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Cognitive Ability in Four Breeds of Domestic Sheep

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Abstract: **Understanding cognition, the mechanisms by which animals acquire, process, store, and act on information from the environment is important because it can provide insight into animal's affective and welfare state. In farm animals it may have links to production parameters of value but for this possibility to be explored cognitive ability have to be first established in the animals. Four breeds of sheep (n=15/breed) were subjected to two operant learning tasks using visual stimuli in an operant system to test their cognitive abilities in two experiments. Data on the response time, response time during right (I-R) and left (I-L) screen image presentations, number first time correct choice and number of corrections required to complete the task were collected and subjected to ANOVA. In experiment I, significant differences (p<0.01) in response time was found between breeds and also during right or left screen image presentations. However in experiment II, there was no significant difference between breeds in response time to complete task. Suffolk ewes made significantly (p<0.05) higher number of accurate responses and required fewer corrections in completing the task than the other breeds. There was also significant differences between sessions in response times, accurate responses, and corrections required to complete the task. The results demonstrate that ewes of the four breeds can master the cognitive task over time albeit at different rates. Tests of cognition in livestock may have application in animal welfare, but more systematic studies are needed to explore possible links with production traits.**

Keywords: **cognitive ability, breed, stimulus, learning task.**

1. INTRODUCTION

It is becoming increasingly important to study animal cognition for a variety of reasons. The most obvious ones are psychology, medicine and lately animal welfare. Human psychiatry and neurology research is reliant animal models to study a plethora of psychiatric and neurological disorders. Common animals used in medical research are rodents [1], [5], [24], [13] and non-human primates [22]. However rats and mice do not make ideal experimental subjects to study brain disorders in humans firstly because they are short-lived but also because they have dissimilar brain architecture with humans [14]. While monkeys do similar brains to humans, their management is not as routine as that of farm animals and their size will make them too cumbersome to manage under laboratory conditions if it were to come down with the clinical symptoms of neurological disease such as Huntington's or Alzheimer's. There are also ethical issues that might be raised in dealing with non-human primates. People's attitude to animals are affected by their evaluations of the animal's abilities [3]. If people perceive animals stupid and unaware, they are more likely to treat such animals as objects rather than as individuals with possible consequences for physical and mental aspects of needs, or feelings such as pain, fear, pleasure and environmental stress. As non-verbal beings, it is pertinent to device other means of gaining insight in to how animals feel in the different farm conditions for us to be able to improve their welfare [12]. Recently, animal cognition is found to be useful as an indirect method of assessing their welfare [12]. Studies [2] suggest that animal emotion and cognition have a bi-directional relationship implying that emotive states of animals can be deciphered by measuring their cognitive states. Sheep have credentials that make them suitable for studying cognitive functions. They are long-lived, unlike rodent and have large brains with similar morphology to that of humans, a human like basal ganglia and a well-developed and convoluted cerebral cortices [7], an impressive ability to remember the faces of others for up to two years [8], suggesting potentials for learning and memory. Despite this remarkable similarity to the human brain, studies to characterize cognitive functions in the sheep are lacking and would be essential if their emotions and welfare state are to be understood. Studying cognition in sheep may possibly allow for indirect selection of production traits to improve livestock if cognitive ability have correlation with a particular trait. Links between productions and behavioral traits have been demonstrated in previous studies. Findings by Kilgour & Szantar-Coddington [9] suggest that fear, a behavioral trait,

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is correlated to maternal ability in sheep, [11]. The authors compared merino sheep from a line selected for lamb rearing ability and a random flock at 6, 12 and 20 months of age and found the total distance travelled differed significantly between the fertility flock and the random flock. Subsequent studies on this phenomenon by Kigour and Szantar-Coddington, [9], and [10] also reported similar findings suggesting that arena behavior, specifically fear can be a candidate for indirect genetic selection for lamb rearing ability at least in the merino sheep. It is possibly that similar links between production and behavioral can be discovered. For any trait to be a valuable selection tool it should be easily measurable at a young age and measurable in both rams and ewes and heritable. Discrimination learning in operant conditioning which test cognitive functions can be designed to meet these requirements. Cognitive functions, like other biological parameters is variable and their determinants heritable [21]. Cognitive ability has also been reported to differ in closely related wild and domestic species [4], [16] and may therefore be genetically related or inherited. However, cognitive function is seldom investigated in farm animals. Thus there is the need to study, characterize and quantify this ability as they may serve as useful tool for indirect selection in both sexes [3]

Before we develop husbandry protocols that can improve animal welfare and also to explore possible links between cognitive ability and production trait(s), there is need to first demonstrate cognitive ability in various breeds of the species. This study therefore is aimed at testing the cognitive ability in ewes of four breeds of sheep using a set of operant learning tasks for that purpose. If the test is appropriate for the species we expect all the ewes of the four breeds to learn the task and vice versa.

2. MATERIALS AND METHODS

Experimental animals

The experiments was conducted with 60 ewes from the same farm, 15 each from the Beulah Speckle-faced (Beulah), Bluefaced Leicester (Bluefaced), Suffolk and Texel breeds. The ewes had no prior cognitive testing experience and had no prior training or handling apart from routine farm husbandry practices. The sheep were housed in four large straw-bedded pens according to breed in the same indoor facility and offered ad libitum access to water and hay plus once a day supplementary concentrate feed. On experiment days, concentrate feed was offered only after the experiments to ensure sufficient appetite [20] during testing. Studies were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986. No licensed procedures were carried out in the course of these experiments.

Testing facility

A purpose built semi- automated mobile operant system designed by University of Cambridge (see figures 1, 2, and 3 below) was used for the experiment. The system is designed to be ethologically relevant for medium sided quadrupeds that have strong locomotor characteristics. Stimulus presentation, reward (5 grams commercial pellet) and data entry are controlled by a MATLAB computer program coded for this study.

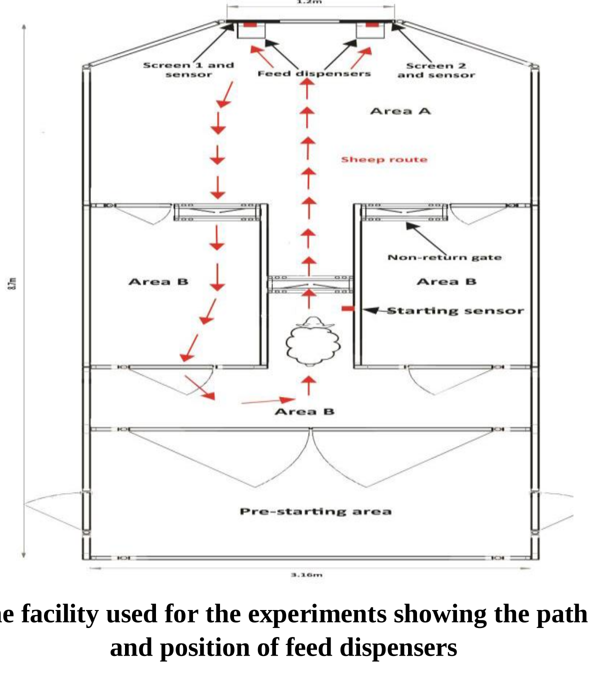

Fig. 1: Schematic diagram of the facility used for the experiments showing the path to be followed by test subject and position of feed dispensers

Operant task

The operant task involves a series of test similar to the simple reaction time (SRT) test which measure simple reaction time through the delivery of a known stimulus in one of two possible locations to elicit a known response and choice reaction time (CRT) which is a two-choice reaction time test where there is a stimulus and two possible responses. It is useful for testing associative learning, visual memory, general alertness, memory and motor speed, response accuracy. Details of the experiments are described as follows

Pre-experiment habituation to the testing facility

The sheep were first subjected to a pre-testing phase where animals are held and fed in the operant system (figure 1) as a whole group for 15 minutes once and then in groups of 6 and then 3 both twice for 15 minutes before the commencement of the experiment with the objective of familiarizing them with the facility layout, location of feed dispensers, and visual and audio stimulus [6].

With the sheep held in area A for 6 minutes, one randomly selected image from a library of ten is selected on each screen with the simultaneous presentation of an audible medium-pitched tone and the delivery of food from two dispensers located directly below the screens every ten seconds. No operant response is required from the subject to elicit food delivery and no data is collected. The subjects go through this habituation phase in pairs and then individually for two sessions each lasting 6 minutes.

Experimental design

Experiment 1: The aim of the first experiment is to determine the rate of operant learning in the ewes of the four breeds by measuring their speed of decision making. A random image from a library of 10 wingding images is presented on either of two screens as stimulus with simultaneous presentation of an audible medium-pitched tone. Each of the experimental subjects is required to respond to the stimulus by moving to the screen with the image to activate a feed dispensing mechanism and receive a food reward. The aforesaid sequence (called a trial) is iterated 10 times at an inter-trial interval time of 10 seconds counting from the delivery of reward while the sheep held in area A (see figure 1). In every trial, the response time in seconds (T) i.e. time interval between presentation of image and appropriate response from the subject, the side of image presentation, - image on left (I-L) or image on right (I-R) screen - as well as subjects tag no are automatically recorded by a MATLAB computer program.

Fig. 2: Photograph illustrating layout of the mobile operant system used for the operant task

Fig. 3: Photograph of a sheep using the operant system.

Experiment II: This experiment is aimed at measuring attentiveness and short term memory. The stimulus presentation and activation of reward is same as in experiment 1 however, the subject is required to move out of area A into B and return back to area A (see red arrows in figure 1) after responding to the stimulus regardless of whether subject's response is correct or wrong. In the event subject makes a wrong response by going to the screen without image during a trial, the image on the other screen is removed, a high-pitched tone is presented and the subject is required to takes a correction trial which is a repetition of the sequence of procedure described above. There is no time limit within which the test subject is required to make a response but subject is required to make correct responses in ten consecutive trials to end the experiment. Experiment II was conducted four times on nonconsecutive days. The response time in seconds (T) i.e. time interval between presentation of image and appropriate response from the subject, the number of correct responses (accuracy) the number of correction trials (errors) as well as the side of image presentation (Left I-P or Right I-P) for successful trials in each session are automatically recorded by a MATLAB computer program.

Data transformations & Statistical analysis

Datasets for the number of accuracies and errors failed the Shapiro-Wilk and Kolmogorov-Smirnov tests of normality and was subjected to inverse transformation prior to analysis. All the data was analyzed by one-way analysis of variance using SPSS software version 22 from IBM Corporation. Where the ANOVA reveal significant differences between breeds, Fisher's LSD was used to pinpoint which means differ significantly from the other.

3. RESULTS

Experiment 1

One-way ANOVA results for experiment 1 that measures response times presented in table 1 show a significant difference between breeds in total response time to make a choice following stimulus presentation F (3, 52) = 6.14, p=0.001 with the Beulah speckle-faced taking significantly longer time to make a choice than the three other breeds whose response times did not significantly differ from one another. This trend is repeated when image was presented on the left screen F (3, 52) = 4.66, p = 0.006. The Beulah and Bluefaced Leicester breeds are significantly slower ($p < 0.05$) than the Suffolk & Texel breeds.

TABLE 1: Mean (±sem) latencies to make a choice in cognitive test during acquisition of trial and error behavior

	Beulah	Bluefaced	Suffolk	Texel	
	n=13	*n=14*	*n=14*	*n=15*	*P* value
Response time	1.29 ± 0.15^{a}	0.93 ± 0.08^{b}	0.83 ± 0.08^{b}	0.73 ± 0.06^{b}	0.001
Right I-P	1.27 ± 0.13^{a}	1.03 ± 0.10^{a}	0.86 ± 0.11^{b}	0.75 ± 0.07^{b}	0.004
Left I-P	1.18 ± 0.19^{a}	0.73 ± 0.08^{b}	0.73 ± 0.06^{b}	0.67 ± 0.06^{b}	0.006

Data was log-transformed to ensure normality

Values with different superscripts within a row are significantly different ($p<0.05$)

Experiment II

Response time

The mixed ANOVA comparing breeds (fig. 4) and sessions (fig. 5) in speed of response during the operant task show a significant effect of session of when the test was conducted $F(2.639, 137.25) = 2.82$, $p = .048$, partial eta squared = .051 but not breed $F(3, 52) = .67$, $p = .57$, $\eta p2 = .38$. However, ewes of Blue-faced Leicester breed are much faster in responding during the operant task than ewes of the other three breeds while ewes of the Beulah breed are slower than those of the Texel & Suffolk breeds. The interaction between breed and session was also not significant, $F(7.92, 137.25) = 1.374$, $p = .214$, $\eta p2 = .073$.

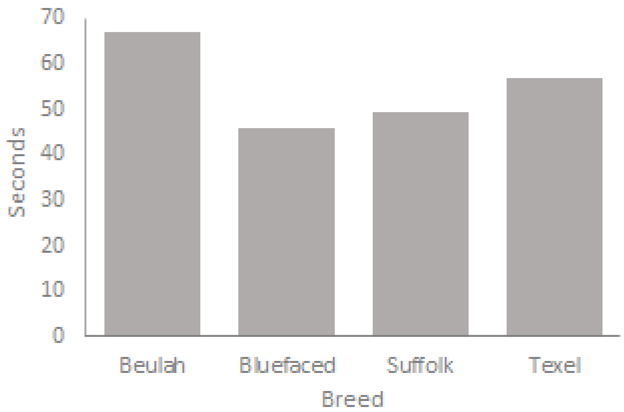

Figure 4: Average time taken to complete operant task between breeds

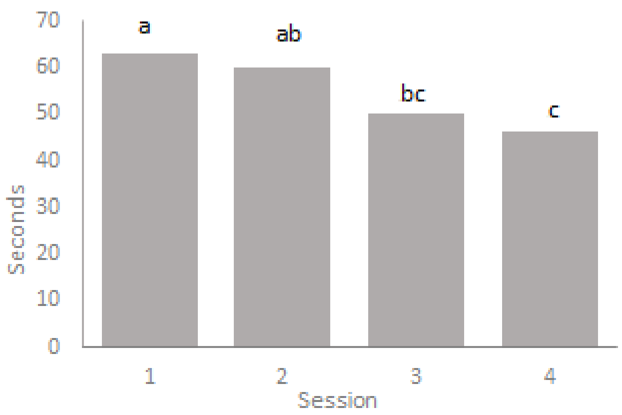

Figure 5: Average response time for all breeds in completing operant task over four sessions in experiment II. Means with different letters are significantly different (p<0.05)

Accuracy

There was a significant effect of both session when test was conducted and breed of ewe in accuracy when the number of correct operant response is compared between the four breeds (table 2). But there was no interaction between session of test and breeds. The Ewes of Suffolks made significantly accurate choices $F(3, 52) = 9.87$, $P < 0.05$, $\eta p2 = .363$ compared to the other three breeds. Expectedly, the number of first time accurate responses between the sessions is significantly higher in the fourth session $F(3, 156) = 5.217$, $P < .05$, $\eta p2 = .091$ than in the three previous sessions. The accuracies in the three previous sessions are not significantly different from one another.

TABLE 2: Mixed ANOVA comparison of accuracy in executing operant task between breeds over four sessions

	Session				
	First	Second	Third	Fourth	Mean
Beulah	5.9±0.55[a]	5.7±0.54[a]	6.6±0.70[abc]	6.7±0.66	6.27±0.33[a]
Bluefaced	5.1±0.32[a]	5.4±0.34[a]	5.4±0.54 [bc]	6.6±0.58	5.60±0.33[a]
Suffolk	7.0±0.65[b]	8.3±0.54[b]	8.6±0.51 [ac]	8.1±0.59	8.00±0.33[b]
Texel	5.4±0.37[ab]	5.9±0.49[a]	6.8±0.56 [c]	7.1±0.53	6.32±0.33[a]
Mean	5.8±0.25[A]	6.4±0.24[A]	6.8±0.29[A]	7.1±0.30[B]	
SEM			±0.16		

[a, b] and [A], [B] used when comparing means between breeds ad between sessions respectively.

Number of corrections

Figure 6. below show a significant difference between breeds F(3, 53) = 5.118, p=0.003 in the number of corrections trials required to complete a session of ten tests with Bluefaced Leicester ewes requiring more correction test while Suffolk ewes required fewer corrections to complete a session. Although the mean error rates of the Beulah and Texel breeds are not significantly different, they make significantly fewer errors than the Bluefaced Leicester. But the mean error rate of the latter two breeds did not differ significantly between 10 successful trials. There was a significant difference in the number of corrections between sessions (fig. 7.), there was no statistically significant difference between any two consecutive sessions. However, there is a statistically significant interaction between breed and session, $F(9, 156) = 2.098$, $p < .05$), partial $\eta 2 = .108$. The main effects of session and breed were also statistically significant $F(3, 156) = 7.516$, $p < 0.05$, partial $\eta 2 = .126$ and $F(3, 52) = 312.5$, $p < .05$, partial $\eta 2 = .339$ respectively.

Fig. 6: Mean number of corrections made by ewes of the four breeds

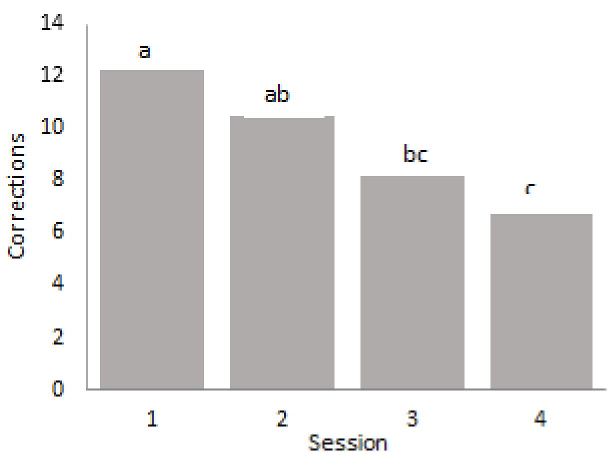

Figure 7: Mean number of corrections sponses (errors) made by all ewes over four session of experiment II

4. DISCUSSION

We investigated the potential of using sheep for systematic cognitive studies. We show that not only can normal healthy sheep perform cognitively demanding discrimination learning tasks but that the rate at which they learn vary between the Beulah, Bluefaced Leicester, Suffolk and Texel breeds of sheep. While ewes of all four breeds used in this study were raised and reared under similar conditions of husbandry prior to the experiment, ewes of the Beulah breeds tending to display a more reactive and flighty disposition typical of wild animal than the rest. Unsurprisingly, performance of the Beulah's differ from the other ewes in experiment I. But contrary to expectations however they took longer time to respond to the operant task. This finding is somewhat surprising is at variance with Price [26] suggesting that wild individuals may show greater attention towards the environment unlike their domestic congeners.The Beulah originated from and is predominantly a hill or upland breeds adapted to extensive system of management. Its natural home range certainly places selection pressure for cognitive skills like learning where to find food and memorizing locations. It is therefore reasonable to expect this uplands home environment to confer the Beulah a superior level of performance than ewes of the other three breed that are of more benign lowland origin. Furthermore, it has been suggested previously that dietary complexity is a major driver of cognitive evolution and ability in non-primate species [29]. Hill breeds of sheep have access to heterogeneous grassland communities with more than 22 species of forage that they actively graze [28]. Despite all this, the Beulah, performed the task at a much slower rate. But studies by Brust & Guenther [4] also found superior performance by guinea pigs over wild cavies in an association learning task, however both lineages performed equally well in reversing the learnt association. The visual discrimination reversal task is a cognitively challenging behavioural tests in which the animals first learnt to associate a symbol and a reward and, once this association is reliably formed, animal were rewarded for adjusting their learnt choice flexibly to one of the previously non-rewarded symbols that then served as the new rewarded cue. It is un-like the relatively strait forward visual discrimination task or SRT/CRT test employed in this study. Perhaps it is this complexity of the reversal task that increases its sensitivity to accentuate differences in cognitive abilities allowing only individuals that exert greater mental effort to succeed. McBride & Morton [25] show that sheep ranked just below humans and gorillas in a two choice discrimination reversal index comparing eleven species which also includes dogs (fifth), pigs (ninth) and mice (eleventh). Meaning that they are very much capable of learning visual discriminations and their reversals than many species.

In experiment II, the significant difference between breeds in mean response time to complete the operant task earlier observed in experiment I disappeared when it was tested again showing no significant difference between breeds at this stage. Although there was no significant difference in the response time the Beulah and Texel took longer to complete the task. The experiment also reveal the effect of session when task was conducted with the response time decreasing as the sessions increase Figure 5. This trend was also seen in the increasing number accurate responses, Table 2, and decreasing number of correction trials Figure 7. The data for number of correction trials (Fig. 6) show the Beulahs and Suffolks made significantly fewer errors than the Bluefaced Leicesters & Texels. But this observation is conformity with findings from a similar study [25] which reported no significant differences between breeds in the number of trials to learn a visual discrimination task but breed differences manifested during the reversal phase with the Bluefaced Leicester ewes requiring significantly more trials than the other three breeds to reach a learning criterion. In other studies domestic guinea pigs are reported outperform wild cavies in an associative learning task [4] and also on a spatial learning task [23]. These workers attributed the superior performance of the guinea pigs to their long periods of domestication a process which confer the ability to cope with man-made environments and handling procedures. Using that logic one, should therefore expect the Beulah ewes to being an upland breed to be outperformed by the other three breeds. However, only the Suffolk required fewer (about 5) corrections. But the Bluefaced Leicester required over 12 corrections to complete the task. While the number of corrections for Texel was at par with that of Beulah breeds. Possibly, the upland, extensive system often hostile where the breed originates from may have enabled them to evolve a greater sense of attentiveness than the lowland breed.

Because the recap of the experiment over four sessions are separated by at least 48 hours intervals, it is likely that maturation effect is at play hence performance of the test subjects gradually improves over time irrespective of treatment. The data for the repeated measure analysis of variance was collected four times only. Some studies of visual learning tasks [8] suggest that sheep could also discriminate 25 face pairs for up to 600 days. It would have been interesting to see for how long the associations learnt are retained by the ewes. Lastly, ewes of the Suffolk breed performed better than the other 3 breeds in both number of accurate responses & number of corrections with the Bluefaced Leicester lagging behind in both parameters. Recent studies of cognitive capabilities in mammals [27] suggests that absolute numbers of neurons and glial cells in the cerebral cortex are good correlates of cognitive function: the more the neurons, regardless of brain or body size, the better the performance at a task. But without accompanying data, the best proxies are morphometric. The size of the head do give some indication of absolute brain size. In a post-mortem study of rams of the Texel and Bluefaced Leicester breeds, Waine and colleagues [28] found head length to be 240cm and 238cm for the two breeds respectively. This difference although small may have played a role in the less than impressive performance of the Bluefaced Leicester ewe

5. CONCLUSION

The current study attempts to measure cognitive ability in four breeds of domestic sheep. Although all the breeds were able to eventually learn to work with the operant system, the rate with which they did so vary depending on breed. The study also demonstrate that learning and memory in operant tasks increase with number of trials. Now that sheep cognitive ability can be tested, one side of the equation has been taken care of; what the livestock sector may needs next are more systematic studies that can be validated so as to predict more accurately sheep cognitive capabilities and also to explore possible links between cognitive ability and production traits of in the domestic sheep. Such a link is not impossible. Indeed, investigations successfully demonstrating links between behavior in an arena test & lamb rearing ability [9], [10], [11] have been documented previously.

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