on measuring behavioural and physiological components of animals affective states (Mendl et al., 2009).

2.2 Measuring affective states

Previous welfare research has acknowledged the advantages of using a range of physiological and behavioural indicators of welfare (Lay et al., 1992; Duncan, 2005). In addition to the well-established analysis of behaviour, more physiological parameters have been used such as heart rate variability (HRV), a non-invasive method of assessing animals' affective states. HRV analysis is a technique used to investigate the functioning of the autonomic nervous system (Mohr et al., 2002). To better understand the meaning of HRV we should refer to Reefmann et. al. (2009) that related high levels of HRV in sheep with low frequency of ear posture changes during grooming by a familiar handler (positive stimuli) compared with low HRV and high ear posture changes during social isolation (negative stimuli). Over the past decade, the use of HRV provides a method to assess changes in sympatho-vagal balance in animals, changes that can be connected with diseases, environmental stressors and psychological or individual characteristics, as temperament and coping strategies (von Borell et al., 2007). Data from past research on sheep and cattle (Mohr et al., 2002; Désiré et al., 2004) demonstrate the value of HRV measure on the evaluation of stress and affective states in animals. It is indicated that HRV could potentially contribute to the assessment of the underlying neurophysiological processes of different welfare states in farm animals (von Borell et al., 2007). The collection of behavioural and physiological (as HRV) data during anticipation of rewards could be a potential basis for the study of animal emotions (Spruijt et al., 2001).

2.3 Anticipation and reward

Expectancy or anticipation can be explained by the concept of a specific subject that learns that a certain stimulus (signal, or in our study location change) predicts the occurrence of another event (Ursin and Eriksen, 2004). In a classical conditioning paradigm, anticipatory behaviour is induced when an initially neutral stimulus is repeatedly paired with the arrival of a rewarding event and as a consequence of an established association between the stimulus and the reward (van der Harst & Spruijt, 2007). Subsequently, the stimulus acts as an announcement and the animal can display anticipatory behaviour at/after the presentation of the stimulus. Previous research has shown this behavioural reaction during the interval between the announcement and the actual arrival of the reward is distinguished by an activity increase (van der Harst and Spruijt, 2007). This means that the amount of the movements and the number of different activities that the animal performs during anticipation increases in number and kind compared with baseline activity (Spruijt et al., 2001). In general, this activity increase was found in the research by Van der Harst *et al.* (2003) where the number of performed behaviours in rats increased during the time between the announcement and the reward. Other studies on anticipation (van den Bos et al., 2003; Moe et al., 2006) found increase in activity and exploratory behaviour in, mink and silver foxes while anticipating various types of reward. Moreover the type of the expected reward could affect the behaviours expressed during anticipation (to that reward) as animals expess (during anticipation), behaviours that are expected to be experienced during the reward consumption (Spruijt et al., 2001). Knutson et al., 2009 found that rats anticipating play expressed during anticipation vocalizations similar to those that were expersing during play-reward experience. Anticipatory behaviour in response to positive stimuli has been identified as a potential indicator of emotion (Spruijt et al., 2001; van de Bos *et al*., 2003) as it can differ according to the type of reward that it is related to since it is not expressed in the same way under all conditions and in all species (Mason and Mendl, 1997, Dudink et al., 2006). The 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 (reward) (Manteuffel et al., 2009).

Announcement and presentation of rewards can be a useful method to assess and improve the welfare of animals, because it is based on the natural behavioural response of animals therefore is a non-invasive method (van der Harst & Spruijt, 2007). Another important aspect for the experience and expression of anticipatory behaviour is the sensitivity that animals can potentially have to the expected event. This sensitivity can be measured by the (spontaneous) behavioural responses that an animal could express when expecting a so called reward (van der Harst & Spruijt, 2007). Previous research states that any difference in reduction between the current state and the desired state of a motivational system is regarded as rewarding (Spruijt et al 2001; van der Harst & Spruijt, 2007). It is also suggested by Spruijt et al. (2001) that certain (species-specific) behaviour can be rewarding, and this is based on the evolutionary theory that vital behaviours for survival of the animal activate the neural reward system, thereby stimulating their display. For example, Moe et al. (2006) investigated the behavioural activity and emotional expressions in foxes during anticipation

of either positive (food or toy) or negative experience (capture with neck tong) and found that the activity levels, time spent in the front part of the cage and the forward erect ear position were increased when anticipating a positive reward compared with anticipating aversive stimuli. Providing the opportunity to interact with a toy (thus play) was found to be rewarding for foxes (Moe et al., 2006). Play could be beneficial (psychologically and socially) and can potentially convey the concept that animals get both "rewarding and relaxing" experiences during its performance (Held and Spinka, 2011).

2.4 Play as a reward

To understand better the essence of play, a presentation of a list of the characteristics of play is essential. According to Burghardt (2005), play is a behaviour that does not contribute to the current survival of the animal so it is characterised as not 'fully functional'. Play is 'autotelic' meaning its purpose is in and not apart from the action itself, in other words self-rewarding. Additionally play's structure and timing differs from the adult form of behaviour (e.g. mounting for mating or play), is performed 'repeatedly', but not stereotypically; it occurs when the animal fitness is not at immediate risk. According to Boissy et al. (2007) "Play along with affiliative behaviours and some vocalizations appear to be the most promising and convenient indicators for assessing positive experiences in laboratory and farm animals under commercial conditions". Boissy et al. (2007) also suggests that providing animals with the opportunity to play could induce positive experiences. Play is also a measurable tool of positive welfare because animals will not perform play if their basic needs are not fulfilled or if they face fitness threats (e.g. Held & Spinka, 2011). Farm animals (that are motivated to play) often face limitations to express play behaviour due to of lack of space, play partners and play objects (Jensen et al., 1998). One of the methods for the promotion of positive experiences in farm and laboratory animals is environmental enrichment (Boissy et al., 2007).

2.5 Play and lambs

The main types of play in lambs (and mamals in general) are usually described as "object", "locomotor" and "social" play (Dugatkin, 2009). Healthy lambs are usually very active during the times when they are not asleep. Lambs usually perform social and locomotor play as they perform jumping and running in groups. They also climb and seek out wood piles and small hills as well as their mother to reach higher ground (Gill, 2004). The most often recorded play behaviours in lambs are mounting, butts, threats, running and gambolling (Orgeur, 1995). The most common motor patterns during play that were observed in a study by Sachs and Harris (1978) were butting, mounting and gambolling. Pawing the ground and repeated head lowering were observed before butting. Regarding the sex differences in play expression, male lambs were found to be engaged in more butting and mounting and female lambs in more gambolling behaviour (Sachs and Harris, 1978).

2.6 Research on affective states in lambs

Research that focuses on behavioural indicators of positive emotion in sheep is very limited, especially studies around anticipation-reward physiological and behavioural mechanisms. It is possible that valuable information can be gained for the affective state of sheep by observing their behaviour under different conditions and in response to different stimuli. The study of positive emotions is a relatively new area for sheep behaviour research very little published data is available (Chapagain, 2011, submitted; Anderson et al., 2013, submitted). We based our study on previous research that suggests that access to an arena provides opportunity to play, play can act as a reward and consequently its anticipation, experience and failure to experience it could potentially induce certain behavioural and physiological responses indicative of the lambs' possible affective states.

3. Aim

This study aimed to investigate the behaviours (and heart rate) of lambs that where conditioned to anticipate access to an enriched arena and weather play was performed in the arena. Additionally we were interested whether an anticipation period would affect the performance of play in the arena and what was the behavioural and physiological response when an expected access to the arena was denied.

The questions we aimed to investigate were the following:

1. Will lambs conditioned to anticipate access to an enriched arena show more walking, exploring, playing and behavioural transitions compared to prior the establishment of this association?

2. Will lambs experiencing an anticipation period prior to entering the arena express higher levels of play compared to when they were not offered an anticipation period?

3. How will lambs' behaviour be affected when an expected access to the arena is denied?

We predicted that lambs will express behavioural signs of anticipation to an opportunity to access an enriched arena. Additionally we predicted that lambs that experience an anticipation period prior to arena access will perform more play compared to when they were offered access with no previous indication of what is following. We also predicted that denied access to the anticipated arena would result in behaviour indicative of stress as something unexpected will occur when expected access is denied.

4. Materials and methods

4.1 Animals, housing and management

The Swedish Ethical Committee of Experimental Animals of Gothenburg (Dnr. 97-2013) authorized approval for this experiment.The study took place at Götala Research farm in Skara, in the West Gothland region of Sweden. Twenty uncastrated male lambs of Dorset x Swedish fine wool were borrowed from a local farmer. Prior to the experiment the lambs were kept exclusively on pasture with their mothers. They were weaned from their mother at the day of transportation to the Research Farm. Their average age on arrival to Götala was 16 weeks (ranging from 15-17 weeks) and the average lamb weight was 24.5 kg (ranging from 19 to 32 kg). They were paired according to their body weight (as similar as possible). There were no siblings amongst the experimental lambs. The lambs were kept in the same pairs throughout the whole experiment (Table 1) and after the study, lambs were sent to slaughter at a slaughter house in Skara when they had reached the desirable slaughter weight.

Table 1. Pen number, birth dates and weights (kg) of lambs at arrival to the Research Farm (in) and at the end of the experiment (out)

Pen	Lamb date of birth	Lamb weight in	Lamb weight out
1	09/03/2013	27.8	38.0
1	02/03/2013	28.2	44.6
2	07/03/2013	26.6	42.4
2	02/03/2013	26.8	39.0
3	06/03/2013	27.0	38.6
3	06/03/2013	27.6	37.0
4	14/03/2013	18.9	34.4
4	05/03/2013	20.8	32.0
5	14/03/2013	29.0	43.4
5	28/02/2013	29.6	41.8
6	02/03/2013	23.6	35.6
6	04/03/2013	23.8	39.0
7	08/03/2013	21.2	35.0
7	01/03/2013	23.6	35.6
8	06/03/2013	30.0	41.6
8	04/03/2013	30.8	43.8
9	09/03/2013	31.0	42.0
9	06/03/2013	31.2	42.6
10	06/03/2013	25.8	38.6
10	14/03/2013	26.0	35.2

The lambs were kept in 10 home pens (HM, 6 m^2 each) built by galvanized steel gates. Six pens were built on the left and four on the right side of the experimental area (Figure 1). In between the pens, an arena was placed, covered on all sides with playwood panels (Figure 1). The arena was accessible from both sides of the experimental area through two holding pens built on each side of it (Figure 1). The two holding pens were also covered with pan-

els. The uneven number of pens on each side was due to the limitations caused by the watering system of the farm. Pens were placed adjacent to each other so that animals from neighbouring pens could see and touch each other between the gate gaps. The floor of the HM was covered with straw bedding and this straw was renewed approx. every 3-4 days. Before the lambs were moved into HM, fly larvae dust *(Neporex WSG2, Novartis, Denmark*) was applied. The straw bed was not removed during the experiment.

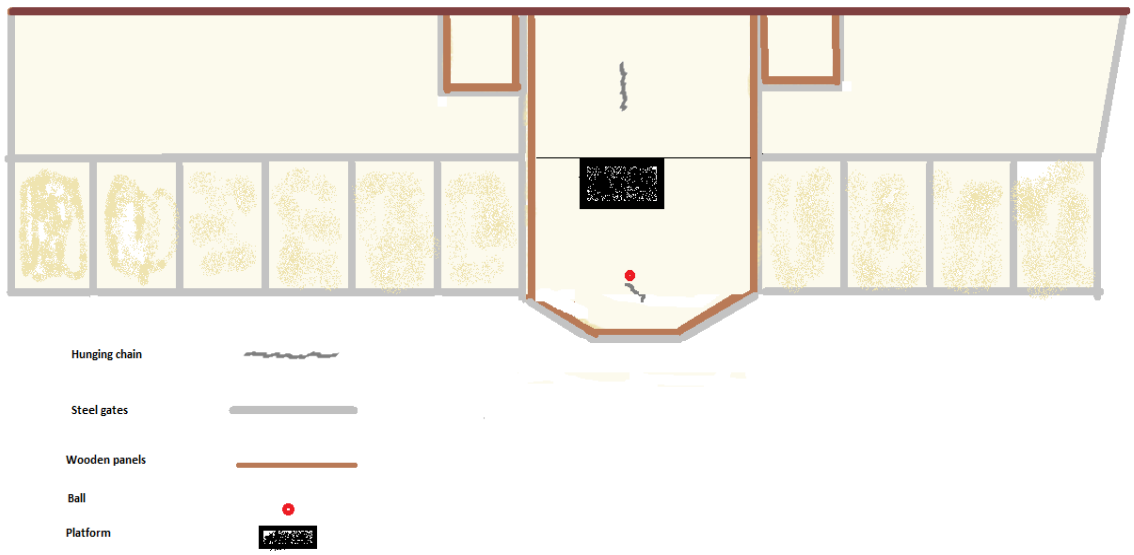

Figure 1. Housing and experimental area for 10 pairs of lambs in the barn. Ten home pens (six on the left and four on the right side of the arena, covered with straw bedding), two holding pens (one on each side of the arena) and the arena (containing two chains, a platform and a ball).

Feed was delivered each day, at approximately 7.00 a.m. in a plastic feed trough hanging outside their home pen. The feed consisted of grass silage and a commercial concentrate mixture (Lamn500, Lantmännen, Lantbruk, Sweden) in amounts that could cover the lambs' needs for a good growth. Additionally, mineral supplement ("Effekt Får utan Cu", Lantmännen Lantbruk, Sweden) including Ca, P, K, Mg, Zn, Mn, I, Se, Co, Mo, and vitamins A, D3, E was offered and a block of salt was always available in the trough to cover the lambs' needs of sodium chloride (NaCl). Water was provided *ad libitum* by an automatic water cup (one for every two pens) placed between the home pens.

4.2 Experimental area setting

The experimental area was built before the arrival of the lambs at the research station. It was built by galvanized steel gates and in some parts wooden panels were used to block visual access of some experimental areas and procedures from lambs in the test or from lambs that were staying in their home pens. The experimental area consisted of two holding pens one on each side of the arena and an enriched arena of 22 m^2 (Figure 1). The arena was enriched with a wooden platform (size 1.2 m^2 and 40 cm high) which was covered with a black rubber mat, two hanging chains and a ball. The two holding pens measuring 2.8 m^2 each were also covered by wooden panels and had two doors, one as entrance for the pen area and one as entrance to the arena. The ball was always placed in the same position after the completion of each session and the arena was brushed to remove droppings and straw brought from the lambs that had been tested. The space was lit by natural light as well as artificial lights that were always on during the experimental procedures regardless of the intensity of the natural light.

4.3 Experimental treatments

4.3.1 Arrival and habituation

After their arrival, lambs were group housed (in four pens, five lambs in each pen) for two-days. Prior to being moved into their home pen, ten of them were sheared (one lamb selected randomly from each pen) around the chest due to heart rate equipment requirements (to achieve skin contact). During the first four days, after their arrival, lambs were habituated to the presence of their main handlers as they were present near their pens for a few hours every day. Lambs were also habituated with the application and heart rate equipment (applied five times during five days of habituation) in order to get accustomed to the procedure and the handlers.

4.3.2 Treatments

All animals underwent all treatments which are numbered by chronological order in the following text (Appendix 1).Training for each treatment differed as lambs needed to be trained to undergo the different procedures.

1. HP-control (Holding pen- Home pen)

In the first treatment (that was designed to be the control treatment), HP-control, lambs from one pen at a time were moved to the holding pen and stayed there for 5 minutes. Afterwards they were moved back to their home pen. Prior to this treatment lambs were trained for 5 days to walk and stay in the holding pen for 3 minutes the first day, 4 minutes

the second and 5 minutes the third, fourth and fifth day. The actual recording of the treatment was repeated every other day for three days. The purpose of this treatment was to have recordings on how lambs behaved in the holding pen without having the play arena experience.

2. HP-A (Holding pen – Arena- Home pen)

After the end of HP-control the lambs were trained for five days on the routine of holding pen-arena, meaning that they were led into the holding pen, stayed there for five minutes , followed by access to the arena for 15 minutes. After the five days of continuous training followed 3 sessions of recordings taking part every other day.

3. A (Arena- Home pen)

In treatment A, lambs were led directly into the arena where they stayed for 15 minutes without spending five minutes in the holding pen before. Four sessions, one every other day were recorded for this treatment instead of three due to environmental disturbances that occurred in the second session (this session was not used in the analysis).

4. HP-A(2) (Holding pen – Play arena- Home pen)

For treatment HP-A(2) the procedure followed was identical to HP-A treatment, and was repeated for 3 sessions, one every other day. Prior HP-A (2) treatment animals went through re-training to the holding pen-arena routine for three days.

5. H (Holding pen – Home pen)

For treatment H the procedure was identical to HP-control (led to holding pen, stayed for 5 minutes, followed by return to home pen) and was conducted in two sessions (only one analysed in this thesis), one every other day.

4.4 Data collection

4.4.1 Recordings of behaviour and physiology

During all treatments, video recordings took place in the holding pens, arena and home pens in order not to disturbe the lambs during their play sessions and to get more detailed recordings of their behaviours. Four cameras (Avtech, US) were mounted in the ceiling, one in the ceiling above each holding pen and two covering the arena from opposing sides.

The cameras were connected to a desktop that had installed the Media recorder software (Noldus Information Technology, The Netherlands) for recording. For video recordings in the home pen, we used two portable cameras set on tripodes in the aisle outside the home pen (Panasonic HC-V100 Full HD Digital Camcorder).

Heart rate equipment was applied in order to record the lambs' heart rate during each treatment session. Polar equine equipment was used, two electrodes with transmitter (Polar Equine RS 800c) and a heart rate sensor (Equine H3 heart rate set) was placed on an elastic belt and applied around the lambs chest (Figure 2). Each time the belt was applied, the skin was soaked with water and a blue gel in order to enable optimum contact between the skin and the electrodes. The watch-receiver (RS800c) was placed on the belt and remained there throughout the session. The equipment was placed by two handlers, one immobilizing the animal and the other placing the equipment. After a few applications most of the animals looked undisturbed by the procedure and stood almost still during the equipment application 15 minutes prior the beginning of procedures. Physiological data (HRV) obtained in this study have not been analysed or presented in the present thesis, and will be published elsewhere.

Figure 2. Lamb in the play arena wearing the Polar HR equipment (Photo: Aikaterini Zachopoulou).

4.4.2 Video analysis

Duration and frequency of defined behaviours (Table 1 and 2) were recorded by Observer XT version 11 (Noldus Technology Systems, The Netherlands). Behaviours were defined and scored in the holding pen, the arena, and the home pens for the videos. All videos were analysed single-blind (Martin and Bateson, 2007). After the behavioural coding, excel data

sheets were extracted from the program resulting in data on percentage of durations of behaviours (all pens) and number of behaviours (holding pen) (Table 1 and 2).

Table 1. Ethogram of general behaviours, definitions *(modified after Sachs & Harris, 1978; Hass & Jenni, 1993)* and location where they were recorded (holding pen(Hp), arena(Ar), home pen(Hm)

Behaviours	Definitions	Location
Walking	Move a few or many steps at a regular and fairly slow pace	Hp, Ar, Hm
Standing	Standing having the body (the head could move) still facing any direction	Hp, Ar, Hm
Eating from feeder/or ground	Standing in front of the feeder with their head placed in the feeder/ picking parts from the straw on the floor of the pen	Hm
Ruminating (standing/lying)	Mouth making chewing movement for re- chewing regurgitated fermented ingesta	Hm
Sniffing ground	Placing/moving mouth and nose to a close distance or picking parts from the straw on the floor of the pen	Hm
Sniffing/licking pen/arena	Placing/moving mouth and nose to a close distance from the wall or the floor of the pen	Hp, Ar, Hm
Sniffing/licking lamb	Placing/moving mouth and nose to a close distance from the other lambs face or body	Hp, Ar, Hm
Biting/licking HR equipment	Touching or having mouth/nose close to HR equipment, or pulling it with teeth	Hp, Ar, Hm
Climbing	Placing the front legs on the sides of the pen, stretching head and neck and looking outside the pen	Hp, Ar
Scratching/ rubbing	Moving body or head repeatedly along the surface of the walls/ lifting hind leg and moving the hind claws repeatedly along the head/neck	Hp, Ar, Hm
Avoiding	Trying to move away from the other lamb when being mounted or butted	Hp, Ar, Hm
Kicking/hitting	Moving head, legs or body hard against the pen walls	Hp
Lying	Lying on the floor, with curled legs, head either lifted or touching the ground	Hm
Pawing	Scrape/hit the floor or the wall with paw (hoof)	Hp, Ar

Table 2. Ethogram of play behaviours definitions *(modified after Sachs & Harris, 1978; Hass & Jenni, 1993)* and location where they were recorded (holding pen(Hp), arena(Ar), home pen(Hm))

Behaviours	Definitions	Location
Butting	Mutual head hitting of lambs after moving backwards and then quickly forward	Hp, Ar, Hm
Mounting	Standing on hind legs and placing front legs on back of the other lamb	Hp, Ar, Hm
Pushing	Pushing head or body against other lambs body	Hp, Ar, Hm
Running	Running around, usually both lambs at the same time	Ar
Inviting	Backing of from the other lamb by moving backwards, lowering the head, looking at the lamb and standing still for some time	Hp, Ar, Hm
Jumping	Lifting forelegs from ground and elevating front part of body	Hp, Ar
Object manupulation	Licking/ chewing or pulling and pushing the hanging chains or the volley ball	Ar

4.5 Data analysis

For statistical analysis of the data we performed comparisons of the treatments according to our hypothesis. Since each treatment (excluding treatment H) was repeated in three sessions, we calculated the mean percentage of the behaviours (per lamb) over the three sessions for each treatment. These mean values were then analysed using Wilcoxon Signed Ranks test (as data were not normally distributed) by hand (Petrie and Watson, 1999) for significant differences between treatments. For treatment H, data were derived from one session, since this treatment could not be repeated. Results were accepted as significant if $p<0.05$. For analysis purposes a number of behaviours were grouped. In play arena sessions the interval analysed for behaviours started 30 sec after entering into the arena and finished 30 sec before leaving the arena (14 minutes) and for holding pen sessions all five minutes were used.

Total play behaviours in the holding pen was derived by the sum of all play behaviours (butting, mounting, pushing, inviting). Jumping was not included due to that it was never recorded in the holding pen. Total exploring was the sum of sniffing/licking the pen, climbing and pawing.

Total play behaviours in the play arena was derived by the sum of all recorded play behaviours performed in the play arena (butting, mounting, pushing, running, inviting, jumping, object manipulation).

In the home pen the behaviours were grouped for analysis purposes. Total eating was created by grouping eating from the feeder and the ground. Moreover we grouped ruminating while standing and lying into total ruminating, and butting, pushing, mounting and inviting into total play. Each category of behaviours was calculated for all ten focal lambs. Mean number of behaviours was used for the H, HP-A and HP-A(2) calculation of the behavioural transitions. Mean percentage duration and standard error (SE) of each category of behaviours for all ten subject lambs for the first 7 minutes and last 7 minutes in the play arena sessions was also calculated. Additionally the latency of food consumption (how fast lambs started to eat after entering their home pen) was calculated.

5. Results

5.1 Holding pen

There were no significant differences in any of the behaviours *(total play Z=-1.27, total exploring Z =-0.25, walking Z=-0.66, $p \geq 0.05$)* performed by lambs in the holding pen when being there for 5 minutes and then being brought back to the home pen (HP-control) compared to when being there for 5 minutes and then being let into the arena for 15 minutes after which they were brought back to their home pen (HP-A) ($p \geq 0.05$). The mean percentage of durations of walking, total exploring and total play for the treatments HP-control, HP-A and HP-A(2) and H are shown in Figure 3. Additionally no significant differences were found comparing total play *(Z=-0.76),* and walking *(Z=-0.97)* performed by lambs in the holding pen for treatments HP-A and HP-A(2)*($p \geq 0.05$)* but significant difference was found in total exploring *(Z=-2.19, p=0.029).* Data on standing in the holding pen was not analysed due to that it could not help answering the specific questions for the holding pen.

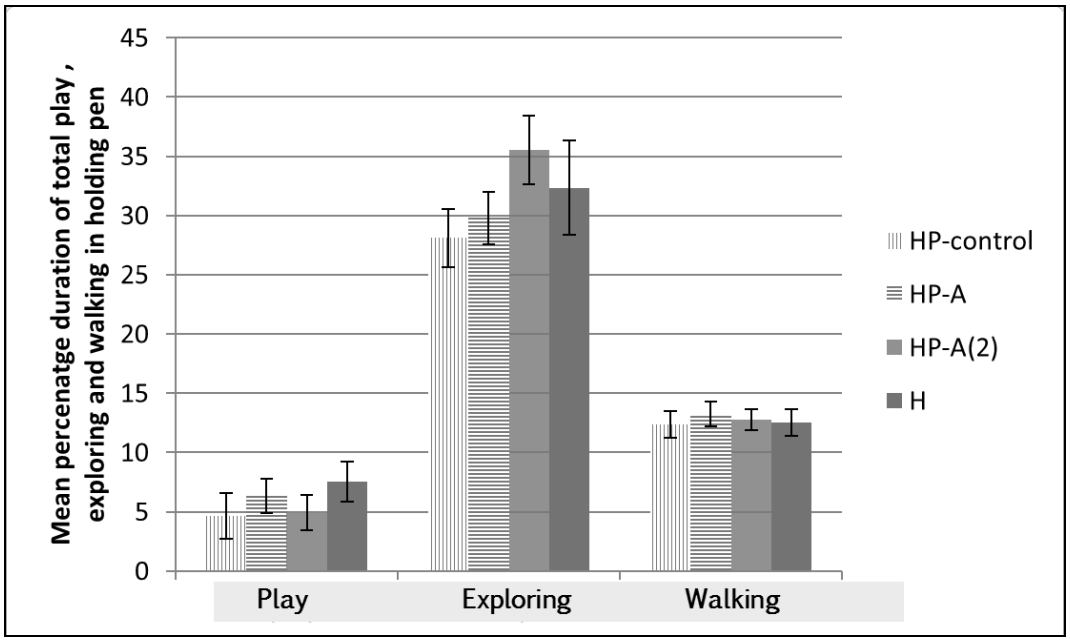

Figure 3. Mean percentage duration (± SE) of total play, total exploring and walking expressed in the holding pen for treatments HP-control, HP-A and HP-A (2) and H (n=10).

There was no difference in number of behavioural transitions (number of behaviours performed per session) between HP-control and HP-A *(Z=-0.61, p=0.542)* or between HP-control and HP-A(2) *(Z=-0.31, p ≥ 0.05)* (Figure 4).

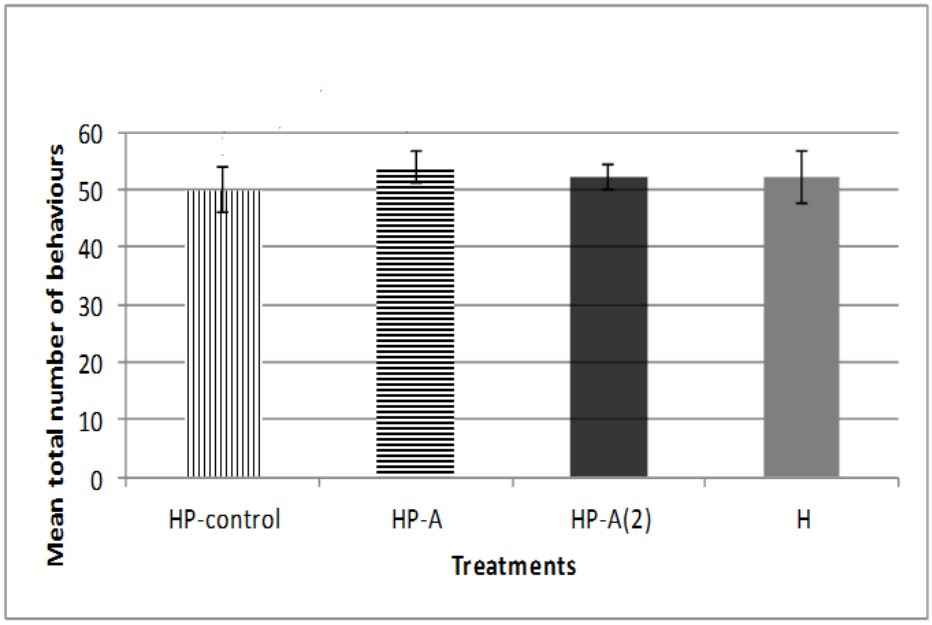

Figure 4. Mean total number of behaviours (behavioural transitions) expressed in the holding pen (± SE) for control treatment HP-control, and treatments HP-A and HP-A (2) and H that assumed that arena access was expected (n=10).

5.2 Arena

When lambs walked directly into the arena without a previous stay in the holding pen (A) they had a significantly higher percentage duration of performing play behaviours than when they stayed five min in the holding pen before entering into the arena (treatment HP-A) *(Z=-1.99, p=0.047).* Mean percentage duration of total play in HP-A(2) tended to be higher than in HP-A *(Z =-1.89, p=0.059)* (Figure 5).

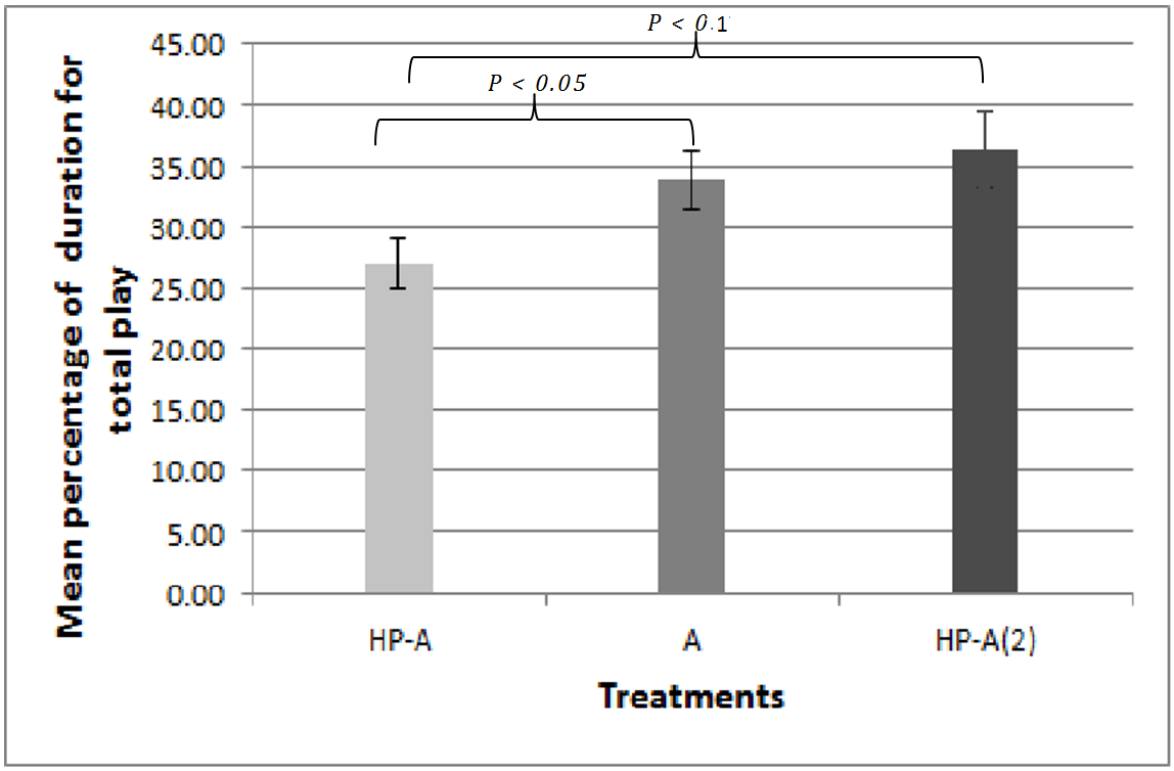

Figure 5. Mean percentage duration (± SE) of total play in the arena for treatments HP-A, HP-A(2) and A (n=10, Wilcoxon Signed Ranks test).

In treatment HP-A lambs were engaged in play for 27 % of their time during the first day in the arena, 22% during the second day and 32% during the third day. In A the lambs were engaged in play 31% on the first day, 35 % on the second day and 36% on the third day (Figure 6). In treatment HP-A(2) lambs were engaged in play on the first day for 44% of their time in the arena, 32% for the second day and for 34% on the third day (Figure 6).

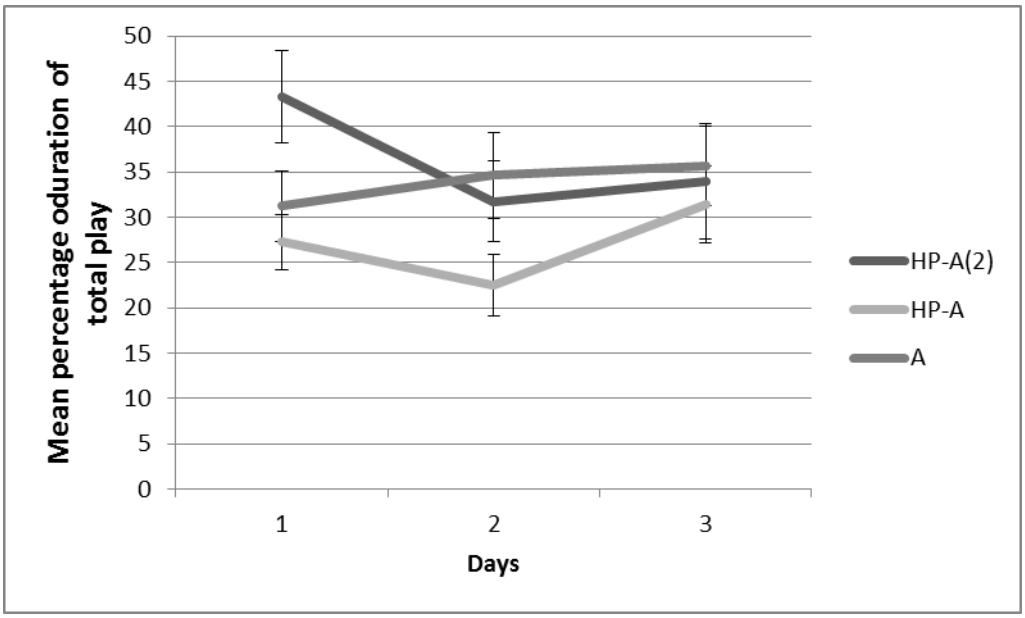

Figure 6. Mean percentage duration (± SE) of total play per day for treatments HP-A, HP-A (2) and A (n=10) showing how duration of play evolved through time within the treatment sessions/days.

On HP-A treatment lambs performed total play behaviours significantly longer during the first 7 minutes of the play arena session than during the last 7 minutes of the arena session *(Z=-1.99, p=0.047)* (Figure 7). Performing the same comparison for the first and second half of treatments A and HP-A(2) no significant difference was found ($p \geq 0.05$) (Figure 7).

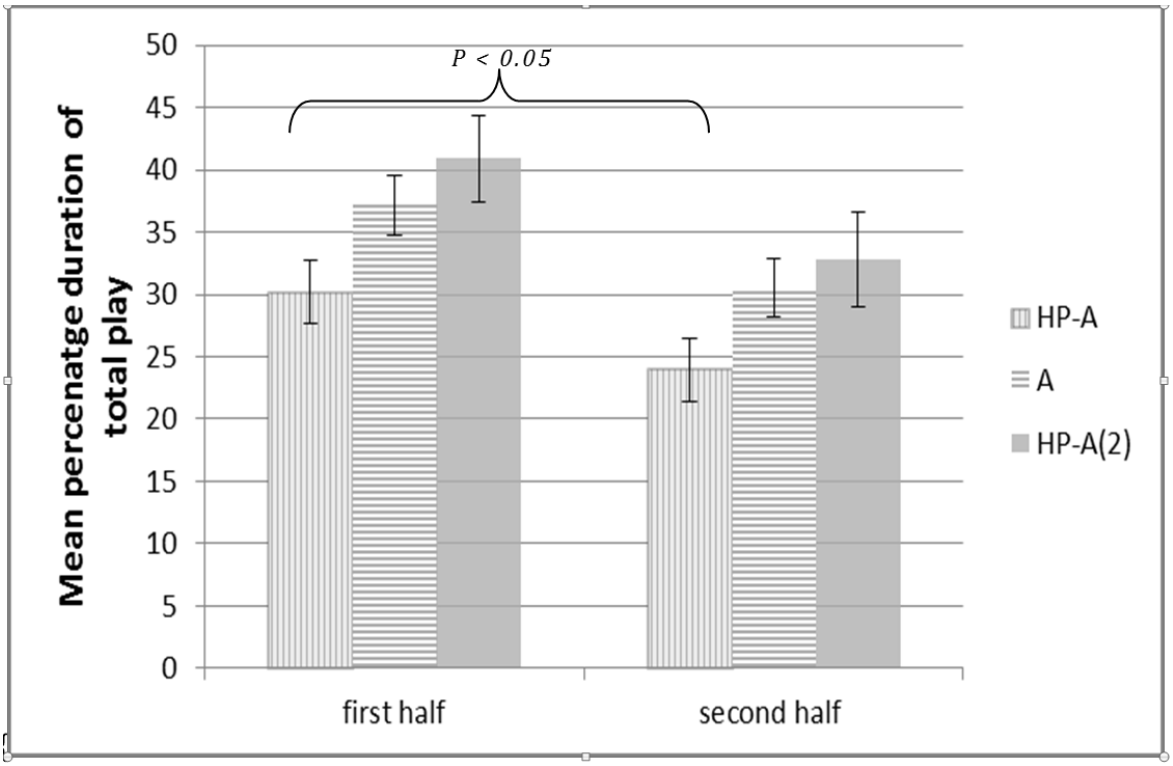

Figure 7. Mean percentage duration (± SE) of total play for the first half (0-7 min) and second half (8-14 min) of arena sessions for treatments HP-A, HP-A(2) and A (n=10, Wilcoxon Signed Ranks test).

5.3 Home pen

The lambs performed a significantly higher percentage duration of total eating in H compared to HP-control treatment *(Z=-2.70, p=0.007,* Figure 8*)*. Rumination was performed significantly less in H compared to HP-control *(Z=-2.80, p=0.005,* Figure 8*)*. No significant difference was found when testing total play, rumination and lying between HP-control and HP-A (*p ≥ 0.05,* Figure 8). No significant difference was detected (p≥0.05) comparing time spent in total play in HP-control with H (Figure 8). The mean percentage duration of lying in HP-A treatment tended to be higher than in treatment H *(Z=-1.82, p=0.1,* Figure 8).

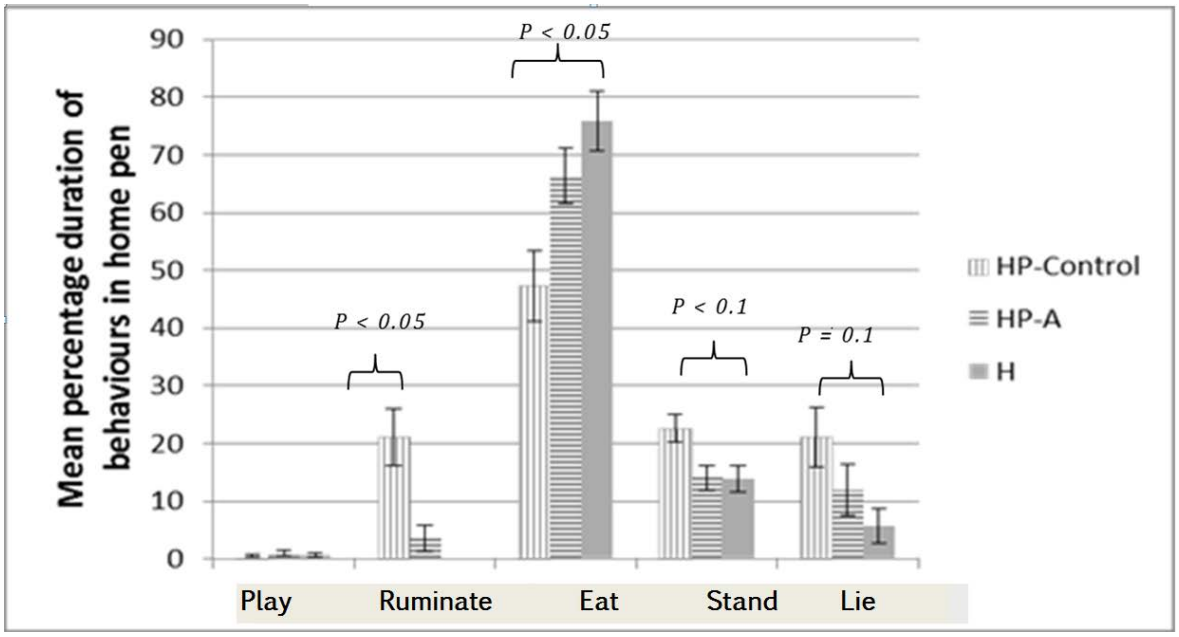

Figure 8. Mean percentage duration (± SE) of behaviours perfrormed in the home pen for HP-control , HP-A and HP-A(2) and H treatments (n=10, Wilcoxon Signed Ranks test).

A tendency for standing a longer percentage duration in HP-control treatment compared to treatment *H* was found *(Z=-1.89, p=0.575).* In treatment H all ten pairs of lambs remained standing in the holding pen when the door opened and they should walk back to their home pen. After ten seconds they were mildly moved out by the research personell and returned to their home pen. Comparing latyency to start eating between treatments H and HP-A and H and HP-control we did not find any significant differences between the treatments.

6. Discussion

This study was designed to investigate the behaviours (and heart rate variability) of lambs that were conditioned to anticipate a reward, in our case the opportunity to access an enriched arena. Boissy et al. (2007) suggests that providing animals the opportunity to play could induce positive affective states. Assuming that the access to an arena (that included enrichments such as a platform and manipulative objects as volleyball and two hanging chains) and a space larger than the actual home pen of the lambs' usual housing would provide the opportunity of greater behavioural expression and therefore play behaviours. A study on the effects of space allowance on the play behaviour of calves by Jensen and Kyhn (2000) indicate that larger space allowance could result in higher behavioural expression of play (for certain time periods).

Our lambs were tested in five treatments (in chronological order): Access to a holding pen with no arena experience (HP-control), access to an arena after being trained to expect it after remaining in a holding pen for 5 minutes (HP-A), access to the arena with no previous

holding pen session (A), access to the arena after being trained to expect it after remaining in a holding pen for 5 minutes (HP-A(2)) and finally holding pen exposure (5 min) and return to the home pen thus denied access to the arena (H).

The main results were that lambs did not show any difference in their behaviour in the holding pen when they were assumed to expect arena acces to the arena after 5 minutes (HP-A) compared to before they had been trained on this (HP-control). When the holding pen was skipped and the lambs were let directly into the arena (A) they played more in the arena than when being held in the holding pen for 5 minutes before getting in to the arena (HP-A). However, when testing holding pen and arena again (HP-A(2)) the lambs played even more in the arena than during the first arena treatment (HP-A) that followed identical procedures. The lambs played more during the first half or their time in the arena than during the second half for treatment HP-A, but no significant difference was found in the play duration comparing the first with the second half of treatments A and HP-A(2). After returning to their home pen lambs were mainly eating, their rumination activity was reduced and they were standing more (compared to HP-control).

6.1 Holding pen

Previous studies on anticipation (Van Den Bos et al., 2003: Van Der Harst et al., 2003; Moe et al., 2006) found increase in activity and exploratory behaviour in rats, mink and silver foxes while anticipating various types of reward. Additionally Knutson et al. (2009) found that rats anticipating play expressed, during anticipation, vocalizations similar to those that were expersing during play- reward experience. In our study, lambs conditioned to anticipate enriched arena access for 15 minutes after remaining in a holding pen for five minutes (HP-A) did not express significantly higher durations of play, walking and exploring behaviours that could be expected to be performed in the arena and considered indicative of anticipation, compared to the HP-control treatment. Similarly HP-control did not differ significantly with HP-A on the comparison of behavioural transitions (number of behaviours performed per session) which indicates raised activity that is considered indication of anticipation (van der Harst and Spruijt, 2007). A previous study on lambs by Anderson et al. (2013) found that lambs that were conditioned to expect food as reward compared to a control group expressed higher number of behavioural transitions while waiting to be exposed to that reward in a holding pen (3 min). Similarly with our study, no significant difference between the numbers of behavioural transitions was found when they compared lambs in the control group with the group of lambs that were conditioned to expect access to an enriched arena. A possible explanation for the lack of obvious anticipatory behaviour when access to an enriched arena is expected could be that the arena access as a reward is likely expected and anticipated by the animals but not as intensively as animals would anticipate food as a reward.This is supported by research from Spruijt et al. (2001) that argued that behavioural anticipation for arrival of a reward reflects the activation of reward centres in the brain and the level of activation depends on the incentive the reward offers. Another possible explanation could be a prolonged anticipation period, in our case five minutes, that was creating an uncontrollable and unpredictable experience. Therefore, as

indicated by previous research, if expected events have not happened at the appropriate time it could possibly change to frustration if the reward is delayed (Waitt and Buchanan-Smith, 2001). Chapagain et al. (2014) found that when lambs were trained to access an arena after being exposed to a holding pen for five minutes the behaviour during that period was altered from anticipatory to frustration. Similar effects could have occurred in our study as we used the same anticipation period. Nevertheless anticipation through holding pen exposure seems to have affected the performance of play in the arena as lambs in HP-A played more during the first part of the arena session compared to the second part. Interestingly when the treatment was repeated (HP-A(2)) the play duration comparison of the first and the second part did not seem to differ significantly. This could mean that the holding pen session in our study did have an effect on the lambs behaviour, a finding that could be more thoroughly investigated with the analysis of collected physiological data (heart rate that will be published later) that might show that anticipation is experienced but not expressed behaviourally.

6.2 Arena

Jensen and Kyhn (2000) suggest that raised play activity that was observed in calves subjected to a larger space might have been elicited by the mere release in a new enriched environment. In our study we cannot compare the play activity in the home pen with that expressed in the arena, nevertheless we can say that play was indeed expressed in the enriched arena.

Comparing treatment HP-A with treatment A, play occurred significantly more on treatment A, a finding that is opposing our hypothesis based on the theory that 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 (reward) (Manteuffel et al., 2009). Our study´s outcome could be due to an extended anticipation period that instead of enhancing the power of the reward through anticipation would have made it to wear out 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, Chapagain et al., 2014 (submitted)). Another explanation for the increase of play could be the creation of a better ‘mood’ in general since animals were continuously exposed to an enriched arena providing them with the opportunity to express a larger variety of behaviours (Reefmann et al., 2012). So the more times lambs were accessing the arena the more motivated they were to perform play.

Comparison of the first and the second part of the stay in the enriched arena for HP-A showed that lambs were engaged more in playing during the first seven minutes (out of 14) than during the last seven minutes. That could be due to hedonic activation that is maximum after presentation of a reward and is reflected as pleasure with the gradual consumption of reward (Berridge and Robinson, 1998). Since the lambs received a signal of access to the arena by remaining in the holding pen for 5 minutes, they might have been more excited and ceased the opportunity of playing 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 part of arena session. This can also be supported by the comparison of the first and the second half of the play sessions for treatment A where no significant difference was found.

6.3 Home pen

When access to the arena was denied in H treatment we found differences in the lambs behaviour compared to HP-A and HP-control treatments. It is likely that these changes in behaviour have been caused by the occurrence of a novel and unpredictable event as previous research has shown that unpredictability triggers stress (Porges, 1995). In H treatment lambs spent significantly more time eating (from floor or feeder) compared to the lambs of the HP-control treatment. Similar behaviour was observed when lambs were returning to their home pen after having accessed the arena in treatment HP-A. This could be due to a learned pattern to behave similarly as when they actually received the reward (although they have stayed away from the food for less time) or that they possibly needed some sort of compensation for the fact that they had not received a reward at all. This could also be supported by the results in treatment H where lambs performed rumination for less time compared to HP-A as decrease of ruminaton could be due to stressfull events or conditions (Bristow and Holmes, 2007). The results that lambs tended to stand more after being refused access to the arena in treatment H compared to HP-control treatment could indicate that they likely anticipated to experience arena access.

6.4 Future aspects

The findings of our research could be further investigated by refusing lambs another type of reward, as for example food, and giving them free access to it with no anticipation period. Another suggestion for future research on lambs anticipation-reward related behaviours especially for the part of anticipation could be the Qualitative behaviour assessment (QBA), a ‘whole animal methodology’used to score animals body language using terms as content, anxious and relaxed that was shown to be feasible for cattle (Rousing and Wemelsfelder, 2006), pigs (Wemelsfelder et al., 2009) and sheep (Phytian et al., 2011).

7. Conclusions

The expected arena exposure did not have a significant effect on the display of behaviours that were considered anticipatory and expected to be expressed in the holding pen as lambs (HP-A) did not express significantly more behaviours (considered anticipatory) compared to control treatment where no arena acces was expected (HP-control). Nevertheless holding pen exposure seemed to affect the time play in the enriched arena session was expressed

since lambs that were exposed to the holding pen (HP-A) tended to play for longer during the first part of the arena session compared to the second part. This holding pen exposure though did not have the same effect in the identical treatment (HP-A(2)) as no significant difference was found comparing play duration for the fist and second part of the arena session. Comparing play duration during first and second part of the arena sessions for lambs that were just offered arena access (A) no significant difference was found. These findings may indicate that even though no significant display of behaviours indicative of anticipation was performed (HP-A), it is likely that the holding pen had an effect on when the play behaviours were expessed. The holding pen session affected the overall duration of play performance in the play arena as lambs played for shorter time period in HP-A compared to A and compared to HP-A(2). Finally the reaction on a reward denial did affect lambs behaviour as they were engaged for longer in eating compared to the HP-control treatment and that might actually be indicative of lambs anticipating to be exposed to the arena.

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Appendix 1

June	10	11	12	13	14	15	16
	17	18 **Lamb arrival** Group housing	19 Habituation weight	20 Habituation pen allocation	21 Habituation	22 Habituation shearing	23 Habituation
	24 Holding pen HREq	25 Holding pen HREq	26 Holding pen HREq	27 Holding pen HREq	28	29 Holding pen HREq	30
July	1 **HP-control** HREq + **REC**	2	3 **HP-control** HREq + **REC**	4	5 **HP-control** HREq + **REC**	6	7
	8 Hold pen-Arena HREq	9 Hold pen-Arena HREq	10 Hold pen-Arena HREq	11 Hold pen-Arena HREq	12 Hold pen-Arena HREq	13	14
	15 **HP-A** HREq+**REC**	16	17 **HP-A** HREq+**REC**	18	19 **HP-A** HREq+**REC**	20	21 **A** HREq+REC
	22	23 **A** HREq+REC	24	25 **A** HREq+REC	26	27 Lena **A** HREq+REC	28 Hold pen-Arena
	29 Hold pen-Arena	30 Hold pen-Arena	31	1 Lena **HP-A(2)** HREq+**REC**	2	3 Ida **HP-A(2)** HREq+**REC**	4
	5 Ida **HP-A(2)** HREq+**REC**	6	7 Ida **H** HREq + **REC**	8	9 Lena **H** HREq + **REC**	10	11

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