South African Journal of Animal Science 2023, 53 (No. 2)

Behavioural responses of alpacas *(Vicugna pacos)* to novel objects

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(Submitted 27 May 2021; Accepted 30 May 2022; Published 15 May 2023)

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Abstract

In recent years, keeping alpacas has becoming increasingly popular. However, little research has been conducted on their behaviour. The study aimed to assess behavioural responses of alpacas towards novel objects as a step toward a practical test including novelty and suddenness. Thirty-two alpacas of different age, sex, and coat colour were each exposed to the novel object where four single objects (a cardboard box, a ball, a suddenly-opened umbrella, and a moving toy car) were presented. Behaviour was rated by use of a five-point score. The latency (time between the appearance of the object to the animal's sniffing and/or touching the object) and the frequency of contact with the novel object were recorded. The moving toy car caused the strongest fear reaction in older females. The box was the most explored object by animals of all ages and sex. The lowest latency values and the most frequent contact with the novel object were found in white alpacas exposed to the box. Spearman's correlations indicated a negative relationship between behavioural scores and latency values and a positive correlation between behavioural scores and frequency of contact with the novel object. Alpaca behaviour score, latency, and frequency of contact differed among the novel objects evaluated. A combination of novelty and suddenness can intensify the alpaca's reaction. A relationship between coat colour and behavioural reactivity may occur in alpacas and if confirmed by genetic analysis, could be used for genetic selection. A novel object test can be used to measure fearfulness/curiosity in alpacas and evaluate behavioural predispositions for activities, including alpaca-assisted therapy.

Keywords: alpaca, behaviour, novel stimulus, reactivity
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Introduction

In recent years, an increasing trend of keeping alpacas has been observed (D'Alterio *et al.,* 2006; Stanitznig *et al.*, 2016; Neubert *et al.,* 2021). The alpaca was domesticated 6000–7000 years ago in the Andes Mountains (Altiplano plateau) (Wheeler, 1995) and is phylogenetically related to llama *(Lama pacos),* guanaco *(Lama guanicoe),* and vicuna *(Vicugna vicugna)* (collectively named South American Camelids, SACs). There is a variety of reasons why smallholders and farmers choose to keep these animals. The main reason is high quality fibre production but also alpacas are more frequently kept as therapy animals or a hobby animal in agritourism farms. However, little research has been done on the behaviour of these animals.

Highly reactive individuals may be difficult to handle due to their intense response to an object, as observed in horses (Geringer & Kasprzak, 2000). In SACs, intense motor activity (e.g., attempts to escape), high-pitched screeching sounds, and spitting of large amounts of saliva and rumen contents indicate high reactivity, whereas limited motor activity, none or silent humming sounds, and absence of spitting indicate animals with low reactivity (Arzamendia *et al*., 2010; Taraborelli *et al*., 2011; Waiblinger *et al*., 2020; Kapustka & Budzyńska, 2021). Animals with low reactivity are desirable because they are

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v53i2.07

easier to handle which eases the workload and enhances the safety of human–animal interactions. Gentle, positive contact with young alpacas (before 6 months of age) results in a calmer reaction during handling (e.g., shearing) (Windschnurer *et al*., 2020).

Fear in animals can easily be evoked through separation from the rest of the herd, placement in an unfamiliar environment, or exposure to a novel stimulus. Understanding how animals will respond to these paradigms will help stock-people anticipate animal behaviour in various situations, thus enhancing animal husbandry (Grandin, 1997; Hirata *et al*., 2016; Budzyńska *et al*., 2019). Currently, research on animal behaviour is based on many tests, including assessment of the level of an animal's fear response (Hemsworth *et al*., 1996; Forkman *et al*., 2007; Budzyńska *et al*., 2019). The novel object test consists of placing an animal in a familiar room while exposed to a novel, unknown object (Hemsworth *et al*., 1996; Forkman *et al*., 2007). Understanding the individual alpaca's behavioural reactivity to novel items in their environment may inform animals' managers as to the context in which the animal is best suited (e.g., agrotourism, petting zoo). While multiple behavioural tests have successfully assessed the temperament of animals (Budzyńska *et al*., 2014; Kamieniak *et al*., 2016; Budzyńska *et al*., 2018; Budzyńska *et al*., 2019), as well as llama and alpaca reactivity towards familiar and unfamiliar humans (Taylor & Davis, 1996, Windschnurer *et al*., 2020), no study known to the authors has investigated alpacas' responses to novel objects. The study aimed to assess behavioural responses of alpacas towards different novel objects as an initial step in developing a practical test that includes exposure to a combination of novelty and suddenness.

Materials and methods

The study was conducted from March to April, 2021. The observations involved 32 alpacas (18 females and 14 males) kept on an agritourism farm in Lublin Province, Poland). The animals were assigned into five age and sex groups: group 1 - the cria group consisting of <1-year-old individuals (n = 8, 6 females and 2 males), group 2 – young (3 years old) females (n = 6), group 3 - young (2–3 years old) males (n = 6), group 4 - older (aged 11 years) females (n = 6), and group 5 - older (aged 4–6 years) males (n = 6). Moreover, all studied alpacas were assigned into three colour groups: white (n = 12), brown (n = 12), and fawn (n = 8). Distribution of animals across age and sex, as well as coat colour is presented in Table 1. All the animals were born on the farm, and they were kept under the same housing and feeding conditions. The alpacas had regular, gentle tactile, visual, and auditory contact with humans at an early age in order to habituate the animals to husbandry procedures.

Table 1 Number of animals with regard to age and sex groups and coat colour

Number of animals	<1 year old individuals	Young females	Young males	Older females	Older males	Sum
White	2	4	2	2	2	12
Brown	4	0	4	2	2	12
Fawn	2	2	0	2	2	8
Sum	8	6	6	6	6	32

In this study, non-invasive methods were used and they were approved by the Ethics Committee (Local Ethical Committee of the University of Life Sciences in Lublin, No. 78/2020). The national law on care and use of animals was also followed.

The observations of the alpacas' reactions to the novel objects were carried out in a solid-sided, test room (approximately 40 m^2) that was familiar to the animals (alpacas were exposed several times to this room without novel stimuli but with *ad libitum* access to hay and water). The ground in the room was covered with a thin layer of straw. On the one side of the room was a latrine (the place where alpacas defecate and urinate). Hay and water were available *ad libitum* in the room during testing. Separation from the herd may cause greater stress than the stimulus used in the test (Forkman *et al*., 2007) and may thus affect the results considerably, thus the alpacas were exposed to one of the novel objects in pairs, and the behaviour of one of the animals was observed, while the other served as a companion to avoid social isolation stress. Companion, non-experimental animals were at the same age and of the same sex as the observed alpacas.

Alpacas were each exposed to the novel object test where four single objects were presented. Objects of different colour, shape, and level of mobility were chosen. The objects were: 1. 70 × 50 × 60 cm grey cardboard box; 2. white and black inflatable ball (55 cm in diameter) put on the floor but free to be moved by the animals; 3. navy blue and green umbrella (100 cm in diameter), suddenly opened and

put on the floor, and 4. 25×15×10 cm white, remote-controlled, moving toy car. The objects were presented once to the test animals according to their increasing level of mobility in the following order: the box, the ball, the umbrella, and the toy car. Each object was shown separately after the animals were brought into the room. The umbrella was opened at a distance of approximately 1 m in front of the animals. All objects (box, ball, umbrella after opening, toy car) were placed on the floor approximately 1 m from the animals. The toy car was moved forward and backward at different speeds and then stopped for a moment in the middle of the room. For this experiment, the room where the alpacas were tested was big enough for animals to have an opportunity to ignore or go backward when they were exposed to the novel object. The reactions of the animals were written down by a researcher (familiar to the animals) and recorded with a camera (Sony α68). The experimenter stood out of sight except when introducing an object and changing it to another one during a 1–2 min pause between the objects. The observations of the alpacas' reactions to each of the objects lasted 10 min. The recording was stopped, and the object was removed and replaced by another. Four alpacas were tested per day and each alpaca spent approximately 45 min in the test room. Alpacas were led into the test room by the same researcher that was presenting the objects and observing the animals' reactions.

The animals were observed and scored by a single female researcher. Alpaca behaviour was rated by using a point score (from 1 to 5, according to decreasing fear level and increasing curiosity of test animals; Table 2). During the observation, the latency (time between the appearance of the object and the animal's approach and sniffing and/or touching the object) and the frequency of contact with the novel object (exploring it) were measured. The exploration consisted of sniffing at a short distance (minimum 20 cm) and/or touching the object with the snout. The latency time was measured in seconds. When the animal did not explore the object during the test, the latency was scored as 600 s (60 s * 10 min of presenting the object).

Table 2 Behavioural scoring scale of alpaca reactions to a novel object

Points	Description of animal behaviour
1	The alpaca jumps and runs away from the object. The ears are held flat, tail is raised, head is raised high. The alpaca shows significant motor activity, it avoids the object or observes it carefully at a distance. The animal doesn't come closer to the object during the 10 min of observations.
2	The alpaca walks away from the object. The tail and head are slightly raised, ears are directed at the object. After a few minutes the animal comes closer to the object but does not display direct exploration by sniffing it or touching it by its snout.
3	The alpaca is not interested in the object or looks at the object only immediately after its appearance, and it does not approach to sniff or touch the object within 10 min. The animal performs other activities (e.g., ruminating, lying, standing).
4	The alpaca shudders slightly when the object appears. The ears are directed at the object, tail is lowered loosely. The alpaca stands for a few minutes from a distance. After a few minutes, the alpaca comes up to the object and sniffs it or touches it with its snout.
5	The alpaca does not walk away or shudder when the object appears but stands and observes the object from a short distance. The ears are directed at the object and the tail is lowered loosely. It approaches the object very quickly and sniffs it or touches it with its snout.

The results of the novel object testing (behaviour scored separately for each object, latency value, and frequency of contact with object) in alpacas were statistically analysed using Statistica software (version 13.1). Parameters are given in the tables that follow: median (Med), and the lower and upper quartiles (Q1 and Q3). A multivariate analysis of variance (MANOVA) was conducted to identify differences between behavioural scores obtained within the age and sex groups and the coat colour groups with reference to the type of object used in the test. A similar analysis of significance was performed for the latency values and the frequency of contact with the novel object. Tukey's HSD test was used to identify differences among the behavioural scores, the latency values, and the frequency of contact with the novel object between the groups of animals, depending on the age and sex, the coat colour, and the type of the object (box, ball, umbrella, toy car). Spearman's rank correlation determined the relationships between the behavioural scores, the latency values, and the frequency of contact with the novel object. A *P*-value lower than 0.05 was referred to as significant.

Results

The statistics (Med and Q1–Q3) and P-values (MANOVA) describing measured parameters in the alpacas' responses to the novel objects within the age and sex and coat colour groups are shown in

Tables 3 and 4 (behavioural scores/points), Tables 5 and 6 (latency values, seconds), and Tables 7 and 8 (frequency (number/10 min) of contact with the novel object).

Some differences (MANOVA) in the behavioural scores for the response to the novel object within the age and sex groups (Table 3) and within coat colour groups (Table 4) were found. The lowest behavioural score (the most intensive fear response) was obtained in the group of older females in reaction to the toy car (Table 3). This result differed from the behavioural score to the cardboard box in the same group of alpacas (P = 0.022). It was found that the cardboard box was the least stressful stimulus in animals of all age and sex groups (Table 3). There were also some differences (Tukey's HSD) in behavioural score during the testing novel objects between the coat colour groups. There were differences in behavioural scores in response to the toy car obtained by older females and scores to the box in the cria (P = 0.01) and young males (P = 0.02). There were differences between the behavioural scores achieved by the brown animals in the toy car testing and scores of the same group (P <0.01), white animals (P <0.01), and fawn alpacas (P <0.01) in response to the box. The response to the toy car in the white and brown alpacas also differed (P <0.05). The response of the white animals to the umbrella was different from the reaction to the box in the same group (P <0.05), fawn animals (P <0.01), and brown alpacas (P <0.05). The behavioural scores obtained by the fawn alpacas exposed to contact with the toy car differed from the scores obtained in response to the box in the same group (P <0.01), in white alpacas (P <0.01), and in brown animals (P <0.01).

Table 3 Behavioural scores (points) in the age and sex groups in relation to the type of the object with the lower quartile (Q1), median (Med), and upper quartile (Q3)

	Objects	**Box**	**Ball**	**Umbrella**	**Toy car**	***P*-value**
<1 year old individuals (n = 8)	Med	5.0	3.0	3.5	2.5	>0.05
	(Q1–Q3)	(5.0–5.0)	(3.0–3.5)	(3.0–4.0)	(1.0–4.0)	
Young Males (n = 6)	Med	5.0	5.0	2.0	2.0	>0.05
	(Q1–Q3)	(5.0–5.0)	(3.5–5.0)	(2.0–2.5)	(1.5–3.0)	
Older Males (n = 6)	Med	5.0	5.0	3.0	2.0	>0.05
	(Q1–Q3)	(4.0–5.0)	(3.0–5.0)	(2.5–3.0)	(1.5–3.5)	
Young Females (n = 6)	Med	4.0	2.0	2.0	4.0	>0.05
	(Q1–Q3)	(4.0–4.5)	(2.0–3.0)	(1.5–2.5)	(3.5–4.0)	
Older Females (n = 6)	Med	5.0	4.0	3.0	1.0	0.022
	(Q1–Q3)	(5.0–5.0)	(3.5–4.5)	(2.0–3.0)	(1.0–1.0)	

Table 4 Behavioural scores (points) in the coat colour groups in relation to the type of the object with the lower quartile (Q1), median (Med), and upper quartile (Q3)

	Objects	**Box**	**Ball**	**Umbrella**	**Toy car**	***P*-value**
White (n = 12)	Med	5.0	3.5	2.5	4.0	0.012
	(Q1–Q3)	(4.3–5.0)	(2.3–4.8)	(1.3–3.0)	(4.0–4.8)	
Brown (n = 12)	Med	5.0	4.0	2.5	1.0	0.001
	(Q1–Q3)	(5.0–5.0)	(2.3–5.0)	(2.0–3.0)	(1.0–1.8)	
Fawn (n = 8)	Med	5.0	3.5	3.0	1.0	0.002
	(Q1–Q3)	(5.0–5.0)	(3.0–4.3)	(3.0–3.3)	(1.0–1.5)	

There were no differences (MANOVA) in the latency values within the age and sex groups (Table 5), however differences (MANOVA) between the latency values in reaction to novel objects within the coat colour groups were found (Table 6). There were also some differences (Tukey's HSD) in the latency time during testing novel objects between the coat colour groups. White and fawn animals exposed to the box had the shortest latency to first touch (exploration of the object). The longest latency values were found in white animals exposed to the umbrella and fawn alpacas exposed to the toy car. The white animals' latency value during exposure to the box differed from the latency values noted during the exposure to the umbrella in the same group (P <0.01) and in the fawn alpacas (P <0.01). The latency values to the toy car in the brown (P <0.01) and fawn alpacas (P <0.01) were longer than

in reaction to the box in the white animals. Latency value during response to the toy car in the fawn animals differed from the latency values noted during the box test in the same group (P <0.01). The latency value to the umbrella in the white animals was different from latency value during exposure to the box in fawn animals (P <0.01). It seems that the fawn animals had the longest latency time in reaction to most of the objects (Table 6).

Table 5 Latency values (seconds) in the age and sex groups in relation to the type of the object with the lower quartile (Q1), median (Med), and upper quartile (Q3)

	Objects	Box	Ball	Umbrella	Toy car	*P*-value
<1 year old individuals (n = 8)	Med	31	600	423	533	>0.05
	(Q1–Q3)	(23–80)	(470–600)	(198–600)	(457–600)	
Young Males (n = 6)	Med	133	220	348	600	>0.05
	(Q1–Q3)	(90–188)	(121–410)	(299–474)	(329–600)	
Older Males (n = 6)	Med	6	272	600	489	>0.05
	(Q1–Q3)	(5–303)	(140–436)	(517–600)	(277–545)	
Young Females (n = 6)	Med	80	330	600	488	>0.05
	(Q1–Q3)	(58–146)	(324–378)	(60–600)	(342–544)	
Older Females (n = 6)	Med	83	155	600	600	>0.05
	(Q1–Q3)	(68–115)	(150–378)	(600–600)	(600–600)	

Table 6 Latency values (seconds) in the coat colour groups in relation to the type of the object with the lower quartile (Q1), median (Med), and upper quartile (Q3)

	Objects	Box	Ball	Umbrella	Toy car	*P*-value
White (n = 12)	Med	58	243	600	315	0.0002
	(Q1–Q3)	(33–82)	(55–402)	(600–600)	(98–475)	
Brown (n = 12)	Med	186	410	391	600	>0.05
	(Q1–Q3)	(71–238)	(164–600)	(274–559)	(517–600)	
Fawn (n = 8)	Med	41	459	600	600	0.004
	(Q1–Q3)	(23–93)	(307–600)	(512–600)	(600–600)	

There were no differences (MANOVA) in the frequency of contact with the novel object within the age and sex groups (Table 7). However, differences (MANOVA) between the frequencies of contact in reaction to novel objects within the coat colour groups were observed (Table 8). There were also some differences (Tukey's HSD) between the frequencies of contact in reaction to novel objects between the coat colour groups. The most frequent contact with the novel object was shown by the white alpacas exposed to the box and this result differed from the frequency of contact with the umbrella in the same group (P <0.01) and fawn alpacas (P <0.05). The frequency of contact with the toy car was different in the brown alpacas (P <0.05) and fawn animals (P <0.05), compared to the white alpacas exposed to the box.

The analysis of Spearman's rank correlation showed negative relationships between the latency values and behavioural scores (r = -0.82, P <0.01) as well as frequency of contact with the novel object (r = -0.85, P <0.01). Positive correlation between behavioural scores and frequency of contact with the novel object (r = +0.66, P <0.01) was found. There were no correlations between the test objects for any behavioural variables.

Table 7 Frequency (number/10 min) of contact with the novel object in the age and sex groups in relation to the type of the object with the lower quartile (Q1), median (Med), and upper quartile (Q3)

	Objects	Box	Ball	Umbrella	Toy car	P-value
<1 year old individuals (n = 8)	Med	2.0	0.0	0.5	0.5	>0.05
	(Q1–Q3)	(2.0–2.3)	(0.0–0.8)	(0.0–2.0)	(0.0–1.3)	
Young Males (n = 6)	Med	1.0	1.0	0.0	0.0	>0.05
	(Q1–Q3)	(1.0–1.0)	(1.0–1.5)	(0.0–0.5)	(0.0–1.0)	
Older Males (n = 6)	Med	2.0	1.0	0.0	1.0	>0.05
	(Q1–Q3)	(1.0–2.5)	(0.5–1.5)	(0.0–0.5)	(0.5–1.0)	
Young Females (n = 6)	Med	3.0	2.0	0.0	1.0	>0.05
	(Q1–Q3)	(2.0–3.5)	(2.0–2.0)	(0.0–0.5)	(0.5–1.5)	
Older Females (n = 6)	Med	1.0	1.0	0.0	0.0	>0.05
	(Q1–Q3)	(1.0–1.5)	(0.5–1.5)	(0.0–0.0)	(0.0–0.0)	

Table 8 Frequency (number/10 min) of contact with the novel object in the coat colour groups in relation to the type of object with the lower quartile (Q1), median (Med), and upper quartile (Q3)

	Objects	Box	Ball	Umbrella	Toy car	P-value
White (n = 12)	Med	2.5	2.0	0.0	1.0	0.0077
	(Q1-Q3)	(2.0-3.0)	(1.3-2.0)	(0.0-0.0)	(1.0-1.8)	
Brown (n = 12)	Med	1.0	1.0	0.5	0.0	>0.05
	(Q1-Q3)	(1.0-1.8)	(0.3-1.8)	(0.0-1.0)	(0.0-0.8)	
Fawn (n = 8)	Med	1.5	0.5	0.0	0.0	>0.05
	(Q1-Q3)	(1.0-2.3)	(0.0-1.3)	(0.0-0.3)	(0.0-0.0)	

Discussion

In the present study, most alpacas exposed to novel moving objects (umbrella, toy car) performed abrupt movements (jumped back, attempts to escape), held their ears flat, and slightly raised their tails in response to the appearance of the object, which may suggest that these stimuli evoked fear. Similar signs can be observed during shearing in alpacas (Kapustka & Budzyńska, 2018; Kapustka & Budzyńska, 2020). The mean behavioural scores and frequency of contact with novel object in the toy car test were substantially lower in many cases and the latency values were substantially higher than in the box test. This implies that the moving object evoked a stronger reaction than the stationary one. Similar reactions were reported by Sandem *et al.* (2004) and Gibbons *et al*. (2009) in cattle and by Budzyńska *et al.* (2014; 2018) and Kamieniak *et al.* (2016) in horses. Intense physical activity during separation from the herd is positively correlated with an increase in heart rate in alpacas (Pollard & Littlejohn, 1995; Waiblinger *et al*., 2020); therefore, it can be regarded as an indicator of acute stress (Taraborelli *et al*., 2011). The same relationship was found in a study assessing horses' reactions to a novel stimulus (Budzyńska *et al*., 2014; Kamieniak *et al*., 2016; Budzyńska *et al*., 2018). In the present study, only behavioural responses of alpacas was assessed, without heart rate measurement, but we also observed the intense physical arousal reported by other authors (Pollard & Littlejohn, 1995; Waiblinger *et al*., 2020).

A positive correlation between heart rate and behavioural reaction in previous studies on alpacas (Pollard & Littlejohn, 1995; Waiblinger *et al*., 2020) or other species (i.e., horses), allows us to focus on behaviour as an indicator of stress. Many behaviours that indicate severe stress (intense motor activity, high-pitched alert sounds, attempts to kick, spitting) are observed in alpacas, vicunas, and guanacos during shearing (Arzamendia *et al*., 2010; Taraborelli *et al*., 2011; Waiblinger *et al*., 2020). Investigations conducted by Prágai and Kovács (2020) proved that the level of cortisol in alpaca's saliva increased during shearing. Elimination behaviours such as defecation and urination are considered to be stress reactions (Arzamendia *et al*., 2010; Taraborelli *et al*., 2011). Defecation and urination were observed in this study, especially in females. However, the frequency of this behaviour was very low. The studied alpacas sometimes displayed an alert posture (0%, 25%, 37.5%, 56% of animals exposed to the box, ball, umbrella, and toy car, respectively), which was described by some authors as a 'frozen posture,' with tail raised, head raised high, and ears erect and directed towards the object (Cavalcanti &

Knowlton, 1998; Kapustka & Budzyńska, 2018; Kapustka & Budzyńska, 2020; Kapustka & Budzyńska, 2021). Alert posture can be observed in alpacas and other SACs (llamas, vicunas, guanacos) in various situations that could be potentially dangerous for the herd (Cavalcanti & Knowlton, 1998; Arzamendia *et al*., 2010; Taraborelli *et al*., 2011; Kapustka & Budzyńska, 2018; Kapustka & Budzyńska, 2021).

In the present study, it was noticed that the alpacas observed the novel object carefully but not all animals dared to explore it (no exploration in 6%, 37.5%, 69% and 56% of animals exposed to the box, ball, umbrella, and toy car, respectively). The proportions of animals that did not explore were based on latency time. When the animal did not explore the object during the test, the latency was scored as 600 s (60 s × 10 min of presenting the object).

Some animals in this study showed evident interest in the novel object without any signs of fear (94%, 50%,12.5%, 37.5% of animals exposed to the box, ball, umbrella, and toy car, respectively), as their ears were directed towards the object and the tail was hanging loosely. They quickly approached, sniffed, and touched the object with their snouts. In other studies, if the llama approaches the object, it is measured as interest (Taylor & Davis, 1996; Cavalcanti & Knowlton, 1998). Similar reactions were observed in novel object tests carried out in horses (Bulens *et al*., 2015). The present study showed that a higher behavioural score was associated with more frequent contact with the object and a shorter latency value. Herskin *et al*. (2004) found that the frequency of contact with an umbrella in cows was higher before its opening. In our study, alpacas also preferred contact with static rather than dynamic novel objects. As demonstrated by Grandin (1997), a novel object can be attractive to animals. Cattle or pigs were found to approach and even manipulate a dropped piece of paper. In turn, as reported by Hemsworth *et al.* (1996), pigs prefer new objects to old ones, whereas cattle exhibit neophobia (they prefer known to unknown objects). The present observations suggest that some alpacas prefer novel objects, which in turn can be stressors to the others. The animals that achieved a high behavioural score and short latency reacted to the novel object with curiosity and approach behaviours; low behavioural scores and long latency indicated a stress response and avoidance behaviours. Similar conclusions were drawn by Van Reenen *et al.* (2004). They observed the reactions of calves to a novel object and found that some of the animals reacted with fear and the others with curiosity. In response to a novel stimulus, animals may experience a motivational conflict between fear and curiosity (Herskin *et al*., 2004). This was similar in the present study, as some alpacas initially walked away from the object, but approached it after some time to explore it; the latency was longer than in animals that did not show fear and approached the novel object immediately.

Animal reactions in a new situation can be conditioned by the human–animal relationship. Windschnurer *et al*. (2020) indicated that regular, gentle tactile, visual, and auditory contact between the alpaca and caretaker makes the animal calmer and it show no signs of fear during handling. In contrast, some animals were completely uninterested in the objects and did not show any signs of stress, as they ruminated or kept lying (even next to the object). This was observed in 6%, 25%, 50%, and 6.5% of animals exposed to the box, ball, umbrella, and toy car, respectively. Van den Heede *et al*. (1998) suggested that feeding and keeping close distance to a novel object were not signs of fear in sheep.

Reducing feed intake is a sign of stress in alpacas (Pollard & Littlejohn, 1995). Hence, alpacas that ruminated during the observations were not stressed. In dairy cows, time spent on feed intake and rumination affects milk production (Johnston & De Vries, 2017) and it is associated with comfort of lying (Ito *et al.,* 2009). It can be concluded that prey animals must feel safe when they are eating, ruminating, and lying. Humming sounds were made by almost all the alpacas, regardless of their behavioural score in the test and may suggest a feeling of social isolation and the desire to return to the herd (despite the presence of the other animal during the tests), rather than a reaction to the novel object. Grumbling sounds were observed in guanacos during enclosing and handling (Taraborelli *et al*., 2011) and alpacas during restraint (Waiblinger *et al*., 2020) and shearing (Kapustka & Budzyńska, 2020). As reported by Grandin & Shivley (2015), vocalization in response to separation from the herd is exhibited by sheep and cattle.

To date, there have been no studies regarding the correlations of coat colour with alpaca temperament. The white alpacas achieved substantially higher mean behavioural scores when exposed to the toy car, which suggests that they may be less reactive to stronger stimuli than those with the other coat colours (brown, fawn). The white alpacas were also the most curious and approached the novel object quicker and more frequently than the brown and fawn ones. An opposite relationship was found by Kim *et al.* (2010) in Jindo dogs, where fawn dogs showed a lower level of fear response in behavioural tests than white dogs. Certain gene mutations associated with coat colour can also affect psychological traits, such as excitability or timidity (Brunberg *et al*., 2013). Correlations of the coat colour with certain temperament traits are suggested e.g., in horses (Brunberg *et al*., 2013; Finn *et al*., 2016), cattle (Tőzsér *et al*., 2003), some sheep breeds (Loehr *et al*., 2008), and dogs (Kim *et al*., 2010). A

similar relationship may also occur in alpacas. In our study, white alpacas were more curious and tended to observe and explore novel objects. There are possibly some temperament genes associated with coat colour that could be determined by future genetic analysis. If this relationship is confirmed, it could be used for genetic selection purposes.

Our observations showed that the younger individuals (group of young females) were characterized by a slightly higher level of curiosity to the novel object than the older individuals (especially the older females). It could be useful information for alpaca breeders and animal-assisted therapists to start early handling and training of alpacas when their level of curiosity is higher (especially in young females). An effect of the age on the exploratory behaviour of horses was demonstrated by Finn *et al.* (2016) and Bulens *et al.* (2015). Young horses prefer contact with novel objects more than older ones (Bulens *et al*., 2015). This was also observed in sheep, i.e., older animals exhibited a higher level of stress than younger ones to a novel object (Van den Heede *et al*., 1998).

Conclusions

Based on behavioural observations, it can be concluded that novel objects evoked several responses such as fear, curiosity, or indifference in the alpacas. Fearful alpacas showed more startle behaviours (jumping, running/walking away) and alertness; curious alpacas showed novel object-directed exploratory behaviour (sniffing at a short distance; minimum 20 cm) and/or touching the object with the snout); and indifferent ones ruminated or kept lying, even next to the object. We found some relationships among parameters of the animal's behavioural reactivity, including the latency value, which was negatively correlated with the frequency of contact with the novel object and the behavioural score achieved by the alpacas for their reactions to the novel objects. Therefore, the higher the behavioural score for the response to the novel object, the higher the frequency of contact with the novel object and the shorter the latency of contact with the object. Similar conclusions were presented by Van Reenen *et al.* (2004), who analysed responses of calves to a novel object. We found no correlations between responses to different novel objects. The behavioural response was linked to the type of object. In our study, we noticed that the combination of novelty and suddenness of the object can intensify the alpaca's reaction. The behavioural responses of the analysed alpacas indicate that unknown objects, especially moving objects such as the toy car or a suddenly-opened umbrella, were fear-eliciting stimuli to these animals. A relationship between coat colour and behavioural reactivity may occur in alpacas. If this relationship is confirmed by future genetic analysis, it could be used for genetic selection purposes. Our method of measuring reactions to both types of unknown objects, stationary and moving, can be a useful way to characterize the alpaca's temperament profile. Testing responses to novel objects is an easy and objective method for assessing the suitability of particular individuals for various uses. It can be useful to evaluate behavioural predispositions of animals for many activities, such as trekking and alpaca-assisted therapy. This study confirms that novel object testing is a reliable method to measure behavioural indicators of fearfulness/curiosity in alpacas. It can be a valuable tool to improve animal welfare.

Authors' contributions

JK (ORCID: 0000-0001-8175-7299) made substantial contributions to conception and design of the study, participated in acquisition of data, performed statistical analysis and interpretation of data, and was involved in drafting the manuscript. MB (ORCID: 0000-0002-6586-151X) participated in design and coordination of the study, performed interpretation of data, was involved in drafting the manuscript and revising it critically for important intellectual content. All authors read and approved the final manuscript.

Conflict of interest declaration

The authors declare no conflicts of interest.

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