

### Annotated Bibliography

These articles are organized in the following themes: Background information, Causes and associated influence on isolation behaviour, and reductions of social isolation behaviours.

**Background information:** These articles provide important context about the biology and social nature of sheep and how that affects their behaviour.

Hutson, G.D. (2007). Behaviour principles of sheep handling. In T. Grandin (Ed.), *Livestock handling and transport* (pp. 155-174). CABI. <https://doi.org/10.1079/9780851994093.0175>

Summary:

Sheep (*Ovis aries*) are kept as livestock and periodically require handling by farmers. Hutson (2007) outlines important behavioural background to consider in sheep handling. Firstly, their flocking ability which is an intrinsic behaviour to most sheep species however the specific behaviours and distances between sheep differs across breeds. The flocking behaviour is important because it allows for social bonds to develop among sheep and may be the reason for their increased social needs. As a result of their flocking behaviour they require the ability to recognize their mothers and one another to maintain group cohesion. Following in sheep is also a natural behaviour in which sheep naturally follow one another, and exhibit a trait called mutual imitation in which they eat, sleep, walk, and run synchronously. Vision is extremely important for sheep because of their need to follow and flock together, so they have excellent binocular, stereoscopic, and depth perception vision. Visual behaviours are their main communication with one another. Sheep have well developed olfaction which mainly plays a role in mothers recognizing the smell of their lambs at close distances in social contexts. When assessing auditory abilities of sheep it was found that it plays less of a role in their behaviours. Sheep can quickly adapt to and ignore loud sounds. Sheep hearing is also likely better adapted to hear predators than one another, which makes sense as they have a lack of vocal communication. Vocal communication only occurs in sheep during social isolation, between mother and lambs, and during courtship behaviour. Sheep are also highly intelligent and have a great ability to learn and exceed in operant conditioning. They have good memories for social recognition, spatial information, and food distribution. All of these characteristics are important when considering handling techniques for sheep.

Conclusion:

This book chapter was included in my review as it gives a lot of background information on influences of behaviours in sheep. Sheep flocking, following, vision, and facial recognition ability all help understand why social isolation causes such stress in sheep, and why maintaining a visual connection with familiar individuals is so important for them to offset stress from handling and isolation. These concepts are all consistent with the literature. Further information on the learning ability of sheep could give additional ways to reduce stress behaviours in sheep.

Nowak, R., Porter, R. H., Blache, D., & Dwyer, C. M. (2008). Behaviour and the welfare of the sheep. In C. M. Dwyer (Ed.), *The Welfare of Sheep* (pp. 81–134). Springer Netherlands. [https://doi.org/10.1007/978-1-4020-8553-6\\_3](https://doi.org/10.1007/978-1-4020-8553-6_3)

#### Summary:

This book chapter outlines many important characteristics of social organization and behaviour in sheep (*Ovis aries*), and this summary will be focusing on characteristics that provide relevant background to social isolation in sheep. Firstly, sheep are always using visual cues to maintain particular distance from one another. The cohesion of the flock is maintained by each sheep adjusting their position relative to one another. Visually they can communicate if a predator is near through adopting a rigid posture. Visually sheep are able to recognize their specific breed as well. A sheep's social status is related to visual displays and while sheep display some dominance behaviour, it is usually slight movements of the head or eyes and does not usually become aggressive in nature. Often the dominant sheep will displace the subordinate sheep from feeding and sleeping positions. As a result of their social nature sheep show an increase in negative behaviours when socially isolated, which results in an increase in vocal activity, locomotion, and a reduction of food and water intake, there is also a reduction in circulating lymphocytes and an increase in cortisol levels. The human relationship with the sheep is also important as regular contact with a familiar human is required to maintain positive associations. Sheep can learn to distinguish humans in as little as 3 weeks and become habituated to human contact. These behaviours and social organizations are important to understand when considering why sheep act the way they do and allows assessment of normal behaviours as well as signs of stress in sheep.

#### Conclusion:

This book section was included in my review as it gives important background information on the social organization and interactions of sheep. When considering stress behaviour influences from social isolation it is helpful to consider the normal social behaviour of the sheep. This section also describes the role that humans can play in reducing social isolation stress behaviours in sheep. This is all consistent with other literature showcasing that social isolation is a result of their normal social behaviour and it also outlines the need to further study and understand how utilizing the natural social behaviour of sheep can help reduce their stress.

**Causes and associated influences on social isolation behaviours:** these articles provide information on proximal causes of social isolation stress, and the associated physiological responses.

Barnard, S., Matthews, L. R., Messori, S., Podaliri Vulpiani, M., & Ferri, N. (2015). Behavioural reactivity of ewes and lambs during partial and total social isolation. *Applied Animal Behaviour Science*, 163, 89–97. <https://doi.org/10.1016/j.applanim.2014.11.016>

Summary:

Isolation in sheep (*Ovis aries*) is well documented to cause strong stress responses, and these stress responses can result in reduction of productivity. Stress responses include behavioural and physiological changes. Stress behaviours include increased locomotion, vocalization, and escape attempts. Genetics and developmental factors are contributors to individual variability in stress responses. Maternal experiences can also affect these stress reactions. Barnard et al. (2015) aimed to compare the reactivity of ewes and lambs during two tests: mother-lamb separation and an isolation test. The study also attempted to measure the effect of breed, parity, and sex. They used 18 ewes, 6 male lambs and 12 female lambs of Sarda (primiparous), 17 ewes, 9 males and 8 female lambs of Sarda (multiparous), and 8 ewes, 3 males and 5 females of Dorset Horn (multiparous). Locomotion was measured using an arena test method. In the first test the mother and lamb were separated by a wire fence and their behaviours were recorded. In the second test the mother and lamb were each isolated for 4 minutes and their individual behaviours were recorded. Locomotion, number of vocalizations and escape attempts were recorded, and were considered indicators of fear. They found that multiparous (2 or 3 births) dams were less reactive than dams which had given birth for the first time. Increased fear in the mother was correlated with fear in the lamb. The Dorset Horn had a lower fear score than the Sarda sheep. The lambs had higher fear scores than their mothers. This indicates that variations in breed, age, and parity may all have an effect of the severity of fear response. These results suggest that fear responses according to individual characteristics may be able to be anticipated. Future studies about the difference between different parity in more breeds can be investigated.

Conclusion:

This paper was included in my review as it highlights several important factors in determining sheep behavioural responses to social isolation. It also highlights that there are possibly different management implications and requirements for different parities, ages, and breeds of sheep. These findings are very consistent with other literature on the matter. There is also an important correlation between mother behaviour and lamb behaviour, indicating a genetic and/or developmental influence which should be further investigated.

Price, E. O., & Thos, J. (1980). Behavioral responses to short-term social isolation in sheep and goats. *Applied Animal Ethology*, 6(4), 331–339. [https://doi.org/10.1016/0304-3762\(80\)90133-9](https://doi.org/10.1016/0304-3762(80)90133-9)

#### Summary:

Sheep (*Ovis aries*) and goats (*Capra aegagrus hircus*) are both social animals, and each exhibits negative responses to social isolation. These responses include increased vocalizations (high-pitched bleats), and locomotion (walking, and rearing). The aim of this study was to determine variables affecting stress responses to isolation. They investigated the effect of interspecies pairs, novel vs. strange environments, and the presence of an observer. They used 20 Dorset. And Targhee male lambs and 20 male Alpine, Toggenburg, and Lamancha kids. They paired 10 of the sheep and 10 of the goats with each other, and the rest were paired with the same species. Measurements of vocalization, rearing, frequency and duration of contact with the observer were recorded. In each test one animal remained in its home environment and was recorded for 10 minutes while the other was taken on a truck to a remote location and recorded for 5 minutes. They were tested 4 times in each location. During half of the tests the observer was present. Overall goats vocalized and reared more. Strange environments resulted in more rearings, but not vocalizations in both sheep and goats. This may indicate a relationship between locomotion and novel environments. There was no difference in the interspecies pairs except that they engaged with the human observer more often on average. The presence of the observer reduced vocalizations and rearings in both sheep and goats. Overall, the goats were more stressed by social isolation than sheep, however both exhibited an increase in vocalization and locomotion when isolated. Goats and sheep were both extremely stressed by social isolation. These results also indicate that humans companionship may be a possible way to reduce stress during isolation. A question posed by the author is why did animals raised with other species interact with the human handlers more often?

#### Conclusion:

This article was included in my review because it allows for a comparison between goats and sheep to better understand sheep isolation behaviour. The presence of the observer reducing stress has important implications for management and could be used to reduce sheep isolation stress in the future. This study also indicates that vocalization might be the key behaviour associated with isolation. These findings are consistent with other studies, and indicate that social isolation is stressful in sheep, and vocalization is a major behaviour associated with this stress.

De, K., Saxena, V. K., Balaganur, K., Kumar, D., & Naqvi, S. M. K. (2018). Effect of short-term seclusion of sheep on their welfare indicators. *Journal of Veterinary Behavior*, 27, 1–7. <https://doi.org/10.1016/j.jveb.2018.05.007>

#### Summary:

Sheep (*Ovis aries*) respond behaviourally to social isolation stress which results in increased general activity and vocalizations. Locomotion could be escape attempts and vocalization could be attempts to communicate with the flock that they are separated from. These behaviours lead to metabolic changes particularly in cortisol, t3 and t4. These metabolic changes can have negative consequences on the productivity and welfare of the animal. The objective of this study was to assess changes in welfare indicators including behaviours, physiological indicators, blood biochemical and endocrine parameters as a result of short-term isolation. 16 Malpura ewes were selected. The Malpura breed is bred for its mutton making them stockier, and as such heavier breeds typically are less social. The selected ewes were kept together for a month, then randomly selected to be isolated individually. They were isolated for 24 hours visually and tactilely, and measurements were taken before, during, and after isolation. Food and water intake and body weight were reduced significantly from isolation. Feeding, idle laying, and rumination were significantly decreased, while standing and locomotion were significantly increased. Cortisol, T4 and T3 increased significantly, and there were no changes in other blood biochemical components. Respiration, pulse rate and rectal temperatures all increased significantly and were considered to be signs of homeostasis related to the increase of corticotropic hormones. De et al. (2018) suggested that locomotion could be considered an active coping mechanism and idle standing could be considered a passive coping mechanism. These physiological mechanisms show a complex chain of changes resulting from isolation stress and may be indicators of reduced welfare in isolated sheep. The direct mechanism associating the metabolic changes with isolation stress should be investigated more thoroughly following this study.

#### Conclusion:

This study is included in my review because it links behavioural changes with hormonal changes and metabolic changes. It is important to understand that not only does the behaviour change from isolation stress but that there are also physiological indicators of stress as a result. These results are consistent with the literature indicating that social isolation increases behavioural and physiological stress in sheep. They also provide an insight into the related mechanisms of these behaviours and how this stress can affect the physical health of isolated sheep.

Poindron, P., Soto, R., & Romeyer, A. (1997). Decrease of response to social separation in preparturient ewes. *Behavioural Processes*, *40*(1), 45–51. [https://doi.org/10.1016/S0376-6357\(96\)00767-X](https://doi.org/10.1016/S0376-6357(96)00767-X)

#### Summary:

Sheep (*Ovis aries*) respond strongly to social isolation from their conspecifics. This involves an increase in stress behaviours including increased locomotion, high pitched bleats and elimination behaviour. In contrast to this, ewes that are close to parturition have been documented to seek out isolation and are less responsive to social isolation after lambing. The purpose of this study was to compare the reaction of pregnant ewes near parturition and non-pregnant ewes to social isolation. To do this they used 24 non-pregnant, non-lactating ewes, and 17 pregnant ewes in their fifth month of pregnancy. They were tested three times first at day 148 of pregnancy, then at first signs of lambing (birth) and finally 60 minutes post-partum. 10 additional dry (non-lactating, non-pregnant) ewes were used as companions. The 5 minutes before isolation behaviour were observed, and during the next 5 minutes their conspecifics were removed from the pen and they were observed again. They recorded vocalizations, locomotion activity, eliminations, attempts to jump, latency out of the pen to join conspecifics, and agitation index. They found that there was a significant increase in stress behaviours when conspecifics were withdrawn for both control and experimental groups. Vocalization, locomotion, and agitation was also significantly higher. There was no difference in the first test among the groups. In test 2 there was a higher level of agitation in the control, and in test 2 the experimental ewes had more high- bleats before conspecifics were removed. After conspecifics were removed the control group was always significantly more stressed and had greater agitation scores. Overall, the pregnant ewes were less responsive to social isolation and exhibited less stress behaviours compared to non-pregnant ewes. These results suggest that some aspect of parturition changes the pregnant ewe's response to social isolation. Future studies should investigate the specific mechanisms of parturition which affect ewe's responses to social isolation.

#### Conclusion:

I included this article in my review as it showcases circumstances in which behaviours in response to social isolation will change. It is important to understand that pre-parturitional ewes will not be as stressed by social isolation, and it may be beneficial for them to be isolated. This study is consistent with other literature, however, the results were not entirely conclusive because of low power due to a lower number of replicates, however it brings to light interesting future studies. Going forward, investigating the relationship between reduction in social isolation distress and parturition will be beneficial.

Viérin, M., & Bouissou, M.-F. (2003). Responses of weaned lambs to fear-eliciting situations: Origin of individual differences. *Developmental Psychobiology*, 42(2), 131–147. <https://doi.org/10.1002/dev.1009>

#### Summary:

Fearfulness is a determining trait that predisposes individuals to react in a consistent manner to stressful events. In nature fearfulness has an adaptive value, but in domesticated species like sheep (*Ovis aries*) it can decrease welfare and productivity. Light breeds are reported to be less fearful than heavy breeds, and adult females are typically more fearful than adult males. The objective of this study is to create an objective measure of fear in lambs and to compare the effects of age, sex, breed, and rearing conditions on fear reactions in sheep. They used 441 sheep in age groups of 3-4 months and 5-6 months. They were either dam reared or artificially reared, and either male or female. They measured fearfulness by scoring behaviours such as locomotion, vocalization, latency to feed, and lack of interaction with novel objects. Il-de-france (heavy) and Romanov (light) breeds were used. There were 3 fear eliciting tests. The isolation test, surprise effect test, and the human presence test. The results indicate that young and female sheep were more fearful. Romanov sheep were more fearful in two of the tests but equally fearful in the isolation test. Rearing had no effect on the fearfulness of males. There were contrasting results in rearing effect for females 3-4 months and 5-6 months where artificially weaned sheep were less fearful at a younger age and more fearful at an older age than dam reared. This indicates a complex interaction between fear and rearing. Age, breed, sex, and to some extent rearing conditions had an effect on fear responses in sheep. These results also indicate that individual variables have a lot of effect on sheep fearfulness in isolation. The authors were also able to create behavioural measures of fear using these three tests to be used to assess stress in future studies.

#### Conclusion:

This article was included in my review as it measures proximal influences on stress responses as a result of social isolation (and other fearful events) in sheep. It is helpful to understand how individual differences will affect the way a sheep reacts to being socially isolated. These results are consistent with other literature and indicate that there are many complex interactions between individual traits and behaviour in sheep. Further research to assess how rearing conditions affect stress during isolation in different ages and breeds of sheep would be helpful to assess how they affect social isolation stress.

Papadaki, K., Laliotis, G. P., & Bizelis, I. (2021). Acoustic variables of high-pitched vocalizations in dairy sheep breeds. *Applied Animal Behaviour Science*, 241, 105398.

<https://doi.org/10.1016/j.applanim.2021.105398>

#### Summary:

The theory of voice production can be utilized to investigate animal vocal communication. Vocalization characteristics can be influenced by multiple factors, such as, breed, sex, body size and individual variability. Under stressful conditions sheep produce high pitched bleats. The purpose of this study was to determine ewe and lamb vocal characteristics during a short social isolation period and to determine if these characteristics are related to sex, body size, siblings, and the relationship between ewe and lamb vocalizations. They used four dairy sheep breeds (*Ovis aries*), Chios N=21 ewes, n=30 lambs, Karagouniki N=14 ewes, n=15 lambs, Orino epiurus, N=3 ewes, n=6 lambs, and a synthetic breed which was a mix of other breeds (N=11 ewes and n=14 lambs). The first phase separated ewe and lamb and recorded their vocalizations and behaviours for 3 minutes. The second test the ewes were recorded again 6 months later when they were no longer raising lambs. They used a principal component analysis to categorize different influential characteristics. They determined that breed affected all vocalization characteristics while locomotion, number of offspring, behaviour, presence of sibling all influenced multiple but not all vocalization parameters in ewes and lambs. Vocalizations being affected by behavior suggests that there might be an emotional component that affects vocalizations. Sex did not affect any characteristics. There were some specific findings of interest. Sheep with siblings produced higher frequency bleats, ewe bleats changed in the presence of their lambs, and ewes with two lambs had lower frequency bleats. These results show that many individual traits can affect vocalization in sheep during isolation and measuring vocal characteristics may be able to help understand more about sheep during social isolation. Future studies should attempt to elucidate the relationship of emotional state and vocalization characteristics.

#### Conclusion:

This study was included in my review because it assesses the influencing characteristics in vocalizations which is a well documented stress behaviour for sheep when isolated. The most notable results indicate that sheep may communicate differently depending on breed, if they have a sibling, and in the presence of none, one, and two lambs. Understanding what affects these stress responses helps to better understand the influences of these behaviours. These results are consistent with other studies that suggest that there is a lot of individual variability in vocalizations in sheep. Future studies investigating the effects of influencing vocalizations in sheep in other contexts will be useful.

**Reduction of social isolation behaviours:** Studies that show methods during isolation resulting in a reduction in social isolation stress



Guesdon, V., Meurisse, M., Chesneau, D., Picard, S., Lévy, F., & Chaillou, E. (2015). Behavioral and endocrine evaluation of the stressfulness of single-pen housing compared to group-housing and social isolation conditions. *Physiology & Behavior*, 147, 63–70.

<https://doi.org/10.1016/j.physbeh.2015.04.013>

#### Summary:

Social isolation is known to cause immunological, physiological, and neurobiological impacts on sheep (*Ovis aries*). Locomotion, vocalizations, and water consumption are related to social stress in sheep, as well as levels of prolactin, dopamine and adrenaline and noradrenaline. The objective of this study was to compare the stress of freely roaming and socially isolated ewes to single-pen housed ewes and assess stress levels through behavioural, endocrine and neurological indicators. 18 adult Romanov ewes were tested with 12 familiar conspecifics. They were habituated to the testing location, to daily handling and the human observer to reduce confounding variables. They were randomly allocated to three test groups (single pen, freely moving, socially isolated). In the single pen test an ewe was contained in a single pen with visual and auditory access to freely moving conspecifics. They measured stress behaviours, blood plasma levels of prolactin, cortisol, and plasma amines for a test period of 90 minutes. The results showed that the socially isolated ewes had more signs of behavioural stress (increased vocalizations, locomotion, decreased mastication) than the single-pen ewes which had slightly more stress behaviours than the freely moving ewes. Pen ewes had the lowest prolactin while isolated ewes had the highest. Pen ewes also had high levels of dopamine compared to the free and isolated ewes. There was no difference in adrenaline and noradrenaline in any group. Given these results role prolactin and dopamine plays in social isolation stress is unclear. The socially isolated ewes were considered the stress control and the freely moving ewes were considered the stress-free control. The single pen ewes were considered to be not stressed. Overall, social isolation stress behaviours were reduced by utilizing a single pen during physical isolation. Further research should investigate the role prolactin and dopamine play during social isolation.

#### Conclusion:

This article was included in my review as it provides an alternative to social isolation for management practices. Being able to see its conspecifics greatly reduced stress in sheep, so a single pen may be useful for certain management practices. In circumstances where disease transmission is a concern, single pen housing may not be sufficient as it allows olfactory transmission. These results are consistent with other literature which suggest that social isolation is stressful in sheep, but also contrasts some studies as they show that single pen housing is not significantly more stressful than freely moving conditions.

da Costa, A. P., Leigh, A. E., Man, M.-S., & Kendrick, K. M. (2004). Face pictures reduce behavioural, autonomic, endocrine and neural indices of stress and fear in sheep. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1552), 2077–2084. <https://doi.org/10.1098/rspb.2004.2831>

#### Summary:

Sheep (*Ovis aries*) have been shown to have advanced and specialized neural systems that facilitate socialization and social recognition responses. Previously it has been shown that unfamiliar human faces have a negative response in the sheep's brain, whereas familiar humans have a positive response and are recognized in a similar way that sheep recognize other sheep. The objective of this study was to determine if viewing familiar faces of sheep alleviates stress associated with social isolation. To determine this, they measured characteristics previously determined to be positively related to stress – vocalization (high pitched bleats), locomotion (jumping, attempted to escape, rearing), heart rate, adrenal hormone levels and brain mRNA expression in certain locations of the brain associated with facial recognition. They used 40 adult Clun Forest sheep, 19 of which were used for behavioural analysis, 11 for blood hormone measures, and 21 were euthanized to study their brain mRNA expressions. The sheep were isolated for 30 minutes and shown triangles for the first 15 minutes, then some of them were shown sheep faces and some shown goat faces for the next 15 minutes. When shown sheep faces vocalization, locomotion, and heart rate decreased. mRNA expression increased significantly indicating usage of facial and social recognition brain regions. Cortisol increased slightly when shown sheep face images and increased significantly more when shown goat and triangle images. Adrenal hormones only significantly increased when shown triangles. Being shown images of conspecifics reduces physiological indicators of stress as well as stress behaviours themselves, particularly vocalization and locomotion. These results also indicate that it is possible that sheep process emotional information in a similar manner as humans, which should be further investigated. Further studies on longer periods of isolation will be useful to understand if stress can be reduced in that context.

#### Conclusion:

This article is included in my review because it presents an interesting solution to reduce social isolation stress behaviours in sheep. It also provides important implications for the neural regulation of these behaviours and the triggers of these behaviours. The results in this study are consistent with other literature and indicate that social isolation does increase stress behaviours in sheep, and sheep stress is reduced by seeing images of their conspecifics. These results indicate that it may be possible to reduce stress behaviours from social isolation in sheep as a result of husbandry practices by showing sheep images of conspecifics.