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Influence of rearing method on the behaviour and welfare of Assaf lambs during the neonatal period

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ABSTRACT

The present study aimed to determine the influence of rearing method on behaviour and welfare in Assaf lambs during the first two weeks of the neonatal period, in an intensive rearing system. The experiment was conducted with 24 Assaf lambs, in which two methods of management were applied – natural rearing (NR) and artificial rearing (AR). Forced separation of lambs from their mothers caused 74% fewer suckling attempts during the first three days of the neonatal period, which reduced suckling time by 57%. The lambs reared under NR, spent more time moving and less time standing and lying. Overall, it was found that the type of rearing affects the suckling time. Artificial rearing decreased lying and playing time, which is indicative of the improved welfare of NR lambs. The two rearing methods (NR and AR) did not affect blood total protein or albumin, indicating no significant effect of different rearing methods on these haematological variables. Artificial rearing does not disturb the welfare and behaviour of the animals when it is done correctly and stress appears transient and animals quickly adapt with similar growth rates.

Keywords: Artificial rearing, Assaf lambs, Natural rearing, Neonatal period

The artificial rearing of lambs led to the modernization of dairy sheep farming, which broke the relationship between "sheep and lamb" (Kieltyka-Kurc and Gorecki 2015), with the end purpose to increase the amount of milk obtained (Napolitano *et al.* 2008). According to Napolitano *et al.* (1995) this system produces an emotional and nutritional stress, which influences the productive potential of animals.

Dwyer (2003) describes the neonatal period as a critical stage in the development of lambs during which the newborn must adapt to the extrauterine environment (Piccione *et al.* 2007).

The feeding of lambs with milk replacer (MR) is widely practiced in the dairy sheep industry (Mialon *et al.* 2021), for additional revenue generation from sheep milk sale (McCoard *et al.* 2020). This is possible with a pre-arranged plan (Napolitano *et al.*1995), otherwise, animal welfare gets adversely affected which is characterized by negative behaviour, endocrine, and immune responses (Sevi *et al.* 1999, Napolitano *et al.* 2002). Artificial rearing and maternal deprivation can have wide-ranging negative consequences affecting the development of the immune system, behaviour, endocrine development, growth, and cognitive development of lambs (Love *et al.* 2022) influenced by a number of endogenous and exogenous factors (De *et al.* 2015). Most studies have focused on optimizing lamb nutrition to improve growth (Danso *et al.* 2018, Herath *et al.* 2020), but there research is scanty with respect to lamb behaviour during the neonatal period with respect to the method of rearing. Weaning the lambs from their mothers is distressing for them. Hence, finding effective husbandry methods with the goal to alleviate stress and improve animal well-being is one of the important research interests (Lérias *et al.* 2013).

With this backdrop, the present study was designed to investigate the influence of natural and artificial rearing method on the behaviour and welfare in Assaf lambs managed in intensive system during the first two weeks of the neonatal period.

MATERIALS AND METHODS

The experiment was conducted during December 2022 to January 2023 in 24 Assaf lambs, raised in a Livestock farm located at Kostelevo Village, Vratsa Municipality, Bulgaria (43.21659°N, 23.61234°E).

Experimental design: The experimental lambs were paired based on body weight, sex and type of birth and were randomly assigned to two groups (12 animals per group) to equalize these variables between groups. The difference in age between the animals was one day. In the natural rearing (NR) group, the animals were reared alongside their mothers. This group consisted of three ewes with their lambs, with two ewes having single lambs and

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one having twin lambs.

In the artificially reared (AR) group, the lambs were separated from their mothers after birth and moved to separate premises, so that they could not have visual or auditory contact with their mothers. The room in which AR lambs were housed was insulated (no draft) and maintained at an average temperature of 16°C. During the first two days after lambing, the lambs were fed *ad lib*. pooled colostrum collected from their dam. On the third day, colostrum was replaced with milk replacer (Table 1). Milk replacer was dissolved in 43°C warm water (190 g/L) and offered *ad lib*. through an automatic lamb feeder (height-adjustable on four teat). In the NR lambs, the milk replacer was placed in a creep area of 15 m^2 to which the lambs had free access (Alcock 2006). The body weight of the lambs was measured with an electronic scale at birth, then at 3, 7 and 14 days of age.

Table 1. Chemical composition of the milk replacer fed artificially reared lambs

Nutrient analysis, g/kg	
Dry matter	98
Crude protein	25.6
Crude fat	24.4
Crude ash	6.5
Crude fiber	0
Calcium	1.27
Phosphorus	0.63

Both the groups had free access to protein concentrate with 33% CP (37.4% sunflower meal; 30.2% soybean meal; 19.4% peas (grain); 5% molasses; 2.5% calcium carbonate; 2.5% vitamins and minerals; 1.5% sodium chloride; 1.5% sodium bicarbonate), maize grain, alfalfa hay, lukewarm drinking water and rock salt for licking from 4 days of age onwards.

Animal behaviour: The behaviour and well-being of the experimental lambs were monitored by XMART WI-FI cameras with built-in BC202 batteries (made in China) and were installed at 4 m above the floor of the experimental sheds. The lambs were marked with a red spray (OVI-line Ukal Eschbach, FR) for identification during the individual behaviour study.

The activites of each of the individual lambs were observed and then tabulated (Table 2) from 33 total recordings with each recordings lasting for 24 h.

Determining the welfare of lambs: To determine the health status and welfare of the lambs, 6 lambs from each group, (3 males and 3 females) were randomly selected. Blood samples were collected at birth, day 3, 7 and 14 to determine the haematological parameters like total protein (TP), glucose (Gl), albumin (Alb) and cortisol (C). These parameters were used for evaluating health status and welfare of the lambs. Blood samples were collected in the morning from the jugular vein using a 21g needle and a sterilized 5 ml heparin vacuum tube. Subsequently blood samples were subjected to centrifugation for 15 min and plasma was collected and then stored at -20°C until further analysis.

The concentration of TP and Alb were determined using an automated biochemical analyzer Mindray BS-240 (Made in China). Blood Gl concentrations were determined by a Biolabo Diagnostics (France) test using a biochemical analyzer. Cortisol levels in the blood plasma were determined with a commercial kit Cortisol RIA, (Gosling *et al.* 1993).

Statistical analysis: Data for growth, behavioural analysis and blood parameters were subjected to one factor ANOVA and subsequently correlations between the treatment groups were analyzed by Pearsons correlation cefficient by using Statistica 6.0 (2006). Significance was determined by t-test at $P<0.05$ and $P<0.01$.

RESULTS AND DISCUSSION

The body weight of lambs in the NR group was significantly ($P<0.05$) higher at the end of first week compared to AR lambs. Nevertheless, towards the end of 14 days the body weight in between the two groups did not vary significantly (Fig. 1).

The number of attempts to suckle were greater in NR lambs during the first three days of life, along with time spent in suckling and movement as compared to AR lambs ($P<0.01$, Table 3). The lambs in the AR group spent longer duration by standing and lying down ($P<0.01$) and had longer average durations of bouts of sucking ($P<0.05$, Table 3).

Table 2. Description of the lamb behaviour monitored during the experiment

Behaviour	Description	Reference
Suckling (min)	Time during which the lamb was in contact for no less than 5 s with the mother‘s teat or with the teat of the automatic feeder.	Cimen 2007
Attempts to suckle (number/h)	Time during which the lamb attempted to suckle for less than 5 s but was interrupted due to maternal movement or being pushed by another lamb.	Dwyer *et al.* 2004
Average duration of bouts of suckling (number)	Total duration of suckling divided by the number of bouts.	-
Standing (min)	Time when the lamb is on all fours (standing) and not moving.	Broekman 2015
Movement (min)	Time for which the lamb is on all fours and moving (forwards or backwards).	Broekman 2015
Lying (min)	When the lamb's belly and belly are in direct contact with the ground.	Broekman 2015
Play (min)	Building social interaction between lambs in the form of play (running, jumping, stroking, hanging).	-

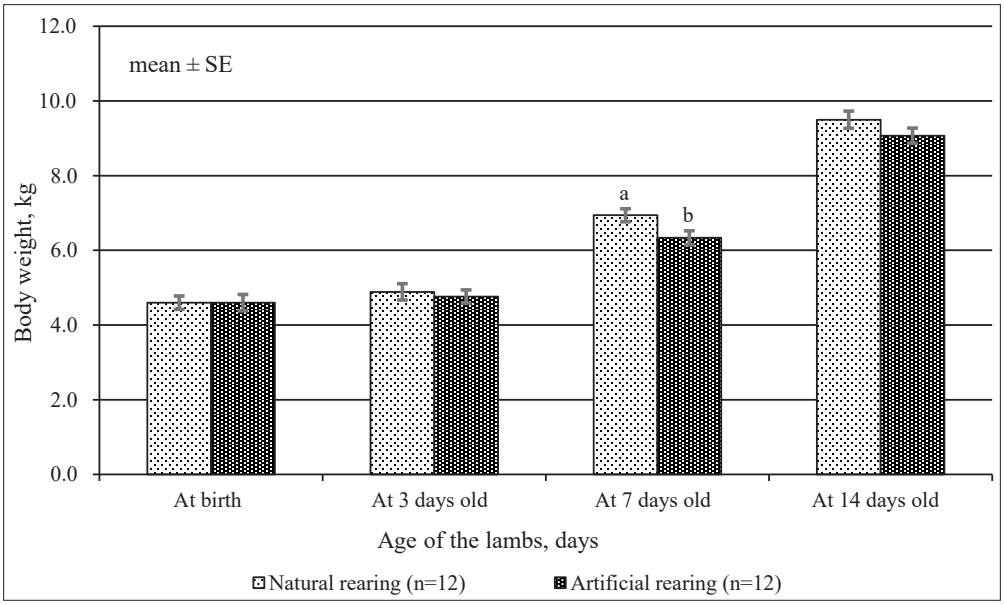

Fig. 1. Lamb growth during the first two weeks after birth ([a, b] P<0.05).

At the end of the second week of birth, more time was reported for petting in NR lambs, with longer durations of petting bouts, playing (P<0.01) and time spent lying down (P<0.05, Table 3). While the lambs in AR group made attempts to suckle (P<0.01), spent more time standing (P<0.05), moving (P<0.01) and consuming feed.

Type of rearing had a positive correlation with suckling, attempts to suckle, movement (P<0.01) and negative with time spent lying down during the first two weeks after birth of the lambs (P<0.01, Table 4). Hours of the day were negatively correlated to suckling, attempts to suckle, movement (P<0.01) and positively with time spent lying down (P<0.01, Table 4).

Naturally reared lambs had higher blood glucose (3.05 mmol/L, P<0.01) and cortisol (29.1 ng/ml, P<0.01, Table 5). However, total protein and albumin content between the two groups did not differ significantly (Table 5).

The findings of the study showed higher growth in lambs during the first week of age if they are reared with mothers (NR lambs), regardless of the fact that the AR lambs were fed with quality milk replacer during that period. During the first three days of life, lambs from both groups grew equally, the possible reason for which may be that during this period the animals received colostrum. However, higher body weight of NR lambs at day seven after birth can be related not only to the amount of colostrum and milk received, but to the differences between the composition of the milk and the MR used. According to Hernández-Castellano *et al.* (2015), the source of milk is one of

Table 3. Behaviour of Assaf lambs reared naturally and artificially

Activities	Natural rearing	Artificial rearing	SEM
First three days of the neonatal period			
Suckling (min/h)	3.24	1.39*	0.06
Attempts to suckling (number/h)	2.27	0.59*	0.04
Average duration of bouts of suckling (number/h)	1.4	2.4*	0.01
Standing (min/h)	7.33	8.27**	0.40
Movement (min/h)	12.1	8.08*	0.42
Lying (min/h)	37.4	42.3*	0.57
First two weeks after birth			
Suckling (min/h)	7.23	5.21*	0.19
Attempts to suckling (number/h)	2.27	2.85*	0.09
Average duration of bouts of suckling (number/h)	3.2	1.8*	0.05
Standing (min/h)	5.05	6.36**	0.35
Movement (min/h)	7.04	12,55*	0.47
Lying (min/h)	33.1	28.5**	0.61
Play (min/h)	5.46	3.45*	0.34
Consumption of fodder (min/h)#	0.54	1.41*	0.20
Consumption of alfalfa hay (min/h)	1.62	2.50**	0.19

SEM, Standard error of the means; *, P<0.01; **, P<0.05; #, Consumption experience includes the protein concentrate and maize.

Table 4. Correlation analysis between factors and lamb behavior during the first two weeks of life

Factor	Suckling	Attempts to suckle	Standing	Movement	Lay
Method of rearing	0.28**	0.48**	0.01^{ns}	0.15**	-0.11**
Hours of day	-0.28**	-0.19**	-0.01^{ns}	-0.08**	0.07**
Type of birth	0.02^{ns}	0.02^{ns}	-0.00^{ns}	-0.02^{ns}	0.004^{ns}
Sex of lamb	0.01^{ns}	0.04^{ns}	-0.02^{ns}	-0.05^{ns}	0.03^{ns}

**, Correlation is significant at the 0.01; ns (non-significant).

several factors influencing growth and the immune system. In the study of Baumrucker and Blum (1993), milk is said to contain insulin-like growth factors (IGFs), which are absent in the MR. In addition, it was reported that longer duration spent on suckling has positive impact on growth performace in NR lambs (Lanza *et al.* 2006) which supports the pesent findings.

When lambs were raised with their mothers during the neonatal period, they not only spent more time suckling, but also showed longer activity compared to AR animals.

This could be due to hormonal changes that occur at birth (Nowak and Poindron *et al.* 2006), which result in the formation of a mutual bond between the mother and the lamb in the first few days of life (Dwyer *et al.* 2016). Besides, under NR the lambs learn to suckle in the first 12 h of their life, which develops a strong motivation to suckle (Margerison *et al.* 2003). In NR, intensive physiological and behavioural stimulation of the newborn occurs after birth (Lagercrantz and Slotkin 1986). This creates an arousal that encourages the newborn to explore the mother's body and through the sensory cues it is directed to the udder by exploring the mother's lower body (Nowak and Poindron *et al.* 2006). In this case, thermo-tactile cues on the mother's side have a crucial role in locating the teats of the udder (Vince 1993).

In the present study, after birth, AR lambs were separated from their mothers, which did not permit mother-young one bonding, and that affected suckling reflex, as well as behaviour. This is also possible cause of occurrence of acute distress in AR lambs (Meagher *et al.* 2019). Hence, forced separation of lambs from their mothers, resulted in 74% fewer suckling attempts, with reduced suckling time by 57% and increased standing time, contributing to decreased growth during the first three days of the neonatal period.

Previous studies (Dwyer 2003 and Nowak *et al.* 2006) reported that body weight, sex of lamb and type of birth can have an impact on the time of suckling. This possibility can be ruled out for the current study since the lambs were grouped homogenously with respect to all those three parameters. In NR lambs, suckling stimulated the expression of maternal behaviour, forming the "sheep/lamb" bond (Nowak *et al.* 2007). According to Nowak and Boivin (2015), colostrum intake activates the insular cortex, focussing the lambs' attention on maternal cues and thus favours cognitive processes, initiating the filial bonding. Hormones like cholecystokinin (CCK) and endogenous opioid peptides are closely related to the animal's behaviour during suckling, further inducing and reinforcing it (Han *et al.* 2023). In addition, these hormones are involved in the ability of lambs to recognize their mother (Goursaud and Nowak 2000). During the first three days after birth, lambs reared with their mothers spent 33% more time moving, resulting in significantly less time spent in standing and lying (11.4 and 11.6%), compared to AR lambs. The present findings confirm the fact from evolutionary viewpoint that parental care immediately after delivery is crucial for offspring survival (Lévy and Keller 2008). The results show that after the first three days of birth, AR lambs make 20.4% more attempts to suckle compared with AR lambs. This affected the time allocated to suckling, which was 28% more in NR lambs and was a clear indication that the rearing pattern influenced the time allocated to suckling.

Table 5. Blood parameters in Assaf lambs under natural and artificial rearing system

Indicator	Natural rearing	Artificial rearing	SEM
Total protein (g/L)	51.7	49.6^{ns}	0.81
Glucose (mmol/L)	3.05	2.5*	0.06
Albumin (g/L)	24.8	24.2^{ns}	0.44
Cortisol (ng/ml)	29.1	19.7*	1.23

*, P<0.01; ^{ns}, non-significant.

After three days of age, NR lambs spent 32% more time lying and 38% more time playing. This, in part, can be explained by the fact that sheep have an intense herd social instinct and exhibit social interactions (Mohapatra *et al.* 2021). Boissy *et al.* (2007) defines it as a category of behaviour generated by locomotor, manipulative and social behavioural characteristics, which is associated with a state of happiness when the animal is in a positive affective state. The possible reason for this is that the lambs are not separated from their mothers, which more quickly engenders social interaction between the animals themselves (Hass and Jenni 1993) and it is an indicator of their welfare (Broekman 2015).

In this study, the absence of a mother caused lambs fed milk replacer to spend more time in moving and standing, an indicator of anxiety or anticipation of caregivers arrival. According to Nowak and Boivin (2015), lambs are looking for specific closeness with the mother or a caregiver who cares for them and becomes the primary attachment figure as early as second day of birth (Boivin *et al.* 2001). In this study, reared lambs were cared for by the same caretaker, positively generating "human–lamb" social contact. However, the anxiety caused by the animals in this group was that their social contact with the caretaker (feeding,

petting, gentle talking) was too short (about 4 h per day) and after separation, the behaviour of the distress resumed. In this case, the lambs fell into a situation of isolation, which is said to reduce the number of neurons that cover the orbitofrontal cortex and the limbic system that regulate emotions and social recognition (Nowak and Boivin 2015). In NR, the lamb-sheep social contact was constant throughout the day. This possibly accounts for the longer lying time followed by longer playing time between lambs in the group, indicative of animal welfare (De *et al.* 2017).

The two methods rearing did not affect the blood total protein or albumin. This is an indicator of good health (Braun *et al.* 2010) and the quality of the MR used.

In AR, frequent human contact reduces stress and positively affects the welfare and future productivity of the animals (Rushen *et al.* 1999). Dhabhar and McEwen (1997) described stress as a complex cascade of events consisting of a stressor that induces the perception of stress and activates its physiological response. A reason for the higher levels of glucose and cortisol in the NR lambs is that they were not used to the presence of people, they did not approach them and when the animals were caught (during manipulation), they showed signs of anxiety. However, this is not an indicator of anxiety, because according to Hemsworth *et al.* (2019) behavioural responses of lambs, such as trying to escape, hitting or bumping into fences, slipping, etc., with elevated cortisol and glucose levels show a risk of compromised welfare. The studies of Sevi *et al.* (2002) have shown that psychological and physical stress increases the levels of cortisol released by the adrenal gland, indicating that even slightly elevated cortisol levels can be associated with stress (Boucher and Plusquellec 2019). In this case, when the animal gets into a stressful situation, epinephrine stimulates glycogen hydrolysis, which releases glucose and lactate into the circulatory systems, where glucose is used as energy for survival and maintaining the animal‘s welfare in a stressful situation.

Lambs grew faster in the first week when reared with their mothers ($P<0.05$), even with quality milk replacer. The main reason is that after birth, lambs‘ early separation from their mothers, as well as the lack of development of a strong motivation to suckle in the first week, indicate that human intervention (prompt to suckle and presence) must be constant as it affects both growth and animal behaviour. During the first three days of the newborn phase, artificially reared lambs made 74% fewer suckling attempts, resulting in a 57% decrease in suckling time ($P<0.01$) and increased standing and lying time. However after the first three days of age AR lambs attempted to suckle more frequently, but this had no effect on the time assigned to suckling, indicating that the method of rearing impacts the time given to sucking. Artificially reared lambs spend more time moving and standing, which may be an indicator of anxiety or anticipation of caregiver contact.

It may be concluded that when lambs are artificially reared correctly and under observation, cortisol and blood glucose levels do not change, indicating their well-being. This allows the animals to become accustomed to their caregivers more rapidly, and it reduces stress during various operations.The stress associated with AR appears transient and animals quickly get adapted which is reflected in their comparable growth rates.

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